ACP01
Effect of sulfadiazine-contaminated pig manure on abundance of genes involved in nitrogen transformations in the rhizosphere of maize and clover

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The antibiotic sulfadiazine (SDZ) can enter the environment by application of manure from antibiotic-treated animals to arable soils. Since antibiotics are explicitly designed to affect microorganisms, they are likely to impair "non-target" microbes in the soil ecosystem so that important soil functions could be compromised and processes in nutrient cycles might be disturbed. Using real-time PCR, the impact of sulfadiazine-contaminated pig manure on the functional microbial communities involved in key processes of the nitrogen cycle (nitrogen fixation, ammonia oxidation, and denitrification) was investigated in a mesocosm experiment in the rhizosphere of two different agricultural crops: maize (*Zea mais*) and berseem clover (*Trifolium alexandrinum*). Manure and manure contaminated with SDZ were applied to the soil surface at concentrations of 40 ml manure and 20mg SDZ kg^{-1} soil, respectively. Sampling was performed 10, 20, and 30 days after the application. The results indicate that sulfadiazine lowered the abundance of all targeted functional genes in the rhizosphere of both plant types, a time-lag being observed between the application of the sulfadiazine contaminated manure and its effect on the microbial biomass. The effect was more pronounced in the rhizosphere of clover. The abundance of transcripts of the respective N-cycling genes was affected as well but to a lower extent. These results demonstrate that the application of manure contaminated with sulfadiazine influences the microbial nitrogen turnover processes in this soil compartment. However, no clear effects on either plant biomass or plant C and N contents were observed.

ACP02
Combined ramR mutation and presence of a Tn1721-associated tet(A) variant in a clinical isolate of *Salmonella enterica* Serovar Hadar resistant to Tigecycline

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A *Salmonella enterica* Serovar Hadar strain resistant to tigecycline (MIC 16 µg/ml) was isolated. Molecular characterization revealed the presence of a plasmid-borne tet(A) variant associated with Tn1721 mediating a rise of the MIC for tigecycline when transferred to Escherichia coli. Additionally, a truncating mutation in ramR was detected. Transformation with wildtype ramR but not with the mutated ramR lowered the MIC for tigecycline. Characterization of this Salmonella isolate thus implicates ramR in resistance to tigecycline.

ACP03
Entomopathogenic bacteria as multiproducers of bioactive natural products

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Genome sequencing projects have revealed bacterial strains of the genus *Xenorhabdus* and *Photorhabdus* as multiproducers of several different secondary metabolites: More than 20 different biosynthesis gene clusters have been identified in the genome sequences currently available. The reason for this wealth of natural product diversity might be due to the complex biological context these bacteria live in: They live in symbiosis with nematodes of the genus *Steinernema* or *Heterorhabditis*, respectively, and together with the nematodes they are involved in infection and killing of soil dwelling insect larvae using insecticidal proteins and small molecules. Nematodes, bacteria and insects are a very useful model system to study the bacterial symbiosis towards nematodes and their pathogenesis towards insects. We have analyzed the natural products produced by these bacteria and could readily identify several new compounds. Among them unusual lipids with a urea moiety which might be involved in inhibition of the insect juvenile hormone III epoxide hydrolase as similar synthetic compounds have already been described as potent inhibitors of this enzyme. Moreover, we could recently identify the biosynthesis gene cluster for the xenocoumacins, antibiotics produced by different Xenorhabdus strains, and could show that a new type of pyrrolidine ring formation is involved in the biosynthesis of xenocoumacin-2. Furthermore, analysis of the regulation of this biosynthesis gene cluster indicate that xenocoumacin-2 might in fact be an degradation product of the more active xenocoumacin-1 for which Xenorhabdus seems to be sensitive.

ACP04
Detection of a novel high–level gentamicin resistance determinant in *Streptococcus agalactiae*

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Streptococcus agalactiae causes life threatening invasive infections in newborn and adult patients. We recently published a *S. agalactiae* isolate originating from a patient with cystic fibrosis that demonstrated high-level gentamicin resistance (HLGR). It is to our knowledge the second publication of HLGR in this species. Molecular characterization of the strain by PCR revealed the presence of the *aacA-aphD* gene, which is frequently associated with a transposon structure. However the flanking sequences in our strain were different and the typical transposon structure of HLGR could not be detected. Plasmid purification of the strain was performed, resulting in the isolation of an extrachromosomal element. For further characterization, the nucleotide sequences flanking the *aacA-aphD* gene in our strain were sequenced and revealed a novel HLGR resistance determinant, harboring various fragments of a large enterococcal plasmid. It has the ability to confer HLGR in streptococci as well as in enterococci, since transfer of the isolated element into an *Enterococcus faecalis* strain caused the gentamicin resistance of this train to increase from 8 mg/l to 512 mg/l. *S. agalactiae* is not routinely tested for HLGR. To evaluate, if other strains with HLGR could be isolated, we tested 532 *S. agalactiae* strains for high level-gentamicin resistance by plating the strains on a screening agar supplemented with 256 mg/l of gentamicin. The strains originated from invasive infections of pediatric patients as well as colonizing strains from adult patients and were collected in Germany and Portugal. In none of these strains HLGR was found. In conclusion we were able to detect a novel HLGR resistance determinant in *S. agalactiae* that has previously not been described. The potential of spread within the *S. agalactiae* population or other species represents a serious clinical threat, since gentamicin is part of the recommended treatment regimen of *S. agalactiae*.

ACP05
Exploring Bacteriocin Diversity in Lactic Acid Bacteria

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Ribosomally synthesized antimicrobial peptides are produced by a wide variety of organisms, from bacteria to humans. Among these, bacteriocins produced by "food grade" lactic acid bacteria (LAB) have attracted great attention, because of their potential application as nontoxic food preservatives and therapeutic agents for the treatment of gastrointestinal infections. Due to the "generally recognized as safe (GRAS)" status of most LAB, bacteriocin producing cells can be used directly as "bioprotective cultures". Alternatively, purified bacteriocin preparations like Nisaplin® may be employed to prevent growth of spoilage and pathogenic bacteria.
We have investigated the production of antimicrobial compounds in field isolates of diverse LAB strains and evaluated them regarding their use in food preservation. On agar plates, more than 10% of the tested strains showed inhibition of at least one of the indicator strains (*Bacillus cereus, Brochothrix thermosphacta, Listeria monocytogenes, Salmonella enterica, Staphylococcus aureus*). Of these, 10% revealed expression levels in liquid culture that were sufficient to be used without further purification. Preliminary analysis of the active substances indicated that most of them belong to class II bacteriocines i.e. small, heatstable, antilisterial proteins.
Cell free culture supernatants of eight selected strains were used in challenge tests to prove their efficiency in food. Unexpectedly, when tested in milk or in minced meat, only few turned out to be effective in reducing cell counts of Listeria monocytogenes. To identify the active components, we followed a biochemical approach of purification and analysis by mass spectrometry. In parallel, we isolated genes of class IIa bacteriocines by PCR. The differences in structure and activity as well as the consequences for novel bacteriocin screening programs are discussed.

ACP06
Genetic reconstruction of protozoal rRNA decoding sites in bacteria reveals that the mitochondrial ribosome is not a suitable target for antiprotozoal drug development

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Infections with protozoal pathogens, e.g. *Plasmodium* spp., *Leishmania* spp., *Trypanosoma* spp., affect hundreds of millions of the world's population. Only a limited number of compounds are available for treatment of corresponding infections, testifying for the need to identify suitable drug targets. Protozoa have up to three ribosomes: cytoplasmic, mitochondrial and plastidial. Both the protozoal cytoplasmic and the plastidial ribosome are validated drug targets. It is, however, unclear whether the protozoal mitochondrial ribosome is a suitable target for development of compounds with antiprotozoal activity. We have used genetic means to construct bacterial-protozoal hybrid ribosomes by transplanting various versions of the eukaryotic small subunit's *rrn* decoding A-site into bacterial ribosomes. We did this with the view to establish an experimental model for the study of protozoal A-site function and its interaction with aminoglycoside antibiotics. We also generated bacterial ribosomes with single point mutations representative of the drug pocket's polymorphic rRNA residues, so as to allow reconstruction of the various protozoal drug binding sites (cytoplasmic, mitochondrial, plastidial). Relating in-vitro drug susceptibility of the genetically reconstructed drug binding sites to that of in-vivo efficacy indicates that the mitochondrial ribosome is not a suitable target for antiprotozoal drug development.

ACP07
Whole genome sequencing of clinical isolates of *Streptococcus mutans* gives insights into the genetic basis for susceptibility to the biofilm inhibitor carolacton

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Streptococcus mutans is a member of the dental plaque biofilm community and etiological agent of caries, the most widespread infectious disease in humans. A screen for compounds which inhibit biofilm formation, but not growth, discovered carolacton, a secondary metabolite produced by the myxobacterium *Sorangium cellulosum*. Carolacton apparently interferes with the density dependent signalling network of *S. mutans*. To determine the genetic basis for the susceptibility to carolacton, we sequenced the genomes of nine Streptococci with different sensitivities, among them six isolates of *S. mutans* with different clinical background, as well as the closely related species *S. sobrinus* and *S. rattus*, using Solexa technology. The genomes will be assembled and annotated and a comparative genome analysis using a data warehouse designed for this study will be conducted. The data will form the basis for setting up regulatory networks related to quorum sensing and biofilm growth. In combination with a transcriptome study aimed at identifying the molecular target of carolacton, these data should allow to understand on a systems biology level how sensitivity to this new biofilm inhibitor is genetically determined.

ACP08
Synthesis and characterization of the bacteriocin produced by the Lactobacillus YR strain in the presence of prebiotics

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The probiotic cultures of lactic bacteria synthesizing bacteriocins are used in the food industry to obtain yoghurt and other dairy products. These peptides have a special practical importance due to the thermostability, which is demonstrated even for small concentrations, in case of peptides isolated by precipitation. This is one of the most important properties of the probiotic strains of lactic bacteria synthesizing bacteriocins. In order to obtain probiotic products, in addition to the capacity of synthesizing bacteriocins, the strains must adhere and colonize the intestinal tract.
The aim of the study is to determine the effect of the prebiotics on the synthesis of a bacteriocin, as well as to provide its partial biochemical characterization. The Lactobacillus paracasei YR strain producing bacteriocins was used. Bacillus cereus CMGB 215, Listeria innocua CMGB 218 and Escherichia coli CBAB2 were used as sensitive strains. The synthesis of the bacteriocin was tested by using the MRS medium, even if the carbon source was replaced by other carbohydrates. In order to determine the prebiotics effect on the synthesis of the bacteriocin, MRS was supplemented by 1% prebiotic. The partial biochemical characterization of the bacteriocin was realized by determining the thermostability (at 60, 80, 100 and 1210C, for 15 minutes), pH (2, 5, 7, 9, 11), enzymes (proteolytic and nonproteolytic) and organic solvents with a concentration of 10%. The partial purification of the bacteriocin was made by adding ammonium sulphate and by freeze-drying the resulted precipitation, which was tested in parallel to the freeze-dried and concentrated supernatant.
Due to the resistance to pH and T, the bacteriocin can be used to obtain products acting on the biological control of the human gut flora. The studies indicated that the type of the culture medium and the used prebiotic influenced directly the inhibiting capacity. It was proved that lactulose with a concentration of 1% determined the maximum inhibiting capacity. The bacteriocin was still active in the presence of enzymes.

ACP09
Development of a photometric test system for detecting inhibitory substances from *Pseudoalteromonas citrea* DSM 8771^T and *Pseudoalteromonas aurantia* DSM 6057^T

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Some *Pseusoalteromonas* species are producing inhibitory substances, which do not inhibit bacterial growth directly by themselves but by inducing a hydrogen peroxide production in the target organism. With increasing H_2O_2 concentration, this substance is subsequently killing the exposed bacteria. Hence H_2O_2 is involved in the inhibitory mechanism, the antibacterial activity can be neutralised by catalase or peroxidase. This could also be proved during this study for the inhibitors from *Pseudoalteromonas citrea* DSM 8771^T and *Pseudoalteromonas aurantia* DSM 6057^T. To examine, if a bacterial sample has inhibitory activity by using a classical inhibitory plate test system, it is necessary to incubate the test plate at least over night. To overcome such a long incubation period by saving time thereby, a photometric test system for detecting inhibitory substances produced by the organisms mentioned above was developed in the present study in which inhibitory activity could be detected already after 2 h. This test system includes ferrous sulfate which in the presence of hydrogen peroxide is oxidised to ferric sulfate forming a red dye complex with potassium thiocyanate. The colour intensity of this complex is directly proportional to the corresponding concentration of H_2O_2 in the sample.

ACP10
Regulatory aspects of pristinamycin biosynthesis

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Streptomyces pristinaespiralis produces the streptogramin antibiotic pristinamycin which is a mixture of two types of chemically unrelated compounds: pristinamycin PI and PII. Pristinamycin PI is a cyclohexadepsipeptide, belonging to the B-group of streptogramins, while pristinamycin PII has the structure of a polyunsaturated macrolactone of the A-group of streptogramins. Both components inhibit the elongation process of the protein biosynthesis by affecting the peptidyltransferase within the 50S subunit of the ribosome. Each compound alone exhibits only a weak bacteriostatic activity whereas the combination of both substances leads to a synergistic effect that results in the bactericidal activity of pristinamycin. The genes responsible for pristinamycin biosynthesis, regulation and resistance are organized as a single large gene region, in which the genes encoding PI and PII are spread over at least 260 kb. Within this gene region, seven regulatory genes were identified that are probably involved in pristinamycin biosynthesis: spbR, papR1, papR2, papR3, papR4, papR5 and papR6.

SpbR (*S. pristinaespiralis* butyrolactone-responsive transcriptional repressor) is a specific receptor protein for γ-butyrolactones. papR1, papR2 and papR4 encode for proteins that are homologous to SARPs (Streptomyces antibiotic regulatory protein), which are pathway-specific transcriptional activator proteins, whereas papR3 and papR5 encode both for proteins belonging to the family of TetR repressors. papR6 encodes a putative protein similar to the response regulators of bacterial two-component signal transduction systems. To investigate the function of the regulatory genes, deletion and overexpression strains were constructed. The results obtained from analyzing the regulatory mutants and overexpression strains, as well as from RT-PCR- and bandshift-experiments revealed a complex signaling cascade which is responsible for the fine-tuned regulation of pristinamycin production.

ACP11
First description of *Escherichia coli* producing CTX-M-15 extended spectrum beta-lactamase (ESBL) in outpatients from south-eastern Nigeria

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Objectives: The prevalence of extended-spectrum beta-lactamases (ESBL) in *E. coli* is increasing worldwide, mainly due to the spread of ESBLs of type CTX-M-15. Plasmids with CTX-M-15 often also carry *aac(6´)-Ib-cr* and OXA-1 beta-lactamase genes.
>Methods: A total of 44 single patient ESBL *E. coli* strains collected from two hospitals located in Enugu (n = 28) and Abakaliki (n = 16) in south-eastern Nigeria were used in this study.
Confirmation of the ESBL phenotype was done by a double-disk synergy test. Initial species identification was confirmed by MALDI-TOF analysis.
Identification of ESBL genes was performed by specific PCR tests and subsequent sequencing. In addition, PCR for OXA-1 beta-lactamase was performed. Screening for *aac(6´)-Ib-cr* was done by a specific PCR followed by restriction with BseGI.
Results: Of the 44 ESBL *E. coli* strains 34 (77.2%) were isolated from urine, 6 (13.6%) from vaginal swabs and 4 (9.0%) from wound swabs. 30 (68.1%) strains were isolated from female patients and 16 (36.4%) strains were from outpatients. The age range of patients was 7 to 72 years, but the majority of patients (n = 42; 95.5%) were under the age of 30.
Using specific PCRs we could demonstrate CTX-M-1 cluster enzymes in 43 (97.7%) of strains, three of which were selected for sequencing and found to be CTX-M-15. A positive PCR for OXA-1 was found in 40 (90.9%) strains and *aac(6´)-Ib-cr* could be demonstrated in 40 (90%) strains.
Conclusion: For the first time we could demonstrate CTX-M-15, found almost always in association with OXA-1 and *aac(6´)-Ib-cr*, as the dominant ESBL type in *E. coli* strains from south eastern Nigeria. Remarkably a high proportion of the tested strains were from outpatients. For the first time *aac(6´)-Ib-cr* was detected in *E. coli* strains from Africa.

ACP12
Broad range antagonistic activity of complex cheese surface microbial consortia against food-borne pathogens

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The microbial surface ripening consortia of 49 soft cheese samples produced by artisan cheese making techniques were investigated with respect to their potential to inhibit the food borne pathogen *Listeria monocytogenes*. *L. monocytogenes* EGDe (serovar 1/2a) was cultivated in cell-free supernatants (CFS) of the complex consortia. Eight consortia with a bacteriocidal activity reduced an initial *Listeria* inoculum of 5×10^7 cfu/ml to zero after 24 hours of incubation; another 5 displayed a bacteriostatic action. Neither the pH values of the supernatants nor their content of lactate or hydrogen peroxide could account for this strong inhibition. Alternative indicator strains like other *Listeria* species, *Bacillus cereus*, *Staphylococcus aureus* and even *Salmonella enterica* serovar Typhimurium were also killed after exposure to the complex CFS. A taxonomical analysis of two anti-listerial consortia using FT-IR spectroscopy yielded a considerable species diversity with comparatively high fractions of lactic acid bacteria. The active transcription of lactococcin G on the cheese surface by the indigenous microbiota could be demonstrated by RT-PCR in five samples. Individual isolates were tested for inhibition of *L. monocytogenes* on solid and in liquid medium but no pure cultures displaying a strong inhibitory action were found. Two complex CFS were analysed via HPLC and LC-MS to identify inhibitory substances.

ACP13
Partial characterization of the anti-listerial action exerted by the yeast *Pichia norvegensis*

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In contrast to bacteria expressing an anti-bacterial activity, very little is known about the inhibitory action of yeasts towards bacteria, especially *Listeria monocytogenes*. Goerges et al. [1] discovered yeasts with the potential to inhibit the growth of *L. monocytogenes*. To characterize the anti-listerial activity generated by an exceptionally active *Pichia norvegensis* strain, methods and protocols for studying anti-bacterial substances produced by bacteria [e.g. 2, 3] were applied and modified. The anti-listerial activity of the *P. norvegensis* strain was only seen when *Listeria* and *Pichia* grew in a co-culture, either on an agar surface or in liquid media. The yeast exerted a bacteriocidal effect. Inhibition was maximal in the stationary growth phase of the yeast. No inhibition occurred, when *Listeria* were added to a co-culture after 24 h or to a yeast culture after 72 h, respectively, but addition of fructose, glucose or mannose restored the anti-listerial activity, which might indicate that an active yeast metabolism is needed for inhibition. Minimal concentrations of three non-ionic and one zwitterionic detergents effectively abolished the inhibitory activity. While various experiments using the cell-free yeast culture supernatant did not show any inhibitory activity whatsoever, cultivation of *Listeria* and *Pichia* separated by a membrane indicated that the inhibitory activity did not depend on cell-cell contact. It might be possible that the *P. norvegensis* strain produces one or more diffusible and unstable anti-listerial substances that have to be synthesized constantly in order to inhibit *Listeria*.

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ACP14
A hydrolytic enzyme from Bacillus sp. and its application in wine making

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The conversion of must into wine is a complex microbiological process. A great variety of different yeasts and bacteria could be involved. Besides desirable microorganisms such as the ethanol-producing wine yeast Saccharomyces cerevisiae many wild-type yeasts and lactic acid bacteria can be found in fermenting musts. They are able to produce multiple off-flavours leading to a decreased wine quality. In order to reach a high standard of quality there is a need to inhibit the growth of these microbial groups and their unwanted compounds.
One representative of spoilage lactic acid bacteria is *Pediococcus parvulus*. It can possibly produce an exopolysaccharid, consequently the wine turns to a slimy and thick texture (graisse). In this context relative small concentration of this β-1,3-glucan can hamper wine filtration.
Further risks in the wine-making process are film formation, cloudiness, gas production as well as different off-flavour and off-odours caused by several yeasts of the genera Candida, Debaryomyces, Kloeckera, Pichia, Brettanomyces, Schizosaccharomyces and Zygosaccharomyces.
This study presents the isolation and characterization of a novel and applied usable β-1,3-glucanase from Bacillus sp. The enzyme cleaves the reference compound laminarin in an endo-type mode with a specific activity of 10 units per mg protein.
Moreover it is also capable to hydrolyze the exopolysaccharide of *Pediococcus parvulus* and therefore the viscosity of wine is minimized. However, the cell wall of yeasts - mainly consisting of β-1,3-Glucan - can also be degraded, thus spoilage yeast growth is inhibited.
Therefore, the isolated enzyme is a suitable tool to prevent ropiness and the growth of unwanted wild-type yeasts.

ACP15
Comprehensive investigation of *Bacillus subtilis* cellular response toward squalamine

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The emergence of multidrug-resistant microorganisms has motivated major efforts to develop new classes of antibiotics. In recent years, a high amount of low molecular weight antibiotics have been isolated from diverse animal species. Among these substances, squalamine, a water soluble cationic amino sterol, has been isolated from the dogfish shark Squalus acanthias. Squalamine exhibits potent antimicrobial activity against both Gram-positive and Gram-negative bacteria and shows haemolytic activity only at (200 mg/mL) suggesting its potential application in human medicine. [1] Squalamines mode of action is yet unknown although the cell membrane is discussed as a possible target. In order to investigate the cellular response of *Bacillus subtilis* toward squalamine we employed proteomics approaches. Using 2D gel-based protomics we studied the changes in cellular protein synthesis rates caused by sublethal dosis of squalamine [2]. In order to address membrane proteins - which escape detection via 2D gels due to their extreme hydrophobicity - we also performed two complementary MS-based shotgun approaches [3], applied by Hahne *et al.*. [4] One comprises a membrane preparation with carbonate and high-ionic-strength buffers, followed by SDS-PAGE and LC-MS/MS analysis. The other is based on enzymatic trimming of the crude membrane fraction with proteinase K followed by gel-free analysis. Quantification was facilitated by *in vivo* 14N/15N-metabolic labeling of proteins.

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ACP16
Studies on potential drug targets in Gram-positive bacteria - a Manganese Metallo-cofactor in the Native Ribonucleotide Reductase (RNR) from *Bacillus subtilis*

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Ribonucleotide reduction, the unique step in the pathway to DNA synthesis, is catalyzed by enzymes in radical-dependent redox chemistry involving an array of diverse metallo-cofactors. Manganese control of growth and DNA precursor biosynthesis [1] suggest a manganese-type of RNR in *Bacillus subtilis*. A three-step protocol for purification of the small subunit (R2F) of the RNR from *B. subtilis* by fast liquid chromatography was developed. This provided R2F as a native metallo-cofactor with a high manganese and a low iron content easily to discriminate against a recently described iron-treated RNR of Bacillus cereus [2]. Sensitivity of the *B. subtilis* R2F as a potential drug target to different inhibitors will be studied for comparison with Mn-dependent RNR in Actinobacteria [3].

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ACP17
Discovery of new lantibiotic producers by genomic data mining

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Rapid evolution and spread of multidrug-resistant bacteria necessitate searching for new antibiotic substances and sources. Lantibiotics (*lan*thionine containing an*tibiotics*) are small microbial peptide antibiotics that exhibit antibacterial activities against grampositive bacteria including drug-resistant strains like MRSA (methicillin resistant *Staphylococcus aureus*). The gene-encoded and ribosomally synthesized lantibiotic peptides undergo extensive posttranslational modifications that result in the formation of the characteristic non-proteinogenic amino acids lanthionine and methyllanthionine. All genes that are necessary for lantibiotic production and modification (*lanA,M,B,C*), producer self protection (*lanI,E,F,G*), regulation (*lanR,K*) and export (*lanT*) are organized in gene clusters. All lantibiotics described so far are produced by grampositive bacteria.
Blast searches employing the biosynthetic enzymes (LanM,B,C) in the NCBI database showed that the occurrence of ORFs coding for lantibiotic modification enzymes is widespread about bacteria of different phyla. Based on these genomic data mining results, we were able to identify novel lantibiotic gene clusters in the grampositive bacteria *Bacillus licheniformis* DSM 13 and *Anaerocellum thermophilum* DSM 6729 as well as in the gramnegative *Nostoc punctiforme* ATCC 29133 strain.
Further experiments to characterize the two-peptide lantibiotic gene cluster of *B. licheniformis* DSM 13 confirmed the production of an antimicrobial substance that is based on the predicted gene cluster. Insertion mutants of the biosynthetic enzyme genes (*licM1, M2*) were characterized by a loss of activity. Furthermore, subtractive Maldi-TOF analysis of these mutants predicted masses of 3021 Da and 3251 Da for the lichenicidin peptides. In conclusion, the genomic data mining approach resulted in the identification of the new lantibiotic lichenicidin.

ACP18
Antibiotic Mechanism of Action Studies in *Bacillus subtilis*: Proteomic Investigation of Short Cationic Peptides and Fatty Acid Biosynthesis Inhibitors

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The cellular response of bacteria to sublethal doses of antibiotics has been shown to be indicative of the antimicrobial mechanism of action. Therefore, global proteomic studies can be used to identify antibiotic mechanisms by comparing the bacterial response to a reference library of response patterns [1,2]. Using this approach, cationic peptides and ferrocenyl-peptides with known antibacterial activity [3,4] were analyzed to elucidate their mechanisms of action. Similarly, fatty acid biosynthesis inhibitors targeting different enzymes in the pathway were investigated to complement the reference library with proteomic response patterns for fatty acid synthesis inhibition.
Proteomic response patterns of the short cationic peptides revealed that the cytoplasmic membrane is the antibacterial target. Strong similarities with the membrane-active substances triton X-100 and valinomycin as well as the cell wall biosynthesis inhibitor bacitracin, which inhibits a membrane-associated biosynthesis step, were observed. Fatty acid biosynthesis inhibitors induced a number of pathway-specific changes. Additional changes indicative of the inhibited biosynthesis step were observed. These results provide evidence for another regulatory mechanism of fatty acid biosynthesis in *B. subtilis* besides the known regulation by the FapR repressor [5].

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ACP19
Isothermal titration calorimetry and biosensor application to simulate the antibiotic mode of action of peptides interacting with membranes

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Vancomycin and the lantibiotic gallidermin possess the antibiotic activity by targeting lipid II and thus inhibiting cell wall biosynthesis. In the present study we focussed on the mode of action by simulating membrane interactions of both peptides using different model membrane approaches and biosensor techniques.
Quartz crystal microbalance (QCM) and surface acoustic wave (SAW) were applied as different mass-sensitive biosensors to determine the binding affinities of both peptides to model membranes, consisting of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) and DOPC supplemented with 0.1 mol% lipid II. The kinetic binding data indicate that membrane interaction of vancomycin is increased in the presence of the target structure lipid II. However a much stronger binding of gallidermin to pure DOPC membranes is evident and thus may cover the existing lipid II binding ability. Additional data from the isothermal titration calorimetry (ITC)-measurements, which illustrate unspecific ways of peptide-membrane interactions, obviously peptide insertion into the membrane, show clearly that vancomycin has no affinity to membranes, while gallidermin significantly interacts with the membrane. These results might explain the different antibiotic activity of vancomycin and gallidermin towards various bacterial strains, although both peptides share the same target structure.
The combination of biosensor technology and the thermoanalytic ITC allows us to get a deeper insight into membrane-associated processes and the role of non-specific membrane interactions on the efficacy of peptide antibiotics.

ACP20
The simulation of the antibiotic mode of action of the lipopeptide friulimicin B using different model membrane approaches

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Friulimicin B, an amphipathic cyclic lipodepsipeptide possesses antibiotic activity against gram-positive bacteria. It consists of a cyclic decapeptide, which is connected to a C14 fatty acid. Friulimicin shows structural similarity to daptomycin, which was recently approved for the therapy of skin/skin structure infections and shown to interact with bacterial membranes in a Ca^{2+}-dependent way. An insertion of daptomycin into the membranes followed by membrane leakage is assumed. Friulimicin was supposed to act similar. However, recent studies reported the bactoprenol phosphate carrier C_{55}-P as the friulimicin target.
Several aspects of calcium-dependency and C_{55}-P-targeting of friulimicin remain to be elucidated. The present model membrane study focusses on the role of Ca^{2+} and the impact of C_{55}-P for the intensity of friulimicin interaction with model membranes (DOPC±C_{55}-P). Binding kinetics were detected by quartz crystal microbalance (QCM) and supported by atomic force microscopy imaging (AFM). Friulimicin was applied as a calcium-free or calcium-associated lipopeptide.
Initial studies could clearly exclude a membrane solubilization by friulimicin. The necessity of Ca^{2+} for the friulimicin-membrane interaction was shown with QCM and confirmed by AFM. Friulimicin-Ca^{2+} interacted with DOPC membranes. Binding affinity was further increased in the presence of C_{55}-P, confirming C_{55}-P as friulimicin target structure. Findings were supported by AFM - an intensive membrane surface accumulation of the peptide in the presence of Ca^{2+} and C_{55}-P was demonstrated.
Our data provide evidence and support recent findings that friulimicin interferes with the cell wall biosynthesis by interacting with C_{55}-P. The important role of Ca^{2+} for this activity might be interpreted with respect to two synergistic activties: A charge induced contact formation with the membrane and the induction of a conformational change and/or multimerization of friulimicin allowing the interaction with C_{55}-P.

ACP21
Comparison of the *Bacillus subtilis* stress response against two unusual antimicrobial compounds, Rhamnolipid and Hydrophobin

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Soil living bacteria have to fight on several fronts at the same time to survive. They have to adapt to the everchanging biophysical properties of their habitat, as well as nutrient limitations in the face of numerous competitors. Bacteria can access at this point by producing antimicrobial substances. In addition to established antibiotics numerous other less wellknown microbial compounds also have antimicrobial (side-)properties. This extended "collection of antibiotics" includes rhamnolipids or hydrophobins. Rhamnolipids are biosurfactants that are produced by *Pseudomonas aeruginosa*. They are amphiphilic molecules with effective surface-active properties. They are used for the bioremediation of hydrocarbons, heavy-metal-contaminated sites, and treatment of oil spills. To gain insight into the mechanism of rhamnolipid action, we investigated the corresponding stress response of *Bacillus subtilis*, a soil-living, Gram-positive model organism, which is well-understood with regard to its stress response network, which is orchestrated by numerous extracytoplasmic function (ECF) σ-factors and two-component systems (TCS). Genome-wide transcriptional profiling demonstrate that three major players in the cell envelope stress response of *B. subtilis*, the ECF sigma factor SigW, SigM and the TCS LiaRS were strongly induced by rhamnolipids. Furthermore, we investigated the response of *B. subtilis* against a hydrophobic protein of the soil living fungus *Aspergillus nidulans*. These small secreted fungal proteins play a role in growth and development of filamentous fungi. Due to their amphiphilic properties, hydrophobins are used as surfactants, emulsifiers in food processing, surface coating and immobilization applications. Despite some common characteristics between the two compounds, our results indicate an altogether different stress response of *B. subtilis* that involves a complete respiratory switch. The implications of our findings will be discussed.

ACP22
Identification of the molecular mode of action of Carolacton, a novel biofilm inhibitor

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The aim of this study is the investigation of the molecular mode of action of the recently discovered biofilm inhibitor Carolacton on *Streptococcus mutans*, the main causative agent of dental caries.
Virulence properties of *S. mutans*, e.g. acid tolerance, bacteriocin production and biofilm formation are mainly controlled via a species specific quorum sensing system consisting of an autoinducer named competence stimulating peptide (CSP, encoded by comC), the two component signal transduction system ComDE and the downstream regulated alternative sigma factor ComX.
First results derived from a comX luciferase-reporter strain and comCDE knock-out mutants revealed that Carolacton might interfere with this quorum sensing system. Furthermore the effect of Carolacton on all 12 two component signal transduction systems of *S. mutans* was investigated using Live/Dead™ staining of biofilms and the expression of comX for mutants of each of these TCS.
For a transcriptome profiling of the effects caused by Carolacton a whole-genome microarray was designed using a commercially available eArray™ platform. As labelling procedure total RNA of S. mutans was coordinatively bound to Cy5/Cy3-ULS™. The applicability and high reproducibility of the array was verified in a CSP-induction experiment. Finally a comparison of Carolacton treated biofilm-cells with an untreated reference sample was analysed in a time-series. Up to 25% of all 1960 ORFs of *S. mutans* were found to be differentially expressed upon addition of Carolacton. Carolacton-affected genes identified by microarray analysis will be verified using quantitative real-time PCR and by the construction and analysis of knock-out mutants. Furthermore the generation of a detailed model of the CSP-regulated network and for biofilm development is planned.

ACP23
Antimicrobial peptides from marine sediment microorganisms

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The marine habitat is rarely investigated for the production of antimicrobial peptides from microorganisms. With the aim to find and characterize new antimicrobial peptides from marine microorganisms we isolated bacteria from diverse marine sediments (Baltic Sea, Mediterranean Sea, Logatchev hydrothermal vent field, Lilliput vent field, Bransfield Strait, Indian Ocean and Timor Trough). 264 bacterial isolates were obtained. According to the 16S rRNA gene similarity these strains affiliate to the *Firmicutes* (90%), *Actinobacteria* (7%) and *Alphaproteobacteria* (3%). All strains were screened for their ability to produce natural products using antimicrobial bioassays, genetic screening for the presence of non-ribosomal peptide synthetase genes (NRPS), HPLC and mass spectroscopic analysis of culture extracts.
Results of the antimicrobial bioassays against diverse bacteria and fungi showed that about 20% of the strains were active against one or more of the tested microorganisms. In addition molecular screening revealed that 23% of the isolates were PCR positive for the non-ribosomal peptide synthetases genes (NRPS). Moreover LC-MS and MS-MS analysis allowed the putative identification of many known and unknown peptides. Most of the peptides were found within members of the Gram-positive bacteria and especially within the genus *Bacillus*.
Two exemplary strains of *Bacillus* sp. are represented here as producers of a diverse set of antimicrobial peptides. Our results show that the genus *Bacillus* is a promising source for the discovery of new natural products.

ACP24
Differentiation of viable and dead *Legionella* cells in water samples by real-time PCR and pre-treatment with ethidium monoazide

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Legionella pneumophila are pathogen microorganisms how are available in different water system, e.g. in cooling tower, fountains and also warm-water reservoirs. Therefore it is useful to detect the exact count of them in these water systems. In the moment most of the water samples were determined with the culture method but the method with the increasing impact is the real-time-PCR. The problem is, that after a disinfect procedure the dead cells were also detect with the PCR.
Ethidium monoazide (EMA) is a photo activated stain, which penetrates only dead bacterial cells with compromised cell walls and cell membranes. It intercalated into DNA of those cells and also in free extracellular DNA. The covalent binding of EMA to DNA is induced by photo activation. The photo induced cross linking result in the insolubility of DNA. Following this DNA can't be extracted.
For the first experiment we prepared different artificial water samples including *Legionella pneumophila* strain Corby. To get an overview of the affectivity of EMA we tested differed incubation-times, different concentrations and different exposure times. In the most experiments we use 5 min EMA treatment with 50µmol and an exposure-time of 2 min. For the real-time-PCR we use a 529 bp-fragment, because statistically EMA intercalate into DNA only every 10-80bp.
We treated the water samples with heat, silver, sodium hypochlorite, formaldehyde, Ampicilin and UV-light to get a reduction of the viable bacteria. With some of these methods a reduction can be verify with the help of EMA, but in some others it doesn't work. This effect is caused by the different antimicrobial mechanisms of the different disinfect-methods.

ACP25
Structure and function of colicin S4, a colicin with a duplicated receptor binding domain

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Colicins are proteins produced by *E. coli* strains to kill related strains.
The colicin domain architecture can be divided into three parts: The N-terminal translocation domain, flexible and often poorly resolved in crystal structures, followed by the receptor binding domain and the activity domain.
Colicins intrude into the host cell abusing transport, diffusion or efflux systems by first binding to a specific outer membrane protein, followed by translocation of the translocation domain into the periplasm and binding of a specific target protein. Finally, the activity domain is translocated through the outer membrane to reach the target area, which, for pore forming colicins, is the cytoplasmic membrane.
We have solved the structure of colicin S4 by X-ray crystallography. It exhibits a novel feature among colicins: two receptor binding domains with high sequence and structural similarity. The exact receptor binding sites were determined by point mutations. Remarkably large parts of the translocation domain of colicin S4 are resolved.
Single channel measurements show discrete conductance steps similar to those of colicin A. Colicin S4 binds to the outer membrane protein OmpW and is translocated through the general porin OmpF to the periplasm where its N-terminal translocation domain binds to the Tol-proteins, as shown by killing assays with mutant *E. coli* strains.
Colicin S4 provides an example for a recent domain duplication event, illustrating the modular evolution of this protein family. Sequence comparison of pore forming domains of different colicins show smaller evolutionary steps leading to the sequence divergence into three different groups.

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ACP26
Antimicrobial resistance among faecal *Escherichia coli* strains isolated from wild rodents

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To get first insight into the antimicrobial resistance status among *E. coli* from wild small mammals we tested 188 faecal isolates from eight rodent and one shrew species. Preselection of resistant isolates was performed via agar dilution test and selected strains were screened by minimal inhibitory concentration (MIC) testing, agar diffusion test and subsequent PCR analysis of resistance genes.
Ten of the 188 isolates (5.3 %) exhibited resistant phenotypes including resistances against beta-lactams, tetracyclines, aminoglycosides and sulfonamides. Multiple resistances against different classes of antimicrobials were observed for six isolates from two rodent species, the wood mouse (*Apodemus sylvaticus*) and the bank vole (*Myodes glareolus*). All *E. coli* isolates exhibiting multi resistant phenotypes were collected at sampling sites located in areas with high livestock unit/hectare indices and in close proximity to livestock breeding. Compared to other wild animals, such as birds, wild small mammals currently do not appear to be an important reservoir of antimicrobial resistant *E. coli* strains. Nevertheless, they might contribute to the spread of resistant and multi resistant strains into the environment and to their transmission between domestic and wildlife species especially in farmland areas.

ACP27
Extended spectrum beta lactamases producing *Citrobacter freundii* in animals

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Besides ESBL producing *E. coli* other *Enterobacteriaceae* like *Citrobacter freundii* have become a serious problem in the treatment of infectious diseases in veterinary and human medicine. *C. freundii* is a common cause of clinical-associated infections in humans as well as in animals. As ESBL producing *Enterobacteriaceae* - other than *E. coli*- are scarcely investigated in animals, we examined eight clinical *C. freundii* isolates suspected for ESBL production which were mainly obtained from companion animals with urinary tract and wound infections. Strains were collected from domesticated animals in a veterinary diagnostic lab and compared with an ESBL producing *C. freundii* isolate obtained from a Tawny Owl.
Four of the phenotypically ESBL-producing *C. freundii* isolates from companion animals harboured a CTX-M-1 type β-lactamase enzyme while three strains possessed an SHV-type ESBL. The isolates of wild avian origin also revealed an SHV-type ESBL. Furthermore all isolates possessed the TEM-1-like gene and in addition other non beta lactam phenotypic resistances and the respective genes were commonly detected. To the best of our knowledge these findings demonstrate for the first time that ESBL producing *Citrobacter freundii* strains with different genetic mechanisms of resistance seem to be present in companion animals. Likewise, ESBL producing *Citrobacter freundii* originating from wild animals have not been described yet, underlining a possible involvement of wild birds in the spread of multiresistant *Enterobacteriaceae* strains.

ACP28
Mechanistic aspects on the antibacterial activity of novel apidaecin-based peptide derivatives

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In the last decades many human pathogens acquired resistance mechanisms against common antibiotics, especially in hospitals (nosocomial infections). Among these life-threatening pathogens are three Gram-negative bacteria, which are under surveillance in the EU, i.e. *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. As many strains of these bacteria are already multi-resistant, the development of new antimicrobial compounds with novel modes of action appears extremely urgent. Among the different compound classes with antimicrobial activities, antimicrobial peptides (AMP) appear very promising, especially the family of short, proline-rich AMP of the innate immune system of insects, such as apidaecin from honey bees. They target intracellular DnaK and thus have a low intrinsic toxicity against mammalian cells.
Apideacins are 18-20 residue-long peptides and contain two domains for cell penetration and protein inhibition and a highly conserved C-terminal region (PRPPHPR-L/I). They are highly active against Gram-negative bacteria and thus represent a viable treatment option for multi-drug resistant pathogens in urinary tract and systemic infections.
Here, a novel apidaecin-based designer peptide derivative with improved antibacterial activities and pharmacological characteristics was studied. To map the sites important for membrane penetration, inhibition of the DnaK as target protein and its stability in human serum, each residue of the sequence was individually replaced with alanine (alanine scan) or its corresponding D-amino acid derivative (D-residue scan). All peptide derivatives were synthesized on solid phase using the Fmoc/tBu-strategy, purified by reversed phase HPLC and analyzed by MALDI-mass spectrometry. The minimal inhibition concentrations (MIC) of the obtained peptides were determined against the already mentioned Gram-negative bacteria, the uptake of fluorescence labelled peptides into bacterial cells was investigated.

ACP29
Effect of veterinary medicines entering the soil via manure on the abundance and diversity of antibiotic resistance genes and mobile genetic elements

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Considerable amounts of antibiotics reach soils through manure fertilization. Manure was shown to be a reservoir of bacteria carrying antibiotic resistance genes and broad host-range plasmids (Binh *et al.*, 2008; 2009). Thus spreading manure might stimulate the dissemination of antibiotic resistance in soil. Previously soil microcosm experiments performed with two different soils showed that adding sulfadiazine (SDZ) to manure increased not only the abundance of the sulfadiazine resistance genes *sul*1 and sul2 but also their transferability (Heuer and Smalla, 2007). The effect of repeated manure application and of the rhizosphere on the abundance and transferability of *sul* genes is presently investigated under field conditions. The qPCR data indicated that repeated application of manure originating from animals treated with SDZ caused a significant increase of *sul*2 gene abundance and an enhanced transferability. Again, the most frequently captured plasmids from manure-treated soils belonged to the previously discovered novel group of low G+C plasmids (Heuer et al., 2009) and to the promiscuous IncP-1ε plasmids. The results of the field test thus confirm the data of previous microcosm experiments. The results highlight the importance of both plasmid groups for dissemination of *sul* genes.

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ACV01
The Dermcidin-derived antimicrobial peptide DCD-1L forms oligomeric structures and kills bacteria by interaction with the bacterial membrane

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Dermcidin (DCD) is an antimicrobial peptide, which is constitutively expressed in eccrine sweat glands. By postsecretory proteolytic processing in sweat the dermcidin protein gives rise to anionic and cationic DCD-peptides with a broad spectrum of antimicrobial activity. We could show that Dermcidin-derived peptides inhibit significantly bacterial macromolecular synthesis (RNA, DNA, protein) within the first minutes without binding to microbial DNA or RNA. Recent structural analysis indicated that the anionic 48mer peptide DCD-1L forms ion-dependent oligomeric structures which are able to interact with the bacterial cell envelope and perturb the bacterial membrane structure. Further investigations by CD-spectroscopy and conductance measurements with artificial phospholipid membranes suggest that DCD-1L is able to form small pores in the bacterial membrane which leads to ion efflux and bacterial death. These data show for the first time how an antimicrobial peptide present in human eccrine sweat is able to kill efficiently several types of microorganisms.

ACV02
Antibacterial activity of oncocin against Gram-negative pathogens and elucidation of underlying mechanisms

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The incidence of serious infections by multi-drug resistant bacteria is still increasing and has triggered research in different areas, i.e. hygiene, microbiology, biochemistry and chemistry, to overcome such life-threatening diseases. One research line focuses on the search for novel, highly active antibacterial compounds that could represent valid drug leads against Gram-negative pathogens. Gene-encoded antimicrobial peptides (AMPs) produced in vertebrates and invertebrates as part of their innate immunity represent such a promising group. AMPs are usually 10 to 40 residues long and contain cationic residues. Mechanistically, they interact first with the negatively charged bacterial surface and then kill them by either lysing the membrane or inhibiting intracellular targets after entering the cells. Proline-rich, cationic AMPs from insects are typically 18-22 residues long and act on intracellular targets. Typical representatives are drosocin (isolated from *Drosophila melanogaster*) and apidaecin (*Apis mellifera*). They represent very promising drug leads to treat multi-resistant bacteria, such as extended-spectrum beta-lactamase hyper producing (ESBL+) *E. coli* and beta-lactam or Carbapenem-resistant *K. pneumoniae*.
Here, we present a novel designer peptide (oncocin) optimized from the structure of antibacterial peptide 4 isolated from milkweed bugs (*Oncopeltus fasciatus*). Oncocin was active against *E. coli*, *K. pneumoniae*, *S. typhimurium* and *P. aeruginosa* with minimal inhibitory concentrations (MIC) in the nano to micro molar range. Furthermore, it was very stable in mammalian serum and not toxic to mammalian cells. Studies with quartz crystal microbalance indicated that the peptide derivative can freely penetrate biomimetic phospholipid membranes without having a lytic activity, which was further confirmed by fluorescence microscopy on bacterial cells.

ACV03
The antibiotic ADEP inhibits cell division of Gram-positive bacteria

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A novel class of antibacterial acyldepsipeptides (ADEPs) exerts prominent antibacterial activity against Gram-positive bacteria incl. *Staphylococcus aureus in vitro* and *in vivo* [1]. ADEPs act via a yet unprecedented mechanism by dysregulating the caseinolytic protease ClpP. Usually, the activity of ClpP is tightly controlled by ATP-dependent Clp-ATPases and accessory proteins. ADEPs overcome these tight control mechanisms, switching ClpP from a regulated to an uncontrolled protease that predominately targets unfolded or flexible proteins as well as nascent polypeptides at the ribosome in the absence of Clp-ATPases [1, 2]. It is also striking that ADEP treatment leads to considerable cell mass increase in treated cells. However, the specific events that finally lead to bacterial cell death have still to be determined.
In our study, ADEP treatment leads to impressive filamentation of *Bacillus subtilis* and swelling of *S. aureus*. To gain further insights into the underlying molecular mechanism, we followed the events that led to inhibition of cell division. Cell division is concerted by the divisome, which is highly dynamic and is characterised by a time-dependent two-step assembly of specific cell division proteins like FtsZ, FtsA, ZapA and EzrA as well as PBP2B, FtsW, DivIVA, etc. [3]. We observed that ADEP treatment led to the inhibition of septum formation in *B. subtilis*, while chromosome segregation was not affected. Localization studies with GFP-labelled cell division proteins revealed that the ADEP/ClpP complex interferes with key components of early cell division. Accompanying *in vitro* degradation assays showed that the ADEP/ClpP proteolytic complex degrades essential components of the cell division machinery and, hence, destines Gram-positive bacteria to death. ADEPs demonstrate that beside their interesting antibacterial potency they are excellent tools to examine central mechanism of bacterial physiology, like cell division and regulated proteolysis.

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ACV04
Deep red cells of *Staphylococcus aureus* – influence of the antibacterial substance "MT02"

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The prevalence of multidrug resistant strains of *Staphylococcus aureus* in hospitals all over the world is an ongoing problem for public health care systems. This necessitates efforts for the discovery and subsequent investigation of new antibacterial compounds. The bisquaternary bisnaphthalimide "MT02" was found to show high activity against a variety of gram-positive bacteria including staphylococci, streptococci, and *Listeria*. Minimal inhibitory concentrations of tested *S. aureus* strains ranged from 0.15 µg/ml to 5 µg/ml.
By performing radioactive whole cell labeling and DNA band shift experiments, we could show that "MT02" intercalates into DNA in a not sequence specific manner. This was also confirmed by binding studies with surface plasmon resonance.
We were able to maintain a "MT02"-resistant mutant of *S. aureus* strain RN1HG. This was accomplished by cultivation of bacteria in medium with increasing concentrations of "MT02" over a period of 11 weeks. The mutant strain showed retarded growth in the presence of the substance compared to the wild type strain and a deep red phenotype after 8 h of incubation with 100 x MIC of "MT02". This red color is most probably caused by strong upregulation of the synthesis of the carotenoid staphyloxanthin which under normal conditions leads to the orange-golden color of *S. aureus*. It was reported that treatment of bacteria with antibiotic substances causes oxidative stress by production of reactive oxygen species within the cells. Hence we assume that the intercalation of "MT02" into staphylococcal DNA triggers the overproduction of the antioxidant staphyloxanthin in order to survive this high concentration of toxic radicals. Microarray experiments will give more insight into regulatory processes underlying this phenomenon.

ACV05
Exometabolome analysis identifies the bacterial pyruvate dehydrogenase as a new target for antibiotics to combat multiresistant bacterial pathogens

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The desperate need for new therapeutics against notoriously antibiotic-resistant bacteria has led to a quest for novel antibacterial target structures and lead compounds. Here we characterize the antibacterial properties of triphenylbismuthdichloride (TPBC), which has recently been successfully used against device-related infections. We demonstrate that TPBC has potent antimicrobial activity against many bacterial pathogens and combines bactericidal properties with a very low tendency to harm human cells. A metabolomics approach revealed a unique TPBC-mediated change in the exometabolites of *Staphylococcus aureus* indicating that TPBC blocks bacterial pyruvate catabolism. To test this hypothesis, we purified the pyruvate dehydrogenase (PDH), the major pyruvate catabolising enzyme in staphylococci, from cell lysates. Enzymatic analyses confirmed that TPBC is in fact a potent, dose-dependent and non-competitive inhibitor of the staphylococcal (PDHC). Our study demonstrates that inhibition of the bacterial PDHC represents a promising and previously little recognized strategy for combating multi-drug resistant bacteria and furthermore identifies TPBC as a lead compound for the development of new types of antimicrobials.

ACV06
Investigating antibiotic targets and mechanisms of action using proteome analyses

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Most antibiotics lead to growth arrest or death by inhibition of essential cellular functions. However, sublethal doses of antibiotics elicit cellular responses that are indicative of the functions impaired [1]. We can, therefore, use global proteome analyses to elucidate mechanisms of action of structurally novel antibiotics. This process is particularly efficient if other antibiotics with like mechanisms of action have previously been investigated [1,2]. Novel protein patterns, on the other hand, hold the promise of unprecedented mechanisms of action but present a bigger challenge with regards to mechanism elucidation [3]. Here we present proteome analyses of four structurally diverse natural and synthetic compounds with unknown mechanisms of action [4]. We provide reference proteomic response patterns for target areas that are currently not clinically exploited, such as fatty acid biosynthesis inhibition and cell division. These response patterns complement an existing *Bacillus subtilis* reference library that covers most antibiotic classes [1]. To generate reference proteomic patterns for potential new antibiotic targets in the absence of inhibitors, we are using conditional mutants to down-regulate essential cellular functions [5]. We are following this strategy to characterize cell division genes as potential antibiotic targets.

ACV07
Whole genome pooled sequencing of 60 clinical *Pseudomonas aerugionsa* isolates reveals resistance-associated mutations

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The constant increase in development and spread of bacterial resistance poses a serious threat to human health. *Pseudomonas aeruginosa* is known as a major nosocomial pathogen that easily develops antibiotic resistance. Recent publications have aimed at identifying all genetic determinants of antibiotic resistance in *P. aeruginosa* by screening comprehensive transposon mutant libraries for resistance profiles towards several antibiotics. From these studies it has become clear that many previously unidentified genes play a role in antimicrobial resistance. *P. aeruginosa* is assumed to adapt to the exposure of antimicrobial agents with the acquisition of specific step-wise mutations each of them conferring small to medium scale resistance. Genetic variations that represent relevant resistance determinants and that are selected for under antibiotic treatment will thus be enriched in sets of strains that show a specific antibiotic resistance profile.
In this study we demonstrate that pooled sequencing is applicable for the analysis of sequence variations of strain collections. Using a Illumina Genome Analyzer we sequenced 60 clinical *P. aeruginosa* isolates in pools of up to 15 strains, grouped by their resistance profile with respect to the fluoroquinolone antibiotic ciprofloxacin. The distribution of known resistance determining variations (*gyrA*, *parC*) could be successfully determined and additionally variations of genes previously unrelated with resistance were identified.
The adaptive development of antibiotic resistance is a genome-wide phenomenon that needs to be investigated from a whole-genome perspective. Sequencing strain collections opens a window for the detailed and extensive identification of sequence variations as potential bacterial adaptation processes, e.g., during the development of antibiotic resistance in the clinical setting and thus may be the basis to uncover putative targets for novel treatment strategies.

ACV08
Antibiotic resistance in the highly infectious and virulent zoonotic pathogen *Francisella tularensis*

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Background: *Francisella (F.) tularensis* is a small, Gram-negative bacterium causing tularemia in humans and a wide range of mammals. F. tularensis, a category A select agent, is intrinsically resistant against all beta-lactam antibiotics and cotrimoxazole. Currently, aminoglycosides, doxycycline or ciprofloxacin are recommended to treat human tularemia, but single strains resistant against one or more of these antibiotics have been described. Macrolides have sporadically been reported to be efficient in tularemia. Here, we investigated the erythromycin resistance pattern in a large collection of German and European *F. tularensis* strains and tried to explore the molecular mechanisms of antibiotic resistance in *F. tularensis* with special focus on macrolides.
>Methods: We performed a bioinformatic comparison of whole genome sequences including 12 *Francisella* genomes. Potentially new target sequences identified by in silico analysis were amplified by PCR and sequenced. Susceptibility testing was performed by E-test.
Results: We could confirm the presence of *blaA* (beta lactamase class A) genes in the genome of *F. tularensis* explaining its complete resistance against all beta-lactam antibiotics including carbapenems. At position 2059 of the *23S rRNA* gene (*E. coli* numbering) we could detect a single nucleotide polymorphism (SNP) which was highly correlated (100% agreement in 40 strains) with a macrolide resistant phenotype in about 40% of all German strains.
Discussion: Our study revealed for the first time the molecular mechanism for erythromycin resistance in *F. tularensis*. The reason for the stable intrinsic resistance against macrolides that involves a SNP (A to C) affecting the peptidyl transferase center of the large ribosomal subunit is unknown. But our results challenge recent recommendations to explore the use of macrolides in the treatment of tularemia and also the new FDA "Orphan Drug Designation" for the prophylactic treatment of tularemia with cethromycin.

ACV09
Combination of folic acid antagonist and nucleoside analog - a new antiinfective approach

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The antimicrobial activity of folic acid antagonists are supposed to be antagonized by elevated thymidine concentrations in damaged host tissues. Therefore, this study was aimed at (i) determining the concentrations of thymidine and its precursor dTMP in different human specimens, (ii) evaluating the impact of thymidine and dTMP on the antimicrobial activity of folic acid antagonists, (iii) screening for nucleoside analogs that impair bacterial utilization of thymidine, and (iv) analyzing the combined antimicrobial activity of nucleoside analogs and folic acid antagonists in the presence of thymidine. We showed that thymidine and dTMP in different human specimens are present at concentrations that antagonize the antimicrobial activity of folic acid antagonists. Several nucleoside analogs, in particular halogenated derivatives of 2'-deoxyuridine, were found that substantially impair bacterial utilization of extracellular thymidine in S. aureus. Combination with 5-iodo-2'-deoxyuridine substantially improve the antimicrobial activity of folic acid antagonists against S. aureus in the presence of thymidine leading up to bactericidal activity. Finally, 5-iodo-2'-deoxyuridine combined with SXT showed a broad spectrum of activity in the presence of thymidine comprising several gram-positive and gram-negative bacteria.
In conclusion, this study provides evidence that a combined antiinfective agent consisting of SXT and a nucleoside analog shows significantly improved antimicrobial activity against bacterial pathogens in the presence of elevated thymidine concentrations.

ACV10
rRNA sequence polymorphism within the bacterial domain and susceptibility to drugs targeting protein synthesis

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The ribosome is target for many antibacterial agents, e.g. aminoglycosides, tetracyclines, macrolides, ketolides, lincosamides, oxazolidinones. It is unclear whether the in part significant polymorphism present in a drug's binding site affects antibiotic susceptibility or resistance development. The drug binding site of aminoglycosides maps to helix 44 of the 16S rRNA (decoding A-site), that of macrolides/ketolides localizes to domain V of 23S rRNA (peptidyl-transferase center). Sequence polymorphism within the aminoglycoside binding pocket involves 16S rRNA residues 1409/1491 and 1410/1490; sequence polymorphism of the macrolide binding site involves 23S rRNA residues 2057/2611. Sequence alterations corresponding to polymorphic residues were introduced by genetic means into a single rRNA allelic model to result in isogenic recombinants carrying homogenous populations of mutant ribosomes. Subsequent drug susceptibility studies allowed for the following conclusions. 1. Natural sequence variations in the small subunit's A-site of bacteria in part influence aminoglycoside susceptibility, but do not affect the resistance phenotype of the A1408G mutation. 2. Natural sequence variations in the bacterial large subunit's peptidyl-transfer center do not affect macrolide/ketolide susceptibility, but significantly impact on the resistance phenotype of the A2058G mutation.

ACV11
Genotype specific *in vitro* efficacy of PA824, a novel nitroimidazo-oxazine, against *Mycobacterium tuberculosis* complex strains

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Resistance to standard first-line and even second-line drugs applied in tuberculosis (TB) therapy is increasing dramatically, implying an enormous threat for TB control worldwide. Therefore it is of great importance to develop new, effective drugs. As Mycobacterium species causing TB belong to various phylogenetic lineages, which might carry genotype specific polymorphisms in target genes, drug efficacy testing using genotypically diverse strains should be performed.
A total of 65 *M. tuberculosis* complex (MTBC) strains from a reference collection comprising mayor phylogenetic lineages were sequenced in characterized PA824 target genes Rv3547 and Rv0407 to detect possible genotype specific polymorphisms. Determination of minimal inhibitory concentrations (MICs) was done by using the modified proportion method in BACTEC MGIT 960 applying PA824 concentrations of 1, 0.5, 0.25, 0.125, 0.0625 and 0.0312µg/ml.
Sequence analysis revealed genotype specific polymorphisms for *M. tuberculosis* Haarlem (Haarlem), *M. africanum* West African I (WA I) and II (WA II), for "wild animal strains" (*M. pinnipedii, M. microti*) as well as for the Euro-American super lineage. Subsequent drug susceptibility testing resulted in slightly different MICs depending on the respective genotype. Besides *M. canettii* ($MIC_{PA824} \geq 1$µg/ml) MICs for strains belonging to the other genotypes ranged from ≤0.0312µg/ml (WA II) to 0.25µg/ml (Haarlem).
Despite genotype specific polymorphisms in target genes and MIC differences PA824 seems to be quite active against various MTBC strains. Considering the geographical spread of the strains analyzed, it can be concluded that PA824 could, after having passed through all clinical trials, be administered to TB patients in diverse geographical settings.

ACV12
The secodammarane triterpenoid, dammarenolic acid from *Aglaia ignea* demonstrates potent anti-retroviral and anti-respiratory syncytial virus activity *in vitro*

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The 3,4-secodammarane triterpenoid, dammarenolic acid from *Aglaia ignea* bark (family Meliaceae) inhibited HIV-1 and RSV infection potently (IC50 < 1 µg/ml and 0.1 µg/ml respectively), while cytotoxic effects and inhibition of cell proliferation were only observed at concentrations exceeding 10 µg/ml. Methylation of dammarenolic acid results in a complete loss of anti-HIV-1 and anti-RSV activity. Time of addition studies reveals that dammarenolic acid targets the RSV replication at a post-entry stage; the anti-HIV-1 kinetics revealed a post entry target similar to the non-nucleoside RT-inhibitor (NNRTI), nevirapine. Contrary to nevirapine however, dammarenolic acid also potently inhibited the *in vitro* replication of other retroviruses, including Simian immunodeficiency virus and Murine leukemic virus in vector-based antiviral screening studies. These results strongly suggest that this natural product could be a promising lead compound for the development of novel anti-retrovirals and anti-respiratory syncytial virus compounds.

BDP01
Analysis of active microorganisms in the gas reservoir Altmark (Germany) and their potential role in CO_2 turnover

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The almost depleted gas reservoir Altmark is located in the southern edge of the Northeast German Basin and is operated by GDF SUEZ E&P Germany GmbH. The *Rotliegend* formation has a depth of ~3300m, a bottom-hole temperature between 111°C and 120°C and is characterized by high salinity (275-350g/l), low redox potential (up to -300mV) and the absence of sulfate. The Altmark gas fields are of special interest for CO_2 injection because of favourable geological properties.
RECOBIO-2, part of the BMBF-Geotechnologien consortium, investigates the presence of active microbes in formation waters of the Altmark gas fields and their potential role in CO_2 turnover. Two types of samples are analyzed: formation water collected at the well head (Nov 2008) and formation water sampled *in situ* (May 2009). Some of the wells were treated frequently with a foaming agent while others were chemically untreated.
Despite the extreme environmental conditions, RNA of apparently active microorganisms was successfully extracted from all samples. Sequence analysis of 16S rRNA revealed mainly fermentative bacteria (*Actinobacteria*) and those possibly involved in the nitrogen cycle (*α-Proteobacteria*). Cell numbers were calculated using CARD-FISH with universal 16S rRNA-specific probes. The fraction of bacterial cells comprised up to 10^4 cells/ml but archaeal cells were below detection limit. However, archaeal 16S rRNA fragments were amplified from DNA extracts using PCR. Determination of whole cell numbers with a generic DNA stain displayed almost equal cell contents. As a result, cell counting could hardly detect any difference between treated and untreated formation waters and also not between well head and *in situ* samples.
Further investigations will attempt to reveal whether particular metabolic pathways are present in the microbial assemblage. In addition, possible microbe-mineral interactions will be in focus using electron microscopic approaches.

BDP02
Low light adaptation of the green sulfur bacterium *Chlorobium phaeobacteroides* BS1 and its distribution in the Mediterranean basin

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The green sulfur bacterium *C. phaeobacteroides* BS1 was previously enriched from the chemocline of the Black Sea located at 90-150 m depth and represents the most low-light adapted phototroph known so far. Measurements of light intensities in the chemocline and experiments with laboratory cultures revealed that the strain from the Black Sea is adapted to extremely low light intensities of 0.003 µmol Quanta m^{-2} s^{-1}. To analyse the physiological process of low light adaptation in strain BS1, cultures grown at 0.1 and 3 µmol Quanta m^{-2} s^{-1} were compared. The ATP-content from light limited cultures maintained constant for 28 days at 0.0014 µmol Quanta m^{-2} s^{-1}, but dropped significantly in cultures grown under light saturation. This analysis revealed that the cells can decrease maintenance energy requirements below that of other bacteria investigated and thus can be used as a model for bacterial survival in energy poor environments like the deep subsurface. Transcriptional analyses were performed using cDNA subtractive hybridization and deep sequencing. First data show a decrease in transcripts encoding for enzymes involved in chlorophyll biosynthesis and reverse TCA cycle for low light adapted cells. Based on its specific low-light adaptation, strain BS1 also represents a reliable marker for past deep photic zone anoxia. When several sediment samples from the Black and Mediterranean Sea were analyzed through LC-MS^3, isorenieratene, the typical carotenoid of green sulfur bacteria was detected. However, using subfossil DNA sequences of strain BS1, the past environmental conditions could be reconstructed more precisely. Additionally we identified this strain in the chemocline of the saline Lake Faro near Messina (Sicily) confirming its prevalence in the Mediterranean basin and opening new insights in the proliferation and adaptation of green sulfur bacteria.

BDP03
Bacteria in the endorhizosphere of maize: Effects of root zone and cultivar

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Plant roots are known to attract soil bacteria and create a hot spot of microbial activity in their rhizosphere. In addition, the endorhizosphere, the microenvironment inside the plant root but outside of the root cells, represents a potentially attractive habitat for bacteria. However, the access to this habitat is restricted to a small group of bacteria and it seems not yet well understood if different cultivars of the same plant species select for different bacterial communities in their endorhizosphere. Nor, is there any information if different root segments of the same plant harbor different bacteria. This study analyzed surface sterilized pieces of roots obtained from three different field grown maize cultivars (Benicia, DKC 4250 and DKC 5143) for endophytes. Fine and coarse roots were distinguished and the respective bacterial communities were represented as T-RFLP profiles of PCR amplified SSU rRNA genes. A specific PCR was applied which excluded plant plastid and mitochondria DNA from the analyses. The results demonstrate that the endophytic communities were clearly different from the communities of the root surface, indicating a successful surface sterilization. The diversity among the endophytes was rather low with only three to four dominant terminal fragments. No differences were found between endophytic communities of fine and coarse roots while clearly different composed communities were found on the root surfaces of the two different root segments (see contribution by Küting et al.). Endophytes in DKC 5143 differed significantly from those in Benicia and DKC 4250 and differences could be attributed to single terminal fragments. A treatment of DKC 5143 with the insecticide tefluthrin had no effect on the root endophytes. DNA sequence data of the endophytic bacteria indicated the presence of species which affiliated to *Phyllobacterium, Caulobacter* and *Paenibacillus*.

BDP04
Response of soil bacterial diversity to a genetically modified maize cultivar with recombinant insecticidal *Bt*-proteins

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Plants have the capacity to select for specific soil microorganisms in their rhizosphere by root exudation and sloughed off root cells. This study analyzed the microbial diversity in the rhizosphere of a genetically modified (GM) maize cultivar, capable of producing three different insecticidal toxins derived from *Bacillus thuringiensis* (*Bt*-toxins Cry1A.105, Cry2Bb2, Cry3Bb1) and compared it to the diversity found with conventionally bred cultivars. As an additional variable the application of the insecticide tefluthrin was included. Plants were grown in a randomized block design with eight replicate plots for each cultivar and rhizosphere samples were collected when the maize was flowering. The amount of Cry proteins found in the rhizosphere was generally low, e.g. for Cry1A.105 0.3 ng g^{-1} soil, compared to the amount present in the roots (33 µg g^{-1} dry weight in fine roots). Differences between two years of successive cultivation were not significant. Microbial community compositions of the rhizoplane were analyzed as this is the area of the rhizosphere which is closest to the root surface and probably most influenced by root exudation. These communities were characterized independent of cultivation by analysis of PCR-amplified small subunit rRNA genes (SSU rDNA) applying T-RFLP (terminal restriction fragment length polymorphism). The microbial domains *Archaea*, *Bacteria* and *Fungi* were quantified by real-time PCR. Preliminary data analyses suggest cultivar specific microbial communities but no effect of the insecticide application. Differences between fine and coarse roots, included as another variable in this study, were significant for all cultivars. Further studies need to establish whether the differences between the GM maize and the other cultivars are within the natural range for conventionally bred cultivars.

BDP05
Diversity of bacterial communities growing on γ-hexachlorocyclohexane in soils from Egypt

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Hexachlorocyclohexane (HCH)-degrading bacteria are believed to mediate natural attenuation of HCH contamination and have potential for active bioremediation processes. This study addressed the huge diversity of bacteria from 4 soil samples collected around insecticide and pesticide producing factories from Alexandria and Monufia (Egypt). The purpose of this work was to determine the extent of microbial potential for the degradation of γ-HCH in soils. From γ-HCH enrichment cultures, all samples yielded high biodiversity as revealed by the analyses of the 16S rRNA genes of the isolates. γ-HCH degrading bacteria were found to fall within the genera *Acetobacter*, *Achromobacter*, *Bacillus*, *Brevundimonas*, *Cupriavidus*, *Novosphingobium*, *Paenibacillus*, *Pseudomonas* and *Rhodococcus*. Soil and sediment samples were used to inoculate γ-HCH microcrystals on a substratum (Permanox™)in microcosms to grow complex biofilm communities on γ-HCH. The biofilms were monitored for about 37 days by community fingerprinting using single strand conformational polymorphism (SSCP) of 16S rRNA gene amplicons. All soil samples yielded biofilms on γ-HCH and SSCP analyses of the biofilms revealed rather diverse bacterial communities with species of genera closely related to *Pseudomonas*, *Sphingomonas* (as dominant members), *Burkholderia*, *Caulobacter*, *Comamonas*, *Nitrosospira*, *Ochrobactrum*, *Planococcus*, *Parvibaculum* and *Sterolibacterium*. From the soil samples and the biofilms 51 isolates were obtained, 36 of them could use γ-HCH as sole source of carbon in a minimal medium. The majority of these isolates belonged to the genus *Bacillus* but *Paenibacillus* or *Achromobacter* species were also among them. Further characterizations of both biofilm communities and isolates are under way with the aim to elucidate their interactions and to understand the degradation of γ-HCH by complex biofilm communities.

BDP06
Community structure and composition of the drinking water microflora in biofilm and bulkwater of a small-scale network

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Drinking water is the most important food and water related material consumed worldwide in which both potentially pathogenic and non-pathogenic bacteria exist. In many cases, pathogenic bacteria are located not only in bulk water but also in biofilms attaching to the tubes and pipes of the distribution network. Interactions between potentially pathogenic and non-pathogenic bacteria can lead to supression or survival of pontentially pathogenic bacteria. Therefore, a detailed analysis of the whole bacterial microflora in bulk water and corresponding biofilms is of special relevance for public health. Many other studies showed that biofilms do not show a stable microbial community composition during the first year. Thus we concentrated on examining a well established water distribution system that was build over 20 years ago. In this study, we obtained an overview on the total bacterial community by using single strand conformation polymorphism (SSCP) fingerprints based on the 16S rRNA gene. Both DNA and RNA, representing the present and active bacteria respectively, were extracted from biofilm and bulkwater samples. General bacterial primers were used to amplify 16S rRNA genes. Detailed insights into the composition of the community were obtained by sequencing SSCP bands of high intensity, representing one phylotype. Analysis of the small-scale network connected to the local drinking water supply system (Braunschweig) showed that there were pronounced differences between the species composition of bulk water and biofilm. A high diversity between biofilm communities sampled on different sampling points was observed, while nearby sampling points showed similar communities even in spite of different support materials. The active bacterial fraction in the bulk water was substantially different from the present bacterial fraction, while in biofilms both were quite similar to each other, indicating that most species grown in the biofilm are also active.

BDP07
Structural Diversity of Archaeal Surface Layer Proteins

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Background: Most Archaea possess quasi-crystalline proteinaceous surface (S-) layers as sole or major cell wall components. S-layers are made of one or two large (glyco-) proteins directly or indirectly anchored in the cytoplasmic membrane. The amino acid sequences are highly variable; more than one family of S-layer proteins that do not follow phylogenetic relationship exist in various branches of the Archaea. Numerous electron microscopic studies revealed the general architecture of archaeal S-layers, however, little is known about 3D and domain structures at atomic resolution.
Methods: We isolated the S-layer proteins (SlaA and SlaB) of three (hyper-) thermophilic and acidophilic Sulfolobales species. We obtained high-resolution electron microscopic images of the *Acidianus ambivalens* S-layer and we analyzed the transcription patterns. 3D structure and domain predictions were done with threading and homology modeling.
Results and Conclusions: The *slaAB* genes of the Sulfolobales, lying adjacently in the chromosomes, are constitutively transcribed as bicistronic operons. 15-20% of the trancripts are read through to a terminator downstream of *slaB* while 80-85% stop at an internal terminator downstream of the *slaA* gene. The operons including termination signals are conserved in the Sulfolobales. Molecular modeling suggests SlaBs to be composed of 2-3 consecutive beta sandwich domains, a coiled-coil domain of 15-17 nm in length and a C-terminal transmembrane helix. Electron microscopy shows crystalline protein arrays with triangular and hexagonal pores. We propose that the mushroom-shaped "unit cells" of the Sulfolobales' S-layers consist of three SlaBs anchoring the complex in the membrane and six SlaAs forming the detergent-resistant outer sacculus. Modelling of other archaeal S-layer proteins showed that many of these might also be composed of a series of beta sandwich domains.

[1] Ref: A Veith *et. al.* (2009) Mol Microbiol 73:58-72

BDP08
Metagenomic study of air borne diversity of mycoparasitic fungus *Trichoderma* (anamorph *Hypocrea*)

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The mitosporic genus *Trichoderma* contains taxa which are important as mycoparasites, secondary decomposers applied in biofuel industry, but also as pests in mushroom farms and pathogens of immunocompromised mammals. To learn the possibility of their transfer by air, we used a metagenomic, non-cultivating approach to investigate the occurrence and diversity of *Trichoderma* spp. in samples from air in Viennese suburban area (Wienerwald, Austria). For this purpose we applied genus-specific PCR primers for internal transcribed spacers 1 and 2 of the rRNA gene cluster and constructed environmental *Hypocrea/Trichoderma* clone libraries corresponding to four seasons. 159 molecular operational taxonomic units (MOTUs) were identified at the species level by the aid of the oligonucleotide barcode for *Trichoderma* and *Hypocrea* to represent 15 known species. The cosmopolitan holomorphic species known for high mycoparasitic activity and association with plants - *H. virens* was the most abundant in all samples (52% of MOTUs) but showing seasonally dependent distribution of ITS1 and 2 alleles. The most surprising was the detection of both obligate tropical species (*H. jecorina*, *H. stromatica* and *T. taxi*) and rare temperate species known from North America (*T. fertile*). The appearance of some of these taxa correlated with the air current from their known habitats. Our data support a panmixis of some taxa by air and the long distanced spore transfer.

BDP09
Characterization of metagenomes derived from volcanic sites of Kamchatka

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Sites of volcanic activity provide a variety of different extreme environments for microorganisms. This study was focused on assessing and exploiting the prokaryotic diversity of two microbial communities derived from Kamchatkian thermal springs by metagenomic approaches. For this purpose, environmental DNA was isolated from collected sediment samples by a direct cell lysis method. The diversity was examined by shotgun sequencing of selected regions of archaeal and bacterial 16S rRNA genes. A total number of 1234 16S rRNA gene sequences were obtained and used for taxonomic classification. The *Proteobacteria* and *Crenarchaeota* represent the dominant phyla in both habitats. The other 16S rRNA gene sequences belonged to the *Aquificae*, the *Dictyoglomi*, the *Euryarchaeota*, the *Firmicutes*, the *Korarchaeota*, the *Thermodesulfobacteria*, the *Thermotogae*, and some potential new phyla (Candidate divisions OP9, OPS8, and EM19). Phylogenetic trees representing the prokaryotic diversity were calculated by employing ARB[1]. In addition to phylogenetic studies, the recovered DNA was amplified by 'whole metagenome amplification' and used for the construction of metagenomic small-insert DNA libraries. Subsequently, a function-driven screening was carried out to identify genes encoding either lipolytic or proteolytic enzymes. So far, three novel genes conferring lipolytic and one gene conferring proteolytic activity were identified.

BDP10
Does tree diversity influence the composition of soil bacterial communities?

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The rhizosphere and the surrounding soil harbor an enormous biological activity. The interaction between plant roots and soil bacteria generates a specific community structure, which is dependent on the plant species and differs from other habitats. The aim of this study is to answer the question if soil microbial diversity depends on tree species diversity. Therefore, mesocosm experiments using *Acer pseudoplatanus* (Sycamore), *Fagus sylvatica* (Beech), *Fraxinus excelsior* (Ash), *Carpinus betulus* (Hornbeam), *Tilia cordata* (Basswood), as tree species have been established in 2002. In addition, mixtures of three and five of these tree species as well as a contol without trees have been employed. A litter overlay was spreaded on another 17 variants. These artificial soil habitats were sampled in October 2008 and June 2009. Samples were taken from three parallels of each of the different variants and pooled in order to minimize abnormalities of the bacterial composition.
The soil prokaryotic community structures present in these 34 types of mesocoms were analyzed using denaturing gradient gel electrophoresis (DGGE). The obtained DGGE profiles were analyzed using GelCompare II software by clustering via the unweight pair group method with mathematical averages (Dice coefficient of similarity). The bands were excised from the DGGE, cloned and sequenced. These sequence data were analyzed and phylogenetic trees were calculated using ARB[1].
The achieved DGGE patterns revealed a high bacterial diversity which is in agreement with the gained sequence data, but there was no correlation between tree diversity and community structure was observed. Nevertheless, the cluster analysis indicated differences in community composition in response to seasonal changes and litter overlay.

BDP11
Investigating the biodiversity of microbial communities in the McMurdo Dry Valleys, Antarctica: an inter-valley comparison study

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Extreme environments provide a unique source of often highly adapted and tolerant organisms. Research on organisms in these habitats has led to the discovery of novel and useful compounds and may assist in understanding the impact of global change on biodiversity. The Dry Valleys of Eastern Antarctica are vast, ice-free regions believed to be the coldest, driest desert on Earth. Despite these harsh conditions, there is an increasing amount of evidence demonstrating that the soil ecosystems of the Dry Valleys sustain a wide diversity of microorganisms. The research presented is an inter-valley comparison study which aimed to scrutinize microbial communities and environmental factors driving their distribution in the Dry Valleys. Automated ribosomal intergenic spacer analysis (ARISA) was used to provide a "snapshot" of bacterial and cyanobacterial communities living in the mineral sands in Miers Valley, Beacon Valley, Upper Wright Valley and at Battleship Promontory. Rigorous analysis of physico-chemical differences between the soils of these four valleys was undertaken in hope to understand the environmental parameters driving the distribution and biodiversity of microbial communities present. Multivariate statistical analysis and ordination of ARISA and physico-chemical data revealed that bacterial communities from each valley form distinctive clusters. Conversely, cyanobacterial communities showed less diversity and a more even distribution between valleys. Furthermore, 454 pyrosequencing using bar-coded primers was undertaken in order to identify the majority of species representatives in each of the four valleys.

BDP12
Genetic diversity of phyllosphere pink-pigmented facultative methylotrophs depending on plant species, geographic location and land-use type

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Phyllosphere methylotrophic bacteria are Gram-negative organisms, which inhabit plant surfaces, mainly the leaves. They use compounds such as methanol as their sole carbon and energy source and play an important role in the methanol cycle by utilizing the methanol emitted by plants. On the other hand, they can produce plant growth promoting substances like auxins, cytokinins and vitamin B12. One objective of this project is to analyse the genetic diversity of phyllosphere methylotrophs in dependence of inhabited plant species, land-use type and geographic location of the sampling sites.
Cultivation of pink-pigmented facultative methylotrophs (PPFM) was performed after isolation from leaf samples of two different plants, *Trifolium repens* and *Cerastium holosteoides*. For each plant, samples were taken from sites with different land-use types. Isolates were grouped using amplified ribosomal DNA restriction analysis (ARDRA). From representative isolates the 16S rRNA gene sequences were analysed and phylogenetic analyses were performed using the neighbour-joining method.
The concentration of PPFM per gram leaf was significantly higher for *Trifolium repens* (2.0×10^7 CFU / g leave) than for *Cerastium holosteoides* (2.0×10^6 CFU / g leave). Geographic location of the sampling site, land-use type and fertilisation had no significant influence on the concentration of PPFM on leaf surfaces. Using ARDRA, 16 different groups of pink-pigmented isolates were found. The main groups of isolates could be found in both sampling periods, all three sampling sites and all land-use types. Analysis of the 16S rRNA gene sequences confirmed that all isolates belong to the genus *Methylobacterium* with similarities between 97.3 and 100% to described species (*M. adhaesivum*, *M. mesophilicum*, *M. organophilicum*, *M. jeotgali*).

BDP13
Microbial diversity on the skin of the medicinal leech *Hirudo verbana*: which bacteria can be found and how can the risk of infection be prevented?

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The beneficial effects of medicinal leeches have been known for thousands of years. Today, medicinal leeches are used mainly post-operatively in the fields of plastic and reconstructive surgery. The saliva of leeches contains hirudin, which has anticoagulant properties. Like all living organisms, leeches harbour communities of microorganisms and their application can lead to a bacterial infection, e.g. with *Aeromonas veronii*, a common inhabitant of the digestive tract, or with microorganisms present on the surface of the leech or near the mouth. Therefore, it is important to reduce the number of microorganisms, especially those that belong to risk group 2 and are potentially harmful to humans. In this study, we analysed the microbial diversity on the medicinal leech *Hirudo verbana* by 16S rRNA gene sequencing of strains isolated from the surface of the leech. Additionally, we tested several methods to reduce the number of microorganisms on the leech, namely washing with water, potassium permanganate, antiseptic agents and antibiotics.
Results show a high microbial diversity on the leeches. Isolated strains had sequence similarities of 99% or 100% with *Ochrobactrum anthropi*, *Aeromonas veronii*, *Acinetobacter calcoaceticus*, *Citrobacter gillenii* (all risk group 2), *Comamonas testosteroni*, *Micrococcus luteus*, *Pseudomonas poea* (all risk group 1), and *Roseomonas terpenica* (not categorized).
Washing of leeches led to varying results, with reduced or, in some cases, increased numbers of microorganisms. Potassium permanganate and antiseptic agents reduced the number of microorganisms, but not always significantly. Interestingly, these treatments facilitate the growth of risk group 2 organism *Ochrobactrum anthropi*. The used antibiotic (ciprofloxacin) negatively affected the vitality of the leeches and is not suitable for treatment.
In further studies, additional washing methods will be tested to significantly lower the number of microorganisms on the medicinal leech.

BDP14
Microbial community composition of biofilms in domestic washing machines

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Biofilms in domestic washing machines are unaesthetic and represent a reservoir for microorganisms that may cause machine and laundry malodor. Moreover, some of them might also have a pathogenic potential. Consequently, these biofilms should be removed. Knowledge about the community composition of biofilms in domestic washing machines is a prerequisite to develop successful strategies for their removal and prevention. In this study, swab samples were taken from soap-dispenser drawer and chamber, bull-eye sealing and sump of eight domestic washing machines. After streaking on agar plates, samples showed bacterial growth (Gram-positive and -negative species) on Caso-agar and also fungal growth (moulds and yeasts) on Wort-agar. Using DNA directly extracted from the swabs as template, bacterial 16S rRNA and fungal ITS genes were amplified by PCR. Subsequently, approximately 650 bacterial 16S rRNA genes and 180 fungal ITS genes stemming from 4 different machines, each, were cloned and sequenced. Preliminary sequence analyses using the RDP-classifier tool and BLAST indicated a distinct and diverse bacterial community composition in all samples, greatly dominated by α- and γ-*Proteobacteria*, suggesting the water used for washing as a major source for microbial recruitment. The majority of fungal ITS-sequences were affiliated with filamentous fungi (moulds) and yeasts.
In conclusion, biofilms in domestic washing machines are highly distinct and diverse and comprise a large fraction of yet-uncultured microbial species. Both cultivation-dependent and –independent analyses clearly proved a washing machine community formed by bacteria and fungi.

BDP15
Identification and characterization of microbial key functions in soils of the German Biodiversity Exploratories Schorfheide-Chorin and Swabian Alb representing different land-use gradients

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Phylogenetic and functional analyses of soil microorganisms present in the German Biodiversity Exploratories Schorfheide-Chorin and Swabian Alb were performed. The research is associated with a network that studies the effect of land-use intensity and vegetation type on biodiversity. The experimental procedure includes the isolation of whole genomic DNA from the A-horizon of selected grassland and forest areas. The prokaryotic diversity present in the different samples was assessed by pyrosequencing of amplified hypervariable V2-V3 16S rRNA gene regions.
The isolated DNAs from 16 different sampling sites were employed for the construction of metagenomic small-insert and large-insert libraries. Comparative screening of the libraries for key microbial functions, such as cellulolytic and lipolytic activities was carried out. Several clones harboring esterase or cellulase activity were obtained during function-driven screening of the libraries. Genes encoding lipolytic activity were recovered from the positive clones and sequenced. So far, the analyzed esterases grouped into superfamilies IV and V of lipolytic enzymes. To allow biochemical characterization of selected esterases the corresponding genes were cloned into expression vectors and expressed. Subsequently, the esterases were purified and the dependence of the esterase activity on the chain length of the fatty acids was investigated by using different p-nitrophenyl esters as substrates.

BDP16
Isolation and characterisation of heterotrophic microorganism from mineral soils of Livingston Island (Antarctica) and Store Koldewey (Northeast-Greenland)

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Microbial communities in extreme habitats like Arctic and Antarctic are still insufficient investigated. Only little information is available about diversity and function in such environments. Because of its special properties and the geographic isolation the arctic regions provide a unique opportunity as a natural laboratory especially for basic research in climatology, geology or biology. The cold-maritime climate of Livingston Island is characterised by low temperatures at -10°C down to -35°C in winter and up to 10°C in summer. The annual precipitation averages around 500 mm and is evenly distributed. Mosses, lichens and algae dominate the vegetation. Environmental conditions and low input of organic material result in slow soil formation processes. The polar climate on Store Koldewey is characterised by low temperature at -24°C up to 4°C and little precipitation around 150mm pa. Because of the harsh climate conditions there is only initial soil formation. In the scope of this work enrichment cultures from both described study sites could be obtained. As a part of the whole microbial community several heterotrophic aerobic bacteria were isolated and eight bacterial strains were elaborately characterised. The molecular genotype was determined by 16S rRNA gene analyses and used for phylogentic characterisation of the strains. Morphological, physiological and biochemical analyses were carried out to describe the phenotype. Considering the current results of our research we propose *Arthrobacter livingstonensis* sp. nov. LI2T and *Arthrobacter cryotolerans* sp. nov. LI3T as two novel species in the genus Arthrobacter. We report the isolation and identification of strain *Cryobacterium arcticum* sp. nov. SK-1T as a novel psychrotolerant species in the genus Cryobacterium. Strain LI-1T could be classified as a novel species in the genus Leifsonia, named *Leifsonia psychrotolerans*.

BDP17
Bacterial communities in industrial water-related applications

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Bacterial contamination is a major problem in many industrial water-related applications. The simple presence of bacteria can either interfere with the working process, or the phenomenom of biofouling causes different problems with industrial surfaces. Biofouling occurs in a wide range of situations, for example contamination of medical devices, fouling of ship hulls, pipelines and reservoirs, in the production of ultra-pure and drinking-water and especially in the use of industrial process-water. In industry biofouling induces high costs due to destruction of materials or machines and cleaning of the concerned areas.
Microbial contamination and biofouling appear in many forms but for the most part there is a common cause, the biofilm, which is the most successful life-form on earth. There is an extremely wide range of high resistance against extreme environmental conditions.
In this study the community-structure of several industrial process-water-basins and the contained process-media were analized by culture-dependent and culture-independent methods. The analized basins contain different chemical composition and represent varying environmental conditions respectively. The main focus of the culture-independent methods was on a 16s rDNA-analysis of the communities of the basins and of the metal surfaces accordingly.
Generally we could identify bacteria belonging to the classes of Alpha-, Beta- and Gammaproteobacteria, Flavobacteria, Sphingobacteria and some other. The predominant bacteria are related to the class of Betaproteobacteria.
Furthermore, the reason of microbial contamination and biofouling of the basins should be located. For this purpose all involved criteria were analized (e.g. raw-water, chemicals, air etc.). Here we could show that the raw-water plays a key-role in inocculation of the basins.

BDP18
Characterization of methanogenic Archaea from two extreme habitats

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Five strains of methanogenic archaea isolated from two extreme habitats had been characterized for their physiological and phylogenetical properties. The strains SMA21 and SMA17 were isolated from the permafrost soil of the Lena river delta, Siberia and the strains MC15, MC20 and MC21 were isolated from the anaerobic sediment of the subsurface lake of Movile Cave, Romania, which contains a chemoautotophically based ecosystem with several endemic species.

The strains SMA21 and SMA17 were irregular cocci whereas strain MC15 formed sarcinalike cocci in aggregates. The strains MC20 and MC21 were rodshaped. All strains grew within a temperature range from 0 up to 44°C, a pH value of 6.2 to 9.9 and salt concentrations from 0.02 to 0.6 M NaCl. Some were also able to grow at lower pH values down to pH 4.8 for SMA21, SMA17 and MC21 and even down to pH 4.1 for MC15. The most of the strains grew also at higher temperatures up to 54°C except MC20. The substrates used for methane formation were H_2/CO_2, methanol and acetate for the coccishaped and H_2/CO_2 and formiate for the rodshaped strains; MC20 could also use 2-propanol. Trypicase peptone and yeast extract were not needed for growth by any strain.
The 16S-rRNA fragment sequences of the strains MC20 and MC21 could be allocated to the genus of Methanobacteria and the sequences of the strains MC15, SMA17 and SMA21 to Methanosarcina. The sequence of MC20 showed only 96.7% similarity to the sequence of the nearest cultivated relative *Methanobacterium oryzae*, which indicates for a discrete species. MC15 and MC21 showed a slightly higher similarity difference of 98.5% to *Methanosarcina barkeri* and 99.4% to *Methanobacterium subterraneum*, respectively. The strains SMA17 and SMA21 showed a high similarity to each other and to their nearest cultivated relative *Methanosarcina mazei* (99.8 and 99.9%), but with significant differences in their physiological properties.

BDP19
Characterization of prokaryotic communities of a two-stage leach-bed biogas plant by means of T-RFLP and qPCR analyses

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Knowledge about structure and function of bacterial and archaeal communities responsible for substrate biodegradation and biogas formation in biogas plants is still rather incomplete. This study analyzes the composition and variability of the prokaryotic community of such a facility in order to better understand the link between the actual diversity of a microbial community and biogas yields. Samples were taken from a two-stage biogas plant consisting of four parallel hydrolytic leach-bed reactors and two methanogenic reactors connected in a circular flow system using mainly maize silage as a substrate. Community structures were characterized independent of cultivation by applying T-RFLP (terminal restriction fragment length polymorphism) of restriction endonuclease digested PCR-amplified 16S rRNA genes. Additionally real-time PCR, targeting the same genes, was conducted for quantification of the bacterial and archaeal domains. T-RFLP profiles revealed high similarities between samples of the leachates and solid substrates even in the early stages of hydrolysis, particularly with regard to the archaea. Real time PCR results showed that the archaeal copy numbers reached a plateau already after a short period of fermentation. Carry-over of methanogenic archaea into the hydrolytic stage may be a reason for lower methane production yields due to unwanted methanogenic activity in the hydrolysis step as well as methanotrophic activitiy in the methanogenic reactor. The counteracting processes of methanogenesis and methanotrophy are now further investigated by phylogenetic analysis of 16S rRNA clone libraries and likewise analyses of functional genes like mcrA (methyl-coenzyme M reductase A) and pmoA (particulate methane monooxygenase).

BDP20
Application of protein based stable isotope probing (Protein-SIP) to unravel anoxic benzene degradation

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Microbial communities play a key role in the Earth's biogeochemical cycles. Microorganisms exist under a vast variety of physiological conditions and are able to metabolize virtually any organic compound for energy production. Furthermore many capabilities of microorganisms can only arise in communities and can't be performed by a single microbial strain, e.g. the anoxic degradation of benzene.
Benzene is a major environmental contaminant of anthropogenic source, belonging to the group of BTEX compounds (benzene, toluene, ethylbenzene, xylene). It is posing a large threat to human health and environment due to its toxic and carcinogenic effects. Although benzene is a widespread pollutant, knowledge about its degradation under anoxic conditions is still meager. One of the reasons for this is a certain lack of suitable methods for analysing complex microbial communities. Classical ways of analysing microorganisms almost entirely rely on pure cultures, even though less than 1 % of the microbial species have been successfully grown in pure culture so far. Hence new methods for unravelling the complex interactions within microbial communities are needed.
Object of our research is a benzene degrading, sulfate reducing culture from a contaminated aquifer near Zeitz, Saxonia-Anhalt. First clues on taxonomic composition of the culture have been acquired by DNA-SIP experiments [1]. To confirm these results and to yield further insights into the culture, but also to open new ways for the analysis of microbial communities, we expanded the classical stable isotope probing methods to metaproteomic analysis. Nonradioactive heavy isotopes are used to label substrates, the metabolization and incorporation into proteins then is analysed by high resolution mass spectrometry. These studies yield taxonomic and functional information of trophic structures, hence offering a powerful tool to study microbial communities.

[1] Herrmann *et al*., Environ Microbiol. 2009 Oct 16

BDP21
Comparative Genomics of representative Members of the *Roseobacter*-Clade: The Genus *Octadecabacter*

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The genus *Octadecabacter* comprises heterotrophic and psychrophilic bacteria belonging to the *Roseobacter*-clade [1]. Members of this genus were found in Arctic and Antarctic sea ice and seawater and appear to be indigeneous to the sea ice microbial communities of both poles. To date, two different species have been described: *Octadecabacter arcticus* and *Octadecabacter antarcticus*. The strains were isolated from the lower 20 cm of annual sea ice of the Arctic and the Antarctic, respectively.
The genomes of *O. arcticus* 238 and *O. antarcticus* 307 were sequenced. To solve problems with misassembled regions caused by repetitive sequences and to close remaining gaps, PCR-based techniques on genomic DNA and fosmids were used. The sequencing results show that the genome of *O. arcticus* is organized in three replicons: a chromosome of 5.2 Mb and two plasmids of 160 kb and 118 kb. In contrast, the genome of *O. antarcticus* consists of one chromosome (4,98 Mb) and only one plasmid (62,8 kb). It was found that the genomes of both strains contain distinct large genetic Islands and an unusually high number of mobile genetic elements, indicating a high potential for horizontal genetransfer. The genomes of both strains are compared with respect to differences which may be caused by geographic separation.

BDP22
Denitrification in soil is influenced by land-use intensity and pH

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Denitrifying prokaryotes are facultative aerobes that catalyse the reduction of nitrate and nitrite to nitrous oxide and molecular nitrogen. Soil denitrification is the main source but also a temporary sink of the greenhouse gas nitrous oxide and is influenced by environmental factors. The potential impact of land-use on denitrification in soils was assessed. Soil parameters as well as denitrification rates and cell numbers of denitrifiers were determined for six grassland and six forest soils under contrasting land-use. Soil pH ranged from 4.0 to 7.6 and nitrate content from 0.25 to 3.35 µmol g_{DW}^{-1}. Forest soils had lower pHs than grassland soils from the same region. MPNs and CFUs were from 100 to 9.3 x 10^9 cells g_{DW}^{-1}. Denitrification rates with 2 mM nitrate varied from 0.005 to 164.73 nmol N_2O h^{-1} g_{DW}^{-1} at pH 3.5 to 9.0. Cell numbers and denitrification rates tended to be lower in acidic soils. Furthermore, maximum denitrification velocities of soils under less intensive land-use tended to be lower than that of soils under intensive land-use. However, maximum denitrification velocities did not correlate linearly with cell numbers of denitrifiers or pH, suggesting putatively different denitrifier communities. The collective data indicate that (i) land-use intensities impact on maximum velocities of denitrification, and (ii) pH is a major effector of denitrification and cell numbers of denitrifiers in soil.

BDP23
Towards functional expression of the magnetostatic metagenome: Cloning and expression analysis of large magnetosome operons from uncultivated magnetotactic bacteria

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In comparison to artificial nanoparticles, magnetic crystals produced by magnetotactic bacteria (MTB) have a number of advantages. Magnetosomes biomineralized by MTB are membrane-enclosed particles of the mineral magnetite (Fe_3O_4) that exhibit well-controlled sizes and morphologies. But by now the genetic determination underlying the impressive morphological diversity of magnetosome biomineralisation is only poorly understood since most MTB are not available in pure culture so far. We have demonstrated before that targeted cloning of large metagenomic magnetosome operons provides a powerful strategy for genetic analysis of magnetosome biomineralization. Functional expression of these large gene clusters in cultivated MTB or other suitable hosts might provide access to the large genetic and morphological variety of magnetosome synthesis observed in environmental MTB. Thus, we developed a strategy to subclone magnetosome operons from metagenomic fosmid libraries and finally tailored these for heterologous expression in diverse hosts. We constructed a 23.4 kbp conjugative plasmid using Red/ET-recombination, which contains a partial metagenomic *mamAB* like operon, completed by a promoter and the *mamI* and *mamH* genes from the cultivated species *Magnetospirillum gryphiswaldense* as a putative minimal gene set required for magnetosome formation. While the plasmid was stable in *E. coli* DH5α, instability was observed via restriction analysis and PCR assay in heterologous hosts such as *Agrobacterium tumefaciens* or MTB strains. In addition, we failed to detect any phenotype conferred by the plasmid in the analysed organisms. Although the question of plasmid stability has yet to be solved, our approach nevertheless has proven suitable for the construction of large chimeric magnetosome operons, which seems to be promising in future "synthetic biology" approaches for the generation of tailored magnetosome operons and functional expression.

BDP24
Bacterial speciation and the evolutionary basis of bacterial diversity

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The role of recombination, adaptation and selection in shaping bacterial diversity was elucidated by searching for different ecotypes within groups of closely related bacterial lineages (up to 100 % 16S rRNA gene sequence identity). Members of the family *Sphingomonadaceae* constituted an abundant fraction of the *Alphaproteobacteria* in the oligotrophic, alpine Walchensee and the mesotrophic, prealpine Starnberger See. Of these, two phylogenetically tight subgroups of *Sphingomonadaceae*, relatives of *Sandarakinorhabdus limnophila* as well as the novel lineage G1A, were identified by a seasonal clone library as the dominant *Sphingomonadaceae*. These two dominant groups were found to be physiologically active throughout the year by DGGE. In parallel, a large number of *Sphingomonadaceae* could be recovered in pure culture by a high throughput cultivation approach followed by a PCR based *Sphingomonadaceae* screening. Among them were 8 isolates corresponding to the *S. limnophila*-cluster whereas 65 isolates were related to the G1A-cluster. Based on the availability of several independent pure culture isolates, their dominance and year-round activity *in situ*, these two bacterial groups will be used as a model systems to elucidate the presence of different ecotypes by qPCR. Their specific ecological niches will be identified by physiological characterisation and *in situ* growth test.

BDP25
Comparative RNA analyses for the characterisation of wastewater treatment plants

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The enhanced biological phosphorous removal (EBPR) is a common technology in wastewater treatment. Despite this fact it is still largely unknown which microbial species are responsible for phosphorus accumulation and release. This acknowledgement is necessary for the improvement and warranty of an efficient and environmentally friendly wastewater treatment process.
The emphasis of this study was to analyze the diversity of the microbial structure and furthermore a comparative analysis of this communities in different municipal wastewater treatment plants.
Therefore the total RNA was isolated from several activated sludge samples. The synthesized cDNA was used for DGGE and furthermore for designing gene libraries.
The different modes of operation resulted in major differences in the bacterial composition of the investigated plants detected by DGGE analysis. Characteristic community fingerprints for each of the investigated plants indicated highly diverse microbial communities in all plants suggesting substantial differences in their microbial structure. This correlation was also proved by statistical evaluation of the results with the software BIONUMERICS.
In our case *Tetrasphaera spp., Microlunatus spp.*, members of the Rhodocyclus group and other PAOs had been also detected by cloning and sequencing of cDNA. These organisms were found in all plants, including those without EBPR, obviously they are constituents of the microbial community structure of activated sludge systems.

BDP26
Molecular tools for detection of ring reductases and ring hydrolases involved in the anaerobic degradation of aromatic compounds

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In anaerobic bacteria most aromatic growth substrates (e.g. benzene, toluene, phenol, cresols, xylenes, ethylbenzenes, benzoate analogues, etc.) are channelled to the central intermediate benzoyl-CoA. This compound is converted to cyclohex-1,5-diene-1-carboxyl-CoA by dearomatizing benzoyl-CoA reductases (BCRs). There are two types of BCRs which yield the same product although they have different compositions and cofactors [1]. Facultative anaerobes were considered to use an ATP-dependent FeS-cluster containing BCR encoded by the *bcrABCD* genes. In contrast obligate anaerobes were proposed to employ a W-/FeS-/Se-/flavo-enzyme complex encoded by the *bamBCDEFGHI* genes. In the benzoyl-CoA degradation pathway the dearomatized ring is subsequently cleaved by a 6-oxocyclohexenoyl-CoA hydrolase. The latter is encoded by *bamA*-like genes, which are highly conserved in all anaerobic bacteria [2]. We constructed degenerate oligonucleotide primers for PCR reactions to amplify conserved regions of the *bcrC*, *bamB* (ring reductases of facultative and obligate anaerobes) and *bamA* genes (ring hydrolase). While the *bamA* gene was detected in all anaerobes, the *bamB* was detected exclusively in obligate, and the *bcrC* was exclusively detected in facultative anaerobes. Transcription of the three genes in all tested organisms was only induced during growth on an aromatic substrate as evidenced by RT-PCR reaction with the degenerated oligonucleotide pairs. By a combination of the three probes a reliable molecular tool is available for the detection of all aromatic compound degrading anaerobic bacteria, which will help to analyze microbial community structure and to monitor bioremediation processes at anoxic sites.

[1] Kung *et al.* (2009), PNAS 106:17687-92
[2] Kuntze *et al.* (2008), Environ. Microbiol. 10(6):1547-56

BDP27
Aerobic methanol-utilizing bacteria in aerated soils are neutrophilic

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Methanol is a reactive volatile organic compound emitted by terrestrial ecosystems and triggers ozone formation. Most soil-derived aerobic methylotrophic prokaryotes utilize methanol as a source of carbon and energy. Little is known about the impact of environmental parameters (e.g., pH) on the abundance of aerobic methanol-utilizing bacteria in soils. Cultured cell-numbers of aerobic methanol-utilizing bacteria were determined in upper mineral soils from 12 sampling sites in the DFG Biodiversity Exploratories Hainich, Schwäbische Alp, and Schorfheide-Chorin. Cultured cell-numbers of aerobic methanol-utilizers were higher in neutral media than in acidic media regardless of *in situ* pH. Soils with neutral *in situ* pH yielded highest cultured numbers (3.0×10^8 cells g^{-1}_{DW}; confidence interval 1.2×10^8 to 6.9×10^8 cells g^{-1}_{DW}). Sequences obtained from a *mxaF* (encodes methanol dehydrogenase) library from a sampling site with an *in situ* pH of 7 were related to neutrophilic species. Nitrate-supplemented media yielded higher cell numbers than media without nitrate. Cell numbers were not correlated with *in situ* nitrate concentrations. Collectively, these results suggest that aerobic methanol-utilizing bacteria in the investigated aerated soils are neutrophilic, the majority of which do not appear able to fix dinitrogen.

BDP28
BSS and beyond: extending molecular tools to unravel the diversity and structure of anaerobic hydrocarbon degrader communities in natural systems

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The microbes responsible for natural attenuation in aquifers contaminated with BTEX, PAH and aliphatic hydrocarbons can be monitored with different methods. One recently established method is the monitoring of the anaerobic hydrocarbon degrader communities via functional gene markers, in this case with the gene encoding the α-subunit of the benzylsuccinate synthase (*bssA*). This enzyme and its homologues are involved in hydrocarbon breakdown via fumarate addition; this particular reaction mechanism has been shown not only to be involved in the activation of toluene, xylenes, cresols, but also for aliphatic compounds and methylated naphtalenes.
In environments such as contaminated groundwater, the diversity of *bssA* and its homologous genes can be extremely valuable to characterize intrinsic degrader populations, and to predict and monitor natural attenuation. However, the true identity and diversity of anaerobic degraders present at such sites is still an enigma. In order to forward our current understanding of the diversity as well as functional and phylogenetic affiliation of fumarate adding genes in the environment, intrinsic degrader populations at different hydrocarbon contaminated habitats as well as selected pure cultures and enrichments were compared and screened for new *bssA*-like sequences. Using novel primer permutations we provide evidence for a previously unrecognized clostridial *bssA* branch and their importance in hydrocarbon degradation in anaerobic habitats. Our new set of *bssA* detection assays is tested for its specificity to recover different lineages of fumarate adding enzyme genes. Thus, an evaluation of the utility of *bssA*-like genes as a specific 'functional marker' will be presented, along with an outlook towards the monitoring of hydrocarbon degrader communities at contaminated sites.

BDP29
Seeking novel enzymes through metagenomics from whole genome amplified deep-sea hydrothermal vent chimney structures

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Life in deep-sea hydrothermal environments is based on chemosynthetic growth. It relies on hydrothermal fluids transporting reduced inorganic compounds (e.g. H2, CH4, H2S) from inner earth to the surface. These reduced inorganic substrates can be oxidized by microbes to yield energy for autotrophic CO2 fixation. As the hot, reduced vent emissions come into contact with the ambient cold, oxygenated seawater minerals precipitate forming the vent chimney. Vent chimney structures constitute steep thermal and chemical gradients ranging from cold, oxygenated conditions characterizing the outer chimney section to extremely hot, highly reduced features in the inner sections. Therefore, the vent chimney matrix offers different types of microhabitats and provides ideal resources for investigating novel metabolisms and enzymes adapted to various oxic/anoxic and thermal regimes. We have collected a deep-sea hydrothermal chimney structure from the actively venting site at Sisters Peak, located on the Southern Mid-Atlantic Ridge. Maximum temperatures of the hydrothermal fluids were 375°C. The effluents exiting from the structure contain 57% end-members, 48 µM H2, 11 µM CH4 and 5.3 mM H2S (Seifert et al., 2009. Cruise report. http://www.ifm-geomar.de/index.php?id=sppnews&L=1). The retrieved chimney structure was vertically divided into three mineralogical distinct sections and DNA was extracted from each one. Whole genome amplification (WGA) was performed with the three sections to yield sufficient material for intended 454 sequencing and construction of metagenome libraries. The latter will be used for function-based screening to discover novel enzymes.

BDP30
Bacteria from the deep subseafloor biosphere - diversity and cultivation

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Sub-seafloor sediments although thousands of years old have been shown to harbour living bacteria [1]. To analyse and cultivate these ancient bacteria we used the deep sea drilling vessel „Chikyu" to obtain a 60 m sediment core at a water depth of 755 m at Suruga Bay (Japan) closely located to Mount Fuji. 120 Sediment samples from different depths were collected for physicochemical and microbial analysis. Age determination of the sediment samples revealed an average age of 5000 years at a core depth of 12 m and an age of 6000 years at a core depth of 40 m. Depending on the depth methane concentration varied with a maximum of up to 2500 ppm around 30 m. Total organic carbon content was 0.5%, showing little variation along the depth profile.
Sediment samples were used as inoculum for anaerobic and aerobic enrichment cultures on different carbon sources such as cellulose, xylan, chitin and glucose. DGGE and 16S rRNA analysis revealed a large diversity up to a depth of 30 m. Depending on the carbon source different microbial communities could be enriched. Furthermore, diversity varied depending on the presence or absence of yeast extract. The cultivated microorganisms were affiliated with the genera *Alishewanella*, *Sulfitobacter*, *Pseudomonas*, *Krokinobacter*, *Glaciecola* and *Lacinutrix*.
[1] Schippers, A. *et al.*, Nature 433: 861-864.

BDP31
Novel spirochetes in the sponge *Clathrina clathrus*

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Though spirochetes have been repeatedly found in marine sponges and other invertebrates, little attention has been paid to the specificity of this association and the interactions between spirochetes and their host animals. We demonstrate that different geno- and morphotypes of spirochetes can reside within the same sponge individual and develop in considerable numbers. Specimens of the calcareous sponge *Clathrina clathrus* collected from the Adriatic Sea off Rovinj (Croatia) were found to harbor spirochete-like bacteria, which we characterized by scanning electron microscopy (SEM), 16S rRNA gene analysis, and catalyzed reporter deposition fluorescence *in situ* hybridization (CARD-FISH). Two novel spirochete sequence types related to *Brachyspiraceae* representatives could be retrieved that showed only ~80% similarity to next uncultivated and cultivated relatives. Using highly specific CARD-FISH probes, we could assign the *C. clathrus*-associated sequences to a linear and a helical spirochete morphotype. Both were located within the sponge mesohyl and resembled the spirochetes identified by SEM. In addition, we also analyzed an aquarium-reared *Clathrina* sp. (most likely *C. coriacea*) that originated from Indonesian coastal waters. Four different spirochete sequence types were recovered from this Indonesian *Clathrina* specimen. Two of these also affiliated with the *Brachyspiraceae*, the other two were found associated with the *Spirochaetaceae* family, one with the genera *Borrelia* and *Cristispira*.

BDP32
A cultivation-independent approach for genetic, cell biological and morphological analysis of *Magnetobacterium bavaricum*

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The giant magnetotactic rod *Magnetobacterium bavaricum* that was previously discovered in sediments of the Lake Chiemsee, Bavaria is highly unusual with respect to its deep branching affiliation with the *Nitrospira* phylum, the up to 1000 bullet-shaped magnetosome crystals, and its unique cell biology. Since all attempts to cultivate *M. bavaricum* failed so far, we used a set of single-cell based tools and strategies combined with the construction of metagenomic fosmid libraries to study the cell biology and the genetic basis of magnetosome biomineralization.
By combining selective magnetic- and mechanical enrichment we were able to yield *M.bavaricum* in high numbers and frequencies as proven by sequence analysis of 16S rRNA gene clone libraries. This enabled the effective genetic analysis of *M. bavaricum*. In addition to a variety of metabolic genes including a putative rubisco-like gene, genes with similarity to magnetosome genes of cultivated magnetic bacteria were identified.
Using light- and electron microscopy, including SEM and FIB milling experiments we demonstrated the presence of multiple chains of magnetosomes and for the first time detected membrane-like structures enclosing magnetite crystals in *M. bavaricum*. Further remarkable features, like the presence of intracellular sulfur globules, a distinct and complex cell wall morphology including a putative additional membrane system, as well as a riffled surface structure indicate a highly unusual cell biology of *M. bavaricum*.

BDP33

Will not be presented and published

BDP34
Anoxygenic phototrophic bacterial community of Salar de Atacama, Chile

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The Chilean Altiplano located 2300 m above sea level harbours a set of extreme habitats characterized by high UV-radiation, partly extreme saline conditions and high diurnal temperature variations. These unexpectedly quite productive habitats are dominated by microbial life. Phototrophic bacteria are assumed to have a major impact. However, little is known about the diversity and composition of the phototrophic prokaryotic communities. To gain insight into the diversity of assumed key players involved in primary production in Chilean Altiplano salares, we studied the phototrophic bacterial communities of different locations around the Salar de Atacama in the Chilean Altiplano.
In order to specifically study the phototrophic prokaryotes of the habitats, we used molecular genetic analyses with group specific primers for the functional genes *fmoA* (for GSB) and *pufLM* (PSB, PNSB and Chloroflexi). GSB were only detected in enrichment cultures by *fmoA* and identified as related to *Prosthecochloris indicum*. PSB as detected by *pufLM* were present in all samples. T-RFLP of the *pufLM* genes indicated high variability between the lagoons and the subsamples thereof. The PSB community represented a highly diverse number of phylotypes identified in *pufLM* clone libraries. Most of these phylotypes were related to known halophilic Chromatiaceae and Ectothiorhodospiraceae, including many so far unknown PSB groups.
This study is the first report on the diversity of phototrophic prokaryotes in the Chilean Altiplano salares determined by both a functional gene approach and a cultivation/isolation approach. We demonstrate the suitability of the functional genes, *fmoA* and *pufLM* for studying communities of phototrophic bacteria including those of unique and extreme environments such as salt lakes of the Chilean highlands around Salar de Atacama.

BDV01
Nitrospira, the most divers and flexible nitrite-oxidizing bacterium

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Nitrite rarely accumulates in aerobic environments due to the activity of nitrite-oxidizing bacteria. The most abundant representatives belong to the deeply branching genus *Nitrospira*, which is denominated as recalcitrant and fastidious. Nevertheless, we were successful in enrichment (and isolation) of numerous laboratory cultures by dilution to extinction in mineral media as well as plating techniques. Samples originated from different locations including extreme habitats with regard to temperature and nitrogen charge. As a consequence, strains differ in their physiological behaviour in adaptation to their special origin. For example, the first thermophilic species *Nitrospira calida*, derived from a hot spring in Russia, is able to grow at temperatures between 35°C and 58°C. Other cultures obtained from sediments of the Laptev Sea in the Siberian Arctic show activity beween 4°C and 28°C. The most susceptible culture of *Nitrospira* inhabited a marine sponge, whereas highly resistant strains were sampled from activated sludge and aquaculture plants. As a result, the substrate tolerance varies between 1 and 25 mM of sodium nitrite. In dependence on the availability of nitrite and accumulation of the endproduct nitrate a transition of aggregated cells occurring in form of microcolonies to single planktonic cells was observed. Such life cycle reflects a morphological adaptation as a reaction to changing environmental conditions. Although regarded as obligate aerobic microorganisms, laboratory cultures of *Nitrospira* oxidize nitrite also under oxygen limitation. This remarkable flexibility provides the potential for colonization of different ecological niches.

BDV02
Lipid chemotaxonomy of *Rhodopirellula* strains

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Bacteria of the genus of *Rhodopirellula* are aerobic, sessile-living bacteria belonging to the phylum of *Planctomycetaceae*. In our laboratory, 70 isolates obtained mainly from Europe were investigated with molecular biological methods such as Box-PCR, Multi Locus Sequence Analysis (MLSA), 16S rRNA studies and DNA-DNA-hybridization studies and classified into different hybridization groups [1].
Based on these results, 17 strains of thirteen different genetic groups were selected for further classification. The focus of the study was the differentiation via chemotaxonomic markers such as phospholipids, fatty acids and quinones using chromatographic and mass spectroscopic methods.
Chemotaxonomic characteristics seem to confirm the genetic diversity resulting in new species within the genus *Rhodopirellula* eventually allowing to define novel genera.

[1] N.Winkelmann et al., Appl. Environ. Microbiol., in press

BDV03
Response of the Siberian Methane Cycle to Climate Changes in the Past and Present

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The currently observed climate change due to global warming is expected to have a strong impact especially on the Arctic permafrost environments. The thawing of permafrost is suggested to be associated with a massive release of greenhouse gases, in particular methane. For the understanding how the system will respond to climate changes it is not only important to investigate the current status of carbon turnover but also how the system reacted to climate changes in the past. Therefore quantitative and qualitative analyses of the variations in composition of bacterial and archaeal communities involved in the Siberian methane cycle in holocene and late pleistocene were conducted, using samples of a permafrost core drilled in central Lena-Delta, Siberia, in 2002.

Our studies on the reconstruction of the methane cycle in deposits of the permafrost affected soils combined methods of biogeochemistry and molecular geomicrobiology. It was possible to recover lipid biomarker and intact DNA continuosly throughout the core. Biomarkers like glycerol dialkyl glycerol tetraethers (GDGTs) were analyzed, whereas highest amounts of ether lipids were found in the upper layer and at the bottom of the core. Generally, the results of GDGTs analyzes fit to measured rates of total organic carbon (TOC) and in-situ methane concentration of the deposits. Furthermore these biomarkers were analyzed and distinguished in biomarkers representing signals of paleo-archaeal and paleo-bacterial communities. To complete information on the qualitative composition of microbial communities DNA analyses were driven using archaeal and methanotrophic specific primer combinations, whereas amplicons were subsequently analyzed by DGGE and clone libraries. Fingerprints of archaeal 16 S rRNA gene sequences of the different soil samples show variations within the vertical profile.

BDV04
Archaean diversity, abundance and potential function in Baltic pelagic redoxclines

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Marine pelagic redoxclines are areas of pronounced biogeochemical cycling and inhabited by distinct functional groups of prokaryotes. Within their suboxic areas, aerobic ammonium oxidation generally plays a crucial role in the nitrogen cycle. For a longer time period this role was especially associated with bacterial ammonium-oxidizing organisms phylogenetically belonging to the *Beta* - or *Gammaproteobacteria*. However, more recent studies indicated that crenarchaea related to *Candidatus* Nitrosopumilus maritimus are abundant organisms within suboxic areas of pelagic Black Sea redoxclines, and are potentially playing a much more important role in the aerobic oxidation of ammonium than proteobacteria (Coolen et al. 2007). We were interested if an analogous phenomenon could be observed for the suboxic, aerobic ammonium oxidation zone of the central Baltic Sea. Thus, we investigated the phylogenetic diversity, distribution and abundance of *Archaea* using 16S rRNA gene cloning, 16S rRNA and rRNA gene fingerprinting, and CARD-FISH within a Baltic pelagic redoxcline. Moreover, the distribution of archaeal and bacterial amoA genes and transcripts within the water column was determined by Arch-*amoA* F/Arch-*amoA*R (Francis et al. 2005), *amoA*-1F/*amoA*-2R, and *amoA*-3F/*amoB*-4R (Purkhold et al. 2000) based clone libraries and AOA-*amoA*-f/AOA-*amoA*-r based DGGE fingerprints (Coolen et al. 2007), respectively. Based on ribosomal fingerprints, several crenarchaeal and euryarchaeal sequences were detected. However, clone libraries were dominated by only one distinct crenarchaeal group with 97 % sequence similarity to *Candidatus* Nitrosopumilus maritimus. Application of specific CARD-FISH probes revealed that this group represented the archaea by practically 100 % and reached up to 25% of total prokaryotic cell numbers slightly above the chemocline. On a functional level, the *amoA* diversity, identity, and distribution in general supported the unique feature of this crenarchaeal group for the suboxic zone. This indicates that aerobic ammonium oxidation in Baltic pelagic redoxclines could mainly be driven by only one crenarchaeal species.

BDV05
Influence of Temperature and Salinity on a Purple Sulfur Bacteria Community Using *pufL* and *pufM* Gene Analysis

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Phototrophic Purple Sulfur Bacteria (PSB) are a physiological group of Gammaproteobacteria and very common in coastal waters and sediments. This is the first time the effect of environmental conditions upon these communities is studied using genetic approaches. The community of PSB from a shallow brackish eutrophic lagoon of the Baltic Sea is studied by using the *pufLM* genes, which are specific for Purple Bacteria and *Chloroflexaceae* and encode for the light and medium subunits of their photosynthetic reaction center, respectively. The influence of temperature and salinity was studied in enrichment cultures in Pfennig's medium under variation of temperature (13°C-56°C) and salinity (0%-12% NaCl).
Differences in communities were followed by *pufLM* RFLP analyses and selected enrichment cultures were analysed by *pufLM* cloning experiments. The natural PSB community was highly diverse and consisted of PSB phylotypes belonging to almost all known PSB genera as well as unassigned new clusters and turned out to be rather stable within the natural occurring ranges of temperature (13-36°C) and salinity (1-3.5%). Changes in the community structures were found at temperatures and salinity values outside these ranges. No growth occurred above 44°C and 10% salinity. We conclude that the natural PSB community of the studied brackish Baltic Sea lagoon is highly diverse and well adapted to the varying temperature and salinity conditions. A large number of generalists dominates the community while also a small number of specialists is present. The functional gene approach using *pufLM* proved to be a powerful tool in analysis of PSB communities and to predict possible changes in the communities upon changes of environmental conditions.

BDV06
An inventory of the global microbial diversity of chloroorganic contaminated aquifers

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Chlorinated ethenes are toxic groundwater pollutants that can be detoxified by dechlorinating bacteria. So far, the focus has been laid on members of e.g. Dehalococcoides, but knowledge about the diversity and ecology of dechlorinators in contaminated environments as well as in the subsurface is far from resolved. To advance bioremediation applications, much deeper knowledge of the microbiology contributing to the detoxification of chlorinated ethenes in the subsurface is needed. Comprehensive knowledge of the key players involved in the chlorinated ethene dechlorination would allow the improved design of hierarchic biomarkers sets for detection and quantification of known and novel species, explore symbiotic relationships for enhanced support of degradative key species, and to predict the stability of a microbial population under different conditions. We have embarked on a systematic, holistic approach to characterize the prokaryotic (Archaea and Bacteria) and eukaryotic biodiversity in chlorinated ethene contaminated aquifers. Two approaches are being pursued: i) a detailed, holistic inventory on a selected number of field sites employing different 16S and 18S rRNA gene-based approaches; and ii) a phylogenetic evaluation of all 16S and 18S rRNA gene sequences retrieved from all so far investigated geographically distinct TCE contaminated sites. Phylogenetic inventories yielded a curated "chloroorganic" ribosomal gene sequence data base. Different statistical parameters regarding the microbial diversity in contaminated aquifers were evaluated, including calculation of the total amount of operational taxonomic units retrieved so far from the aquifers compared to other ecosystems, prediction of how much of the biodiversity has been captured, systematic evaluation of relatedness of all retrieved sequences to those of known isolates and other clone libraries, and systematic evaluations of biogeography of different key groups, including known dechlorinators.

BTP01
Identification and isolation of the alternariol-o-methyltransferase in *Alternaria alternata*

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Black-moulds of the genus *Alternaria* contaminate many foodstuffs and agricultural products. In addition to the economical damage these fungi can produce harmful secondary metabolites, the *Alternaria* toxins. Some of these mycotoxins such as alternariol (AOH), alternariolmonomethylether (AME), altenuene (ALT) and tenuazonic acid (TA) have been described as cytotoxic, genotoxic and mutagenic *in vivo* and *in vitro*. Due to the fact that mycotoxins could be detect in many foodstuffs and that these fungi can grow even at low temperatures it is necessary to know more about parameters and enzymes of the biosynthesis which affect the production of these mycotoxins.
Little is known about the enzymes of the mycotoxin biosynthesis. Alternariol is a polyketide, it consists of acetyl-CoA units which are linked by a polyketide synthase. Alternariolmonomethylether and altenuene are secondary products of alternariol: AME results directly from AOH by methylation of the SAM-dependent alternariol-o-methyltransferase. Enzyme activity can be shown in crude protein extractions, but the gene is not known. The aim of this study is to identify and isolate the gene-sequence of AOH-methyltransferase to get a reliable marker-gene for the mycotoxin biosnythesis pathway. This offers new possibilities to investigate influencing parameters on the molecular level.

BTP02
Pyranose-2-oxidase from white rot fungus *Pycnoporus cinnabarinus* – biochemical and genetic characterization

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Peroxidases involved in lignocellulose degradation depend on the provision of H_2O_2 as a cosubstrate. A number of oxidase enzymes have been postulated as possible donors of H_2O_2 so far. Pyranose 2-oxidase (POx, pyranose: oxygen 2-oxidoreductase EC 1.1.3.10) is widespread among wood-degrading basidiomycetes of the order Aphyllophorales and is proposed to participate in the process of lignin degradation. In the presence of molecular oxygen the enzyme catalyse the oxidation of several aldopyranoses at carbon-2 and sometimes but in lesser extent at carbon-3. Besides oxygen the reduction of some different quinones has also been shown. Pyranose 2-oxidases have been isolated only from a limited number of fungal species. *Pycnoporus cinnabarinus* has not jet been shown to produce this enzyme.
POx had been produced by *Pycnoporus cinnabarinus* in stirred liquid culture. Enzyme production is correlated with idiophasic growth. The enzyme has been isolated from mechanically disrupted mycelium purified to apparent homogeneity using different chromatographic techniques and characterized biochemically. The preferred substrate is D-glucose but other pyranoses (e.g. L-sorbose, D-xylose, cellobiose) can also be oxidized. The protein is a homotetramer with a molecular mass of about 244 kDa containing flavin.
An open reading frame corresponding to the isolated protein could be detected and sequenced using PCR with degenerated primers leading to a partial sequence followed by a genome walking protocol using gene specific primers. A protein model derived from the gene sequence using AUGUSTUS software consists of 661 amino acids on 15 exons. Using ESI-MS-analysis 9 peptides could be found covering 39% of the Protein.

BTP03
StyA1-StyA2B: A novel and highly efficient FAD-dependent monooxygenase from *Rhodococcus opacus* 1CP

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Rhodococcus opacus 1CP harbors a gene cluster of which *styA1* and *styA2B* seem to be involved in styrene degradation. Both genes were cloned and biochemically characterized. Most remarkably, StyA2B was found to combine two activities, an FAD-dependent styrene oxygenase (´StyA´) and an NADH-dependent FAD reductase (´StyB´). StyA2B therefore represents the first active self-sufficient one-component styrene monooxygenase reported (Tischler et al., 2009). However, *i)* low specific oxygenation activity of StyA2B (0.02 U mg-1), *ii)* the proximity of a presumably cotranscribed gene encoding a single oxygenase subunit (StyA1) as well as *iii)* the absence of a single NADH-dependent FAD reductase (StyB) in the above gene cluster raised questions in respect of a concerted action of StyA1 and StyA2B. In that case StyA1-StyA2B would represent a novel and quiet unusual type of two-component monooxygenase.
In order to prove this hypothesis, mixtures of StyA1 and StyA2B were characterized for their oxygenation efficiency and productivity. Moreover, synergistic effects of this combination were identified by exchanging the reductase component StyA2B against FAD reductases from other organisms (StyB, *Pseudomonas* sp. VLB120) or of other types (PheA2, *R. opacus* 1CP). Efficiency which is defined as ratio of NADH consumption per substrate oxygenation was found to be highest for StyA1-StyA2B indicating a low degree of uncoupling and a minimum of site reactions (oxidative stress). In addition, results show that reduced FAD is transferred from reductase to oxygenase subunit by a specific kind of contact rather than by simple diffusion. We conclude that StyA1-StyA2B is a highly efficient oxygenating system with a low NADH shunt which reveals considerable biotechnological potential.

[1] Tischler D., D. Eulberg, S. Lakner, S.R. Kaschabek, W.J.H. van Berkel, and M. Schlömann. 2009. Identification of a novel self-sufficient styrene monooxygenase from *Rhodococcus opacus* 1CP. *J. Bacteriol.* 191: 4996-5009.

BTP04
Identification of microorganisms with matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (Maldi-Tof-MS)

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Most of the current identification tests in microbiological diagnostics are cost- and labour-intensive as well as time-consuming. A new method called Matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (Maldi-Tof-MS) provides a rapid identification of microorganisms from different origins. Maldi-Tof-MS is an analytical tool which is characterized by rapid and simple sample preparation, high accuracy and sensitivity. Microorganisms are cultivated on agar plates, the samples are collected directly from the agar plate, mixed with matrix, air dried and imported into the mass spectrometer for analysis. As result a spectrum is obtain which is characteristic for every microorganism. A comparison with reference spectra in the database (e.g. SARAMIS) allows a correct identification. At the moment only few reference spectra of environmental micro organisms exist in the available databases. The aim of this project is to generate spectra from environmental microorganisms not yet included in the database by Maldi-Tof-MS, identify them by sequencing and upgrade the database. With this extension of the database a fast identification of microorganisms from environmental samples will be possible.

BTP05
Exploring nitrogen sources of wood rotting basidiomycetes

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Wood is a valuable raw material and its protection is of economic interest. With the generation of new wood preservatives a shift from an overall inhibition of the microbial community to a defined inhibition of the saprophytic part is desired. Many basidiomycetes, ascomycetes and diverse soil bacteria are known as wood degrading community, but the *in situ* mechanisms and synergisms in terms of nitrogen turnover are not fully understood. In our research, we focussed on the degradation of sap wood from pine and beech by *Trametes versicolor* and *Oligoporus placenta* and developed an experimental setup to examine the wood rotting activity in a closed *in vitro* system. In these experiments we combined different parameters, such as nitrogen sources in the fluid phase, the composition of the gas phase and wood and fungal species, and investigated the characteristics of fungal wood degradation. The changes in the composition of the gas phase after fungal growth under a synthetic nitrogen-free gas mixture (79.77 % Ar and 20.23 % O_2) and under air (78.08 % N_2, 20.94 % O_2 and 0.93 % Ar) were analysed using molecular-sieve gas chromatography. The contents of N_2, O_2 plus Ar, CO_2, and CH_4 confirmed that N_2 does not effect fungal growth. The evolution of CO_2 and CH_4 was proportional to the total biomass. With elementary analysis isotopic-ratio-mass spectrometry (EA-IRMS) the mycelium and the composition of the medium were investigated. It was revealed that under nutrient limited conditions both fungi generate a mycelium which was characterized by low nitrogen content compared to nutrient non-limited conditions. The isotopic ratio revealed which nitrogen substrates were converted to biomass. Combining this analytical set up with an appropriate experimental design the basis for synergistic studies was established.

BTP06

Characterization of mating-type mutants of the homothallic ascomycete *Sordaria macrospora* by real-Time PCR, cross-species microarray and yeast-two-hybrid analysis

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The filamentous ascomycete *Sordaria macrospora* is homothallic (self-fertile). In the closely related, heterothallic ascomycete *Neurospora crassa* the mating type of the haploid mating-partners is genetically determined by the mating-type locus, which can be present in two different forms (mat *a* and mat *A*) in compatible mating partners.
The mating type of *S. macrospora* consists of four genes (*SmtA-1, SmtA-2, SmtA-3* and *Smta-1*) sharing similarities to mating-type genes from *N. crassa* mat *A*- and mat *a*-strains. These genes code for putative transcription factors (*SmtA-1, Smta-1*) or proteins without characteristic DNA-binding motifs (*SmtA-2, SmtA-3*).
In this study we analysed the role of the mating-type genes *SmtA-1, SmtA-2* and *SmtA-3* in *S. macrospora*. Knockout-mutants of all three mating-type genes were generated and analysed. ΔSmtA-1 and ΔSmtA-3 mutants are fertile, while the ΔSmtA-2 mutant is not able to produce mature fruiting bodies and ascospores. The expression of pheromones and cognate receptors, involved in sexual development, was analysed by Quantitative real-Time PCR. Cross-species microarray analysis using *N. crassa* arrays were performed to identify differentially regulated genes in the mating-type mutants. The data for various genes was confirmed by real-Time PCR: we analysed the expression of genes involved in melanin biosynthesis, copper transport and reactive oxygen regulation and cell cycle control. Furthermore a yeast-two-hybrid screen resulted in new putative interaction partners of the mating-type protein SMTA-2.

BTP07

Fruiting-body development of *Sordaria macrospora* depends on the interaction of PHO1 and PRO11

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Fruiting-body development of the filamentous ascomyete *Sordaria macrospora* depends on a complex regulation network. In this work we focus on the component *pho1* and its interdependency on *pro11* during fruiting-body development. The PHO1 protein shares high homology to vertebrate phocein, especially to the so called pho/mob domain. Little is known about phocein, but in mammals it seems to play an important role in vesicular traffic. The *pro11* gene encodes a multimodular WD40 repeat protein which shows significant sequence and functional homology to the mammalian protein striatin. Proteins belonging to the striatin family are thought to act as scaffolds linking signaling and eukaryotic endocytosis. It was found previously, that striatin forms a complex with the protein phocein. By yeast two-hybrid analysis we showed that the *S. macrospora* homologs PRO11 and PHO1 are also able to interact with each other. Furthermore, knockout strains of *pho1* and *pro11* exhibit both a sterile phenotype. Knockout strains of *pho1* and *pro11* with an additional downregulation of *pro11* or *pho1*, respectively, via RNAi approach, showed intensified sterile phenotypes, which approves the importance of *pro11* and *pho1* during sexual reproduction. Quantitative Real Time PCR revealed similar expression levels of *pho1* and *pro11* during fruiting-body development. In addition, in the *pro11* knockout strain *pho1* is upregulated compared to the wildtype. All in all our results propose a collective role of PRO11 and PHO1 during fruiting-body development.

BTP08

Nutrient content, organic and bacterial composition of sago factory waste water effluents and a conceptual approach for its bioremediation and reuse

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Cassava tubers, produced in tropical and subtropical countries, have high starch contents. A starch based, granulated food product is Sago produced by separating starch from cassava tubers using 30,000 to 40,000 litres groundwater per tonne starch. Sago industries of Salem district, a prominent cassava cropping area in Tamil Nadu, South India, release huge amounts of organically rich waste waters with toxic effects causing ground water pollution and making thousands of agricultural lands unproductive. Sago factory owners have installed biogas plants to purify one third of the produced waste water. Physico-chemical, microbiological and gaschromatographic determinations of in- and out-flowing waste water collected from biogas plant reveal that the purification procedures should be considerably improved. Based on BOD-, COD-, CN- and nutrients- data, a sago factory waste water purification system is designed that interconnects the existing biogas plant with a constructed wetland and a Sterling motor device for electric power gains, heat distillation of the treated effluents, reuse of process water and groundwater saving.

BTP09

Monitoring of a two-stage biogas plant by FISH and enzyme activity measurements of hydrolases

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Biogas is one of the most promising regenerative energies. In biomethanation microorganisms convert biomass in four steps into biogas. Generally, two processes are possible thereby: one- or two-stage biogas plants. Major advantage of the latter is the possibility to independently control hydrolysis- and methane-stage, carried out separately under different conditions in order to enhance the maceration of the substrate.
In this study we analysed the bacterial and archaeal communities as well as the enzyme activities of several hydrolases to monitor such a plant and to identify possible disturbances within various defined stages of the biogas production.
Using fluorescence *in situ* hybridization (FISH) we were able to illustrate the microbial structure to shift with time and an influence of process-related modifications or disturbances. Also, differentiations between solid (substrate) and liquid phases (leachate) were detectable.
Hydrolases esterase, amylase, protease, alanine-aminopeptidase, carboxymethyl cellulase and xylanase represent key enzymes in the first stage of the biogas generation process. For that reason we analysed the corresponding enzyme activities. Similar to the hybridization analyses, activity changed over time. In addition, a distinction between enzyme activities of solid and liquid samples could be demonstrated. Furthermore, activity optima of investigated hydrolases varyied over retention time, allowing a visualisation of the progress of digestion.
Both methods aid in monitoring of two-stage biogas plants, enabling to detect possible process disturbances even before they affect alterations in physical or chemical parameters. Moreover, results based on analysing substrate, on which the major part of microbial biomass is immobilised, and results based on analysing leachate are not equivalent. This proves stand-alone examination of leachate, usually easy to sample, is deficient for understanding the complete process of biomethanation.

BTP10

Molecular evolution of pyranose 2-oxidase from *Peniophora gigantea* for the efficient application in biocatalysis

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Pyranose 2-oxidase (P2Ox) is a biotechnological useful biocatalyst as it catalyses the regioselective oxidation of various sugars to the corresponding dicarbonyl sugars that are effective starting materials for the synthesis of rare pharmaceutical sugars, fine chemicals and drugs. Only recently, we determined the crystal structure of P2Ox from *Peniophora* sp. [1], analysed its complex enzyme-substrate interactions [2], and engineered the enzyme towards improved thermostability and catalytic efficiency [3]. The catalytic efficiency of this variant for 2-Deoxy-D-glucose was optimized via directed evolution and rational design by introducing the substitutions N71Y and S456N and thus raised 30,3-fold. Rational design at the active site at position T169 led to a variant (T169G) with 4,9- fold higher catalytic efficiency for D-Galactose and a 62-fold higher catalytic efficiency for L-Arabinose, another substrate of high interest. Substitution of hydrophobic amino acid residues in the highly exposed head domain by hydrophilic ones lowered the inclusion body formation by 38% and yielded a versatile and stable variant, P2OxB2H1. Coexpression of trigger factor from *E. coli* reduced the inclusion bodies further down to 7,5%.

By establishing a method of high cell density fed batch fermentation of E. coli, enyzme yield of P2OxB2H1 could be raised 12,7-fold to 6333 U/L. By MALDI TOF analysis of P2Ox digested with pepsin prior to and after bioconversion, a crucial methionine for oxidation could be isolated and substituted by a glutamate via saturation mutagenesis. The new variant showed higher operational stability and remained active even after multiple rounds of bioconversion. In conclusion, we were able to produce variants with higher catalytic efficiency, higher expression yield and better operative stability for the efficient use in bioconversions.

BTP11
Marine cyanobacteria as a source of alkaline phytase producers

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Phytate (myo-inositol hexaphosphate) is the major storage of phosphorus in feeds of plant origin. Monogastric animals, such as fish, poultry, and pigs are not able to metabolize phosphate from phytate, because they do not possess the enzyme phytase in their gastrointestinal tract which hydrolyze the phosphate moieties from its substrate. Phytases, which are commercially available as feed additive, are derived from fungi and are active at acidic pH values.
In the present study, we could demonstrate that marine cyanobacteria produce alkaline phytases with high activities.
All marine cyanobacteria tested were grown at 22°C with 1 µE•m-2•s-1PAR in phosphate-free ASN III/2 medium over 4 weeks. The enzyme activity was determined either with the p-nitrophenylphosphate method (pNPP) or the ammoniummolybdate method with phytic acid as substrate.
From 71 different isolates studied, three heterocystous filamentous Anabaena strains (Hi24, Bo25, Hi35) and the unicellular *Chroococcus turgidus* strain Bo1 showed good phytase activities (48-152 mU) under alkaline conditions (> pH 8.0).

BTP12
Improving the thermostability of 1,5-Anhydro-D-fructose-reductase by iterative saturation mutagenesis (ISM)

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The 1,5-Anhydro-D-fructose-Reductase (AFR) from *Sinorhizobium morelense* S-30.7.5 catalyses the stereoselective reduction of 1,5-Anhydro-D-fructose (AF) to 1,5-Anhydro-D-mannitol as well as the conversion of a number of 2-keto aldoses (osones) to the corresponding manno-configurated aldoses. As diverse osones can be readily prepared from gluco-configurated aldoses by regioselective oxidation with pyranose 2-oxidase (P2Ox), rare pharmaceutical sugars become accessible by coupled redox conversions using P2Ox and AFR. In view of the potential of AFR in rare sugar synthesis, we had constructed NADH-accepting variants (AFR-A13G and AFR-A13G-S33D) by protein engineering, which were efficiently employed in bioconversions with co-substrate regeneration.
So far the thermostability of AFR-A13G was low and correlated with poor operative stability. For the application of AFR in bioconversions, it was therefore necessary to significantly increase the enzyme´s thermostability. For this reason, we employed Iterative Saturation Mutagenesis (ISM) using AFR`s crystal structure. B-Fitter [1] was applied to identify amino acids which are responsible for enzyme flexibility and hence thermolability by determination of their B-factors. These sites are subsequently mutated by primers with degeneracy (NNK) and KOD-PCR. In successive steps, the gene of the best hit from the prevenient mutagenesis round serves as template for mutagenesis on the amino acid position of the subsequent most promising mutant and so on.
Concerning AFR-A13G`s structure, the 9 amino acids with the highest B-factors lay in surface loops. After screening of the recent variants in microtiter- and 30 ml-scale, we succeeded in yielding a new AFR-A13G-mutant (A13G-K207P-E208P) which showed an eight times higher residual activity after heat shock (10 min 45°C) than template-A13G. Further promising variants are to be characterised.

[1] Reetz *et al*. 2006. Angew. Chem. Int. Ed. 45: 7745-7751.

BTP13
Conversion of glycerol to poly(3-hydroxypropionate) in recombinant *Escherichia coli*

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The biodiesel industry is very much interested to convert the huge surplus of glycerol, which is obtained during transesterification of the fatty acids from vegetable oils or fats to methanol, into higher value products. One approach is to produce biotechnologically value added products like polyhydroxyalkanoic acids from glycerol. We have developed the conversion of glycerol into thermoplastic poly(3-hydroxypropionate), poly(3HP). For this, the genes for glycerol dehydratase (*dhaB1*) of *Clostridium butyricum*, propionaldehyde dehydrogenase (*pduP*) of *Salmonella enterica* serovar Typhimurium LT2 and PHA synthase (*phaC1*) of *Ralstonia eutropha* were expressed in a recombinant *Escherichia coli*. Poly(3HP) was accumulated up to 11.8 % (wt/wt CDW) in a two step fed-batch fermentation.

BTP14
Improvement of the 1,3-propanediol production in the Colombian strain *Clostridium* sp. IBUN 158B by genetic engineering

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The non-pathogen strains *Clostridium* sp. IBUN 158B and IBUN 13A were isolated from the soil of Colombian tomato and potato fields. These strains belong to a new species, closely related to *C. butyricum*, and are able to transform residual glycerol from biodiesel production into 1,3-propanediol (1,3-PD). In order to establish a competitive 1,3-PD biotechnological industry based on these strains, the yield of IBUN 158B must be improved by genetic engineering, so that the equilibrium between production and use of NADH can be appropriately adjusted. Therefore, the genes coding for hydrogenase, hydroxybutyryl-CoA-dehydrogenase and lactate dehydrogenase of IBUN 158B were sequenced with primers based on the corresponding genes for *C. butyricum*, in order to inactivate them consecutively with the help of the second generation ClosTron system (Heap *et al.*, J. Microbiol. Methods, Epub 2009 Nov 3). Once the NADH equilibrium has been balanced to favor 1,3-PD production, the three enzymes of the reductive pathway from glycerol (contained in the 1,3-PD operon of the Colombian strains) could become limiting factors. Therefore, the 1,3-PD operon of the strain IBUN 13A will be overexpressed in inactivation mutants of the strain IBUN 158B. First, the complete 1,3-PD operon was sequenced with primers designed on the homologous region of *C. butyricum*. Second, the promoterless operon was amplified and ligated downstream the *ptb-buk* promoter of *C. acetobutylicum* into the shuttle vector pBS77, resulting in the plasmid pBS13A, which can be used to overexpress the operon without depending on the transcriptional control of the natural promoter. The effects of genetic inactivation and overexpression on the 1,3-PD yield of IBUN 158B can be evaluated by measuring the concentration of substrate and products of the mutants.

BTP15
StyA1 from *Rhodococcus opacus* 1CP: A single oxygenase component which retrieves reducing equivalents from a self-sufficient monooxygenase StyA2B

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A gene cluster likely to be involved in styrene degradation was recently found on a 8,965-bp genomic fragment of *Rhodococcus opacus* 1CP. Sequence analysis of the deduced proteins of *styA1* and *styA2B* indicated high homology to two-component styrene monooxygenases from pseudomonads. Recombinant expression and biochemical characterization of StyA2B gave evidence for a novel type of fusion protein combining monooxygenase activity of the N-terminal ´´StyA2´´ polypeptide with NADH-oxidoreductase activity of the C-terminal ´´StyB´´ moiety, the latter providing reduced FAD for the oxygenase (Tischler *et al*., 2009). However, very low specific oxygenating activities of StyA2B (0.02 U mg^{-1} towards styrene) raised questions on a functional dependence from other proteins. StyA1, a hypothetical oxygenase encoded on the same genomic fragment is a first candidate in that respect since it might utilize reduced FAD provided by StyA2B.
We report here on the successful expression and biochemical characterization of StyA1. The specific oxygenating activity (0.22 U mg^{-1} towards styrene) was shown to be 10-times higher than that of StyA2B but corresponded to those of two-component monooxygenases from pseudomonads. Substrate specificity of StyA1 and StyA2B were found to be highly similar. (Chloro)styrene(s), dihydronaphthalene, and methylphenylsulfide are converted to the corresponding epoxides and sulfoxide, respectively.
It could be shown that StyA2B supplies enough reduced FAD at the expense of NADH for full activity of both oxygenase units StyA1 and ´´StyA2´´. Moreover, indications of a more efficient FAD transport within StyA1/StyA2B in comparison to other monooxygenases are presented. We conclude StyA1 and StyA2B to be an unusual two-component monooxygenase related to styrene monooxygenases.

[1] Tischler *et al.* 2009. Identification of a novel self-sufficient styrene monooxygenase from *Rhodococcus opacus* 1CP. J. Bacteriol. 191: 4996-5009.

BTP16
Conversion of aromatic amino acids by two fungal peroxygenases

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Some years ago, we discovered a novel group peroxide-consuming enzymes with exceptional catalytic properties – the aromatic peroxygenases (APO) – which oxidize and oxygenate a huge number of aromatic and heterocyclic substrates. These biocatalysts are secreted highly glycosylated heme-thiolate proteins produced by agaric mushrooms (e.g. *Agrocybe aegerita*, AaP and *Coprinellus radians*, CrP) in complex media rich in amino acids and peptides (e.g. soybean/peptone slurries). The aim of this work has been to study the role of free amino acids as potential APO substrates as well as the effects of amphoteric molecules on peroxygenase catalysis. Most amino acids tested turned out to be poor substrates of both peroxygenases, solely tyrosine was converted into a corresponding dimer. Similar reactions have been described for other plant and fungal peroxidases. The conversion of tyrosine coincided with the particular isoelectric point (pI) of this amino acid. Interestingly, merely this reaction took also place in absence of a radical scavenger (ascorbic acid) indicating the possible inactivation of APO by amino acids. To determine which functional group may be responsible for the decline in peroxygenase activity and the slight conversion rates, we analyzed the oxidation of amino acid derivatives lacking either the amino group (3-phenylpropionic acid) or the carboxylic group (3-phenylpropylamine). The rates observed were relatively low as well, in particular in the case of 3-phenylpropylamine while 3-phenylpropionic acid was hydroxylated to moderate extent both at the aromatic ring and at the benzylic position. The fact that aromatic amino acids as well as their mono-functional derivatives (R-NH_2, R-COOH) are poor APO substrates suggests a possible participation of their amino and/or carboxylic groups in substrate binding at the active site.

BTP17
Increasing of zeaxanthine contents in *Escherichia coli* cells by co-production of hydrophobic substances

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Carotenoids are commercially interesting since they are used for many applications. They are produced by chemical synthesis, extraction of plant materials or microbial fermentation; however, carotenoid biosynthesis in *Escherichia coli* in economically feasible amounts could not be established, yet. We describe a new approach to increase the carotenoid amounts in heterologous *E. coli* by simultaneous synthesis with hydrophobic substances like neutral lipids and polyhydroxybutyrate (PHB). For zeaxanthine biosynthesis the plasmid pACCAR25Δ*crtX* carrying genes from *Pantonea ananatis* was used. For lipid biosynthesis the fatty alcohol-producing bifunctional acyl-CoA reductase (*acr1*) from the jojoba plant and the bacterial wax ester synthase (*atfA*) from *Acinetobacter sp*. strain ADP1 were applied. For PHB-synthesis the *phaCAB* operon from *Ralstonia eutropha* was used. Spectrophotometric and HPLC analyses of cells coproducing lipids and zeaxanthine showed an almost two-fold increased accumulation of zeaxanthine with a carotenoid content of about 1.8 mg g^{-1} cell dry weight (CDW). In contrast, coexpression of carotenoid and PHB biosynthesis genes in *E. coli* BL21 (DE3) resulted in a carotenoid content of only about 1.1 mg g^{-1} CDW. These data might initiate further research in this direction to increase the carotenoid contents of engineered *E. coli* strains and possibly also in other bacteria.

BTP18
A periplasmic, pyridoxal-5´-phosphate-dependent amino acid racemase in *Pseudomonas taetrolens*

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The pyridoxal-5´-phosphate (PLP)-dependent amino acid racemases occur in almost every bacterium but may differ considerably with respect to substrate specificity. We here isolated the broad substrate specificity racemase ArgR of *Pseudomonas taetrolens* from *Escherichia coli* by classical procedures. The racemase was biochemically characterized and amongst other aspects it was confirmed that it is mostly active with lysine, arginine and ornithine, but merely weakly active with alanine, whereas the alanine racemase of the same organism studied in comparison acts on alanine only. Unexpectedly, sequencing the amino-terminal end of ArgR revealed processing of the protein, with a signal peptide cleaved off. Subsequent localization studies demonstrated that in both *P. taetrolens* and *E. coli* ArgR activity was almost exclusively present in the periplasm, a feature so far unknown for any amino acid racemase. An ArgR-derivative carrying a carboxy-terminal His-Tag was made and this was demonstrated to localize even in an *E. coli* mutant devoid of the twin-arginine translocation (Tat) pathway in the periplasm. These data indicate that ArgR is synthesized as a prepeptide and translocated in a Tat-independet manner. We therefore propose that ArgR translocation depends on the Sec system and a post-translocational insertion of PLP occurs. As further experiments showed, ArgR of *P. taetrolens* is necessary for the catabolism of D-arginine and D-lysine by *P. taetrolens*.

BTP19

D-sorbose producing L-sorbitol-dehydrogenases (LSDH): enzymes from database screening and directed mutagenesis or molecular evolution of D-sorbitol-dehydrogenase (DSDH) for chiral re-orientation

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The rare sugar D-sorbose is an interesting synthon for pharmaceutical applications and can be produced from easily prepared L-sorbitol by LSDH [1]. BLAST search with the N-terminal amino acid sequence of the *Stenotrophomonas maltophilia* enzyme [1] listed putative enzymes with best similarities for an assumed ribitol-DH of *Bradyrhizobium japonicum*. The gene was amplified, tagged with histidines and heterologously expressed. The enzyme exhibited high activity for D-sorbitol transformation to D-fructose but also reasonable activity with L-sorbitol resulting in D-sorbose as the single product. A cost effective co-factor regeneration system for these procedures can be achieved with electrochemical methods. DSDH from *Rhodobacter sphaeroides* has been shown to be useful as a model enzyme for such electroenzymatic reactions [2]. However, for the production of D-sorbose the chiral properties of the DSDH have to be re-orientated and on the basis of the structural data bioinformatic calculations and modelling were performed. A change of the enantiomeric specificity was proposed by deleting V184 and exchange of Q140G or Q140N. In parallel random mutations with error-prone-PCR were created and improved enzymes were detected using phenazinmethosulfate and nitrobluetetrazoliumchloride, which react with the produced NADH by developing a blue formazan dye [3].

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[2] Gajdzik J., Szamocki R., Natter H., Kohring G.W., Giffhorn F., Hempelmann R., J. Solid State Electrochem. 11, 144-149 (2007).
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BTP20

Generation of D-sorbitol dehydrogenase (DSDH) variants for application in enzyme reactors with electrochemical co-factor regeneration

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Enzyme reactors with electrochemical co-factor regeneration appear as cost effective methods for the production of enantiopure fine chemicals with dehydrogenases, which can be used as synthons in pharmaceutical drug development. Immobilization of all components like the enzyme, the co-factor and the mediator on a gold electrode surface would allow a construction of a flow reactor with minimized product purification. DSDH from *Rhodobacter sphaeroides* Si4 [1] has proven to be active in systems with mediators immobilized on the electrode [2] and can be used as a model enzyme for the development of such reactors. As has been shown for galactitol dehydrogenase [3] also DSDH could be immobilized on electrodes as an active enzyme via cysteine residues in front of an N-terminal His(6)tag. Enzyme variants exhibiting only the His(6)tag were washed from the electrode surface and did not show any activity in cyclovoltammograms, indicating that the cys residues were necessary for stabile attachment of the enzyme.
On the basis of structural data [4] enhanced thermal stability by directed mutagenesis was proposed from bioinformatic calculation and modelling. One of the so far generated variants exhibited a slight increase in thermal stability with preserved activity.
Addition of small amounts of DMSO, methanol, ethanol or 2-propanol for facilitating the solubility of hydrophobic substrates inhibited the enzymatic activity. However, in a biphasic system with buffer and 1-octanol the activity for reduction of 2,3-pentanedione or 3,4-hexanedione increased eight to tenfold as compared to the usual buffer system. Further experiments will focus on optimized stability against solvents and temperature.

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[2] Gajdzik *et al.*, J. Solid State Electrochem. 11, 144-149 (2007)
[3] Kornberger *et al.* Langmuir 25, 12380-12386 (2009)
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BTP21

Constitutive PHB synthesis in mutants of *Ralstonia eutropha* defective in lipopolysaccharide biosynthesis

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Ralstonia eutropha serves as a model organism for the metabolism of the storage compound poly(3-hydroxybutyric acid) (PHB). Six Tn*5*-induced mutants of the strain *R. eutropha* HF39 with defects in genes involved in the synthesis of lipopolysaccharides (LPS) were isolated which appeared to accumulate PHB constitutively in contrast to the wild type *R. eutropha* H16. LPS are part of the Gram-negative outer membrane and play an important role in membrane stability and permeability, and truncated LPS may lead to increased permeability of the membrane for carbon sources located in the medium. The insertions of Tn*5* were detected in the genes *hldA*, *hldC* and *rfaF*, coding for D-β-D-heptose 7-phosphate kinase (HldA), D-β-D-heptose 1-phosphate adenylyltransferase and ADP-heptose:LPS heptosyltransferase, respectively. The Tn*5*-induced mutant HF39 DO10 with a non functional HldA probably resulting in LPS consisting only of lipidA-KDO_2 was characterized in detail. In addition to carbon utilization tests, the accumulation of PHB and the proteoms of these mutants were analyzed and compared with the parent strain. Significantly increased PHB accumulation during exponential cell growth was clearly detected for HF39 DO10 in difference to the parent strain approving the phenotype "PHB constitutive". Proteomic analyses of HF39 DO10 and H16 revealed significantly increased expression levels in the mutant concerning several proteins involved in central carbon metabolism. Based on the obtained results a hypothesis was proposed explaining the observed phenotype: due to the defective LPS in the mutants the uptake of carbon source is most probably facilitated resulting in increased levels of acetyl-CoA caused by an enhanced Entner-Doudoroff pathway and additionally formed NADPH. These higher levels of acetyl-CoA and NADPH obviously promote PHB biosynthesis which are normally not permissive in the parent strain.

BTP22

Regioselective oxygenation of pharmaceutically relevant substances by secreted peroxygenases from *Agrocybe aegerita* and *Coprinellus radians*

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The synthesis of human drug metabolites via selective monooxygenation is one the most challenging reactions in synthetic chemistry. *In vivo* in humans, these reactions are mainly catalyzed within the cytochrome P450-liver metabolism. Here we report that fungal peroxygenases from *Agrocybe aegerita* (AaP) and *Coprinellus radians* (CrP) catalyzed the regio- and stereoselective *in vitro* monooxygenation of the β-blocker metoprolol, the anti-inflammantory drug naproxen, the anticonvulsants carbamazepine, the potassium-channel blocker tolbutamide, the antiviral drug oseltamivir phosphate and the endocrine disruptor 17α-ethinylestradiol. The results demonstrated that the reactions proceed regioselectively and enantioselectively yielding isomeric purities up to 98% of the desired metabolites. For example, chiral HPLC analysis – after AaP-catalyzed oxidation of a racemic naproxen mixture – showed that *(S)*-naproxen was clearly the preferred substrate. The resulting *O*-demethylnaproxen contained a 66% enantiomeric excess (ee) of the *(S)*-enantiomer. Studies using ^{18}O-enriched hydrogen peroxide ($H_2{}^{18}O_2$) as co-substrate revealed that the oxygen incorporated into the reaction product in fact derived form the peroxide, which points to a true peroxygenase mechanism. Thus, mass spectral analysis of the metabolites formed during the AaP-catalyzed oxidation of tolbutamide in the presence of $H_2{}^{18}O_2$ in place of H_2O_2 showed a shift of the principal $[M+H]^+$ ion from *m/z* 287 to *m/z* 289 in case of 4-hydroxytolbutamide. Interestingly, oseltamivir phosphate was only oxidized by CrP (80% conversion into the ester cleavage product oseltamivir carboxylate), whereas AaP was not capable of converting this compound. Our results clearly indicate that fungal peroxygenases may be a useful biocatalytic tool to prepare pharmacologically relevant metabolites.

BTP23
Earthworms Enhance the Microbial Removal of Phenolic Compounds in Soil

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Earthworms play an important role in processing soil organic matter and contribute to the removal of organic pollutants from soil. 2,4-dichlorophenol (2,4-DCP) and 4-chloro-2-methylphenoxyacetic acid (MCPA) are potential soil or groundwater contaminants that persist in the environment. Soil columns were constructed and destructively sampled after 33 days and 35 days, respectively, to determine the potential effect of earthworms (*Aporrectodea caliginosa*) on the fate of 2,4-DCP and MCPA. Burrow wall, cast and bulk soil material were used for subsequent experiments. Soil treated with earthworms showed a 2-fold faster 2,4-DCP and MCPA degradation compared to untreated soil. Furthermore, burrow walls and casts enhanced degradation. Culture-dependent and culture-independent methods were applied for isolation and identification of new key organisms. 250 2,4-DCP- utilizing bacteria were isolated and belonged to 11 genera. Analysis of *tfdA*-like genes (encodes an α-ketoglutarate-dependent dioxygenase involved in MCPA degradation) indicated that hitherto unknown, diverse *tfdA*-like genes are abundant in soil. Stable Isotope Probing (SIP) with ^{13}C-labeled MCPA was performed to identify active microorganisms. 47 families were labeled. *Alphaproteobacteria* (i.e. *Sphingomonadaceae*, *Bradyrhizobiaceae*) were dominant in soil columns. The collective data indicate that (a) earthworms stimulate the degradation of 2,4-DCP and MCPA in the drilosphere by enhancing the activity of 2,4-DCP and MCPA degraders, and (b) soil influenced by earthworms harbors highly diverse and novel potential 2,4-DCP- and MCPA- utilizing microorganisms.

BTP24
Nanopiercing: the ultimate ease of bacterial transformation

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Recently, a unique method for bacterial transformation using nanofibers to inoculate DNA has been developed by Naoto Yoshida and colleagues [1,2]. We have verified the principle, transforming *Escherichia coli*, *Yersinia enterocolitica* and *Acinetobacter baumannii*, and have established a user-friendly protocol. A buffered suspension of sepiolite – an inexpensive, fibrous yet inoffensive mineral – is mixed with bacteria and transforming DNA and the mixture directly spread on selective agar.

[1] Yoshida, N., Ikeda, T., Yoshida, T., Sengoku, T., Ogawa, K., 2001. Chrysotile asbestos fibers mediate transformation of *Escherichia coli* by exogenous plasmid DNA. FEMS Microbiol. Lett. 195, 133-137.
[2] Yoshida, N., Ide, K., 2008. Plasmid DNA is released from nanosized acicular material surface by low molecular weight oligonucleotides: exogenous plasmid acquisition mechanism for penetration intermediates based on the Yoshida effect. Appl. Microbiol. Biotechnol. 80, 813-821.

BTP25
Oxygenation cascade in the conversion of *n*-alkanes to carboxylic acids catalyzed by secreted fungal peroxygenase

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Selective hydroxylation of non-activated aliphatic hydrocarbons is a particular challenging reaction in organic synthesis. On the other hand, numerous microorganisms (bacteria and fungi) are capable of oxidizing and even utilizing *n*-alkanes as carbon source. For this, they use specific intracellular enzymes that transform the inert *n*-alkanes into the corresponding *n*-alkanols, which are further oxidized via aldehydes to fatty acids. Using such enzymes *in vitro* for biocatalytic oxygen transfer reactions would offer an interesting alternative to achieve selective hydroxylation. However, these biocatalysts such as methane monooxygenase (MMO) or certain cytochrome P450 monooxygenases (P450s) are intracellular and less stable enzymes, which are hardly suitable for application in isolated form. Here we report on a secreted peroxygenase from the agaric fungus *Agrocybe aegerita*, which catalyzes the H_2O_2-dependent monooxygenation of C_4-C_{10} alkanes to its corresponding alcohols. Moreover, these alkanols were found to be further oxygenated and oxidized by peroxygenase into aldehydes and fatty acids. The incorporation of ^{18}O from a respectively labeled co-oxidant ($H_2{}^{18}O_2$) into the oxidized products revealed that the reaction proceeds via the typical peroxygenase mechanism, i.e. peroxide-oxygen (R-OOH) - and not dioxygen (O_2) as in case of MMO or P450s – is transferred to the substrate. Unlike intracellular MMO and P450s, *A. aegerita* peroxygenase is highly stable and does not need complex cofactors and electron transport proteins (NAD[P]H, flavin reductases) for function. Thus, our results raise the possibility that fungal peroxygenases may be useful for the selective enzymatic synthesis of alkanols and derived carbonyls as well as acids.

BTP26
Stress response of *Corynebacterium glutamicum* to secretion via twin arginine translocation (Tat)-pathway

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C. glutamicum is a well known host for industrial amino acid production. Recent studies suggested the organism also to be suitable as a host for heterologous protein expression and secretion. Proteins which are translocated to the supernatant are easier to purify than those which need to be purified from cytoplasm. In this study we want to identify cellular factors which are involved in secretion of proteins by the Tat-pathway. To distinguish between expression-related and secretion-related reactions we performed DNA-microarrays with cultures expressing GFP as a model protein fused to differentially effective Tat signal peptides. This led to different regulation patterns. To simulate a perfectly effective signal peptide which would block the Tat system for its native substrates we examined the deletion mutant in *tatAC* which shows a severe growth defect compared to wild type. This seems to be due to impaired use of the central metabolism, for example iron uptake.

BTP27
Biofuel production by the cyanobacterium *Synechocystis* sp. PCC 6803

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To overcome the dependency on fossil fuels, which currently account for almost 80% of the world energy demand, alternative sources of environmentally safe, readily available and renewable energy are urgently needed. Of special interest are the biofuels that allowed to circumvent the extensive use of arable land and to avoid conversion steps of plant biomass into ethanol. Our approach is to combine photosynthesis with the synthesis of ethanol in one genetically manipulated cyanobacterial cell.
The aim of this project is to establish a systemic understanding of the metabolism of the producing strain in order to exploit its potential for biofuel (ethanol) production, based on extensive experimental characterization of photosynthetic growth, metabolomic and transcriptomic analyses as well as mathematical modeling.
The project focuses on *Synechocystis* sp. PCC 6803 as the most suitable model organism with a rich compendium of genomic, biochemical and physiological data available. Its genome was sequenced in 1996 and is well-annotated and a spectrum of methods enables genetic manipulations.
Extensive modification of metabolic pathways demands often the introduction of multiple stable mutations into the organism. Antibiotic-resistance free approaches are specially promising under industrial conditions.
We established two methods for introduction of marker-free mutations into *Synechocystis* sp. PCC6803 using the levansucrase gene *sacB* conferring a sucrose sensitive phenotype (Ried, Collmer 1987) and streptomycin resistant/sensitive alleles of *rps12* gene (Matsuoka, 2001).

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BTP28
Cellulase enzyme production by *Pseudomonas mallei* using rice husk as a substrate

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The study was carried out to asses the potential of rice husk as a feed substrate for the production of cellulase enzyme using *Pseudomonas mallei*. The organism was isolated from an old rice husk dump at Abakaliki rice mill Ebonyi State, Nigeria. Ebonyi State is one of the largest producers of rice in Nigeria with several rice husk dumps all over Abakaliki rice mill, the area with the largest cluster of rice mill in the State. The estimated annual production of rice husk in the State was about sixty thousand tones (60,000).
The fermentation was done on an oscillator (TR-201BD) set at 100rpm for 4 days at 250C and daily analysis of cellulase activity carried out. Cellulase activity and production rate were measured as the ratio of yield of glucose to time.
The result showed the presence of cellulase enzyme in the culture broth for the 4 days of fermentation. The maximum enzyme activity of 5.10 IU occurred at the 1st day of fermentation. Because this organism gave high cellulase yield, it has potential in the commercial production of cellulases using rice husk as a substrate.

BTP29
From nature for nature – bioinspired nanocomposite materials for environmental technology

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Microorganisms like bacteria developed during evolution highly effective mechanisms and structures to survive at the most forbidding, uninviting places on Earth. One example, intensively studied at the Institute of Radiochemistry of the Forschungszentrum Dresden-Rossendorf, is the binding of heavy metals and actinides by cell surface proteins of uranium mining waste pile isolates. The so called surface layer (S-layer) proteins prevent the uptake and any sustainable damage of the cell by toxic and/or radioactive metals. The S-layers itself form highly ordered and mono-molecular envelopes around bacterial and archaeal cells. Noteworthy is their ability to self-assemble in suspension, on surfaces and at interfaces. Furthermore S-layers of different bacteria are able to fulfil different functions and thus may act as immobilization matrix for exoenzymes, as molecular sieve, as ion and molecule trap or they protect the cell from being affected by the immune defence of host organism, by other bacteria or by lytic enzymes. By combining these unique features of S-layer proteins, smart coatings on many different surface can be realized. Currently at the Institute of Radiochemistry, S-layer based functional coatings are under development for the production of (photo)catalytic active materials, metal selective filters and highly specific biosensors. Therefore possible applications are the elimination of pharmaceuticals and germs, the detoxification of metals, the removal of toxic metals, the recovery of noble metals or the detection of pharmaceuticals and other organic matter in water. Additionally, combinations of functionalities are possible using a layer-by-layer technique, offering a wide field for the development of new nanostructured biocomposites for environmental technology.

BTP30
A New Method of Markerless Gene Deletion in *Gluconobacter oxydans*

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Gluconobacter oxydans is an acetic acid bacterium that is well known for its potential to incompletely oxidize a wide variety of sugars, alcohols and polyols. *G. oxydans* can be used in a multitude of biotechnological processes where it performs stereo- and regio-specific oxidation steps that can hardly be achieved by organic chemistry.
Despite its biotechnological relevance methods for markerless gene deletion in *G. oxydans* remain labor intensive. We developed a new system based on the use of uracil phosphoribosyltransferase (UPRTase) as a counter-selection marker in the presence of the toxic pyrimidine analogue 5-fluorouracil (5-FU). Deletion of the *upp* gene coding for the uracil phosphoribosyltransferase confirmed that the mutant is resistant to 5-FU while the wild type of *G. oxydans* 621H is sensitive to 5-FU. When the *upp* gene was introduced in trans into the deletion mutant, sensitivity to 5-FU could be restored. We employ this counter-selection in our deletion strategy.
Up to now we successfully obtained deletion mutants of Gox0310 (NAD(P) transhydrogenase subunit alpha), Gox0431 (phosphogluconate dehydratase) and Gox1711 (transcriptional regulator). Further analysis will unveil a more detailed phenotype of the mutants. Using this method we are in the process of deleting several membrane-bound and soluble dehydrogenases as well as potential regulators. The simplified construction of markerless mutants will ease the functional analysis of *Gluconobacter* genes.

BTP31
Synthesis of aromatic β-amino acids using novel cyclic amidases

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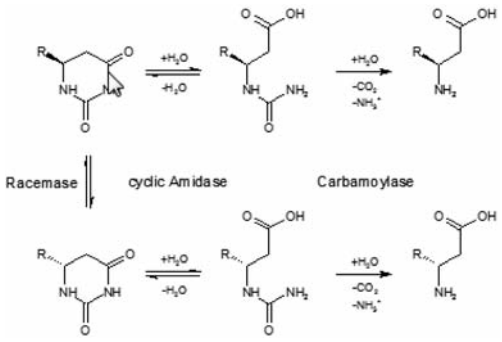
Optical active β-amino acids are important building blocks for pharmaceuticals and fine chemicals.
As their chemical synthesis is still inefficient and costly our approach is a modification of the well studied hydantoinase/carbamoylase system. In order to produce β-amino acids dihydrouracils serve as substrates and a cyclic amidase with hydrolytic activity for these six-ring systems has to be applied.
In a first step phenyldihydrouracil was synthesized from bulk chemicals. This model substrate was applied in whole cell biotransformation experiments using strains with known activity for aryl-substituted hydantoins. Nearly all of the tested bacteria hydrolyzed phenyldihydrouracil to *N*-carbamoyl-β-phenylalanine [1]. Furthermore an analysis of the biotransformation supernatants with chiral HPLC revealed that some strains are able to distinguish between the two subtrates enantiomers while others showed nearly complete conversion. Subsequently biotransformations with crude extracts were carried out to confirm that enantioselectivity relies on the amidase and not on the transport. We started to identify the responsible genes to enable a recombinant expression of several cyclic amidases. A screening for carbamoylases able to catalyze the follow-up reaction to β-amino acids is in progress. Finally a whole cell biocatalytic system using the cyclic amidase/carbamoylase system for the efficient conversion of aromatic dihydrouracils to highly enantiopure aromatic β-amino acids has to be established.



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BTP32
Enzymes in Synthesis: Novel transaminases for the production of enantiopure aromatic β-amino acids

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Transaminases (aminotransferases EC 2.6.1) are a large and diverse group of enzymes that catalyze the reversible transfer of an amino group from amino acids to oxo acids. Transaminases are widely applied in the synthesis of unnatural amino acids and chiral amines due to their high enantioselectivity and broad substrate specificity [1]. Most microorganisms have multiple transaminases and there is a wealth of diversity amongst these enzymes in terms of amine donor specificity and stability.
In this study we applied these versatile enzymes to the production of optically pure aromatic β-amino acids. Several new strains with activity towards aromatic β-amino acids could be isolated from soil samples by enrichment culture. These strains were identified by 16S rDNA sequencing. The conversion of aromatic β-amino acids of these isolates has been characterized [2].

[1] Hwang BY, Cho BK, Yun H (2005), J. Mol. Catal. B- Enzymatic : 37, 47–55.
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BTP33
Diketopiperazines as potential substrates for hydantoinases

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Diketopiperazines (DKPs) are abundant natural compounds produced by a variety of microorganisms for different purposes, e.g. as signaling compounds in quorum sensing [1]. Some of the most important biological activities of DKPs are their antitumor, antiviral, antifungal and antibacterial functions [2].
DKPs as the smallest possible cyclic peptides could serve as a novel source of different dipeptides consisting of unusual (non-proteinogenic) amino acids. This would require a regio- and stereoselective hydrolysis, ideally by a specific proteolytic enzyme; however, DKPs are known to be resistant to proteolysis [2].
In this work certain hydantoinases are tested for DKP hydrolysis activity. Recently we have shown that these cyclic amidases are able to cleave different substituted dihydrouracils [3]. Due to the structural similarity between hydantoins, Dihydrouracil derivatives and DKPs, a selective hydrolysis of DKPs by hydantoinases seems likely.
Additionally, DKPs are reported to be produced by numerous sponge-associated bacteria [4]. Different sponge samples were incubated in minimal media containing certain DKPs as sole carbon and nitrogen sources. With these enrichment cultures more than 20 bacterial cultures were isolated, which are now under investigation for suitable enzymes with the ability to hydrolyze DKPs.

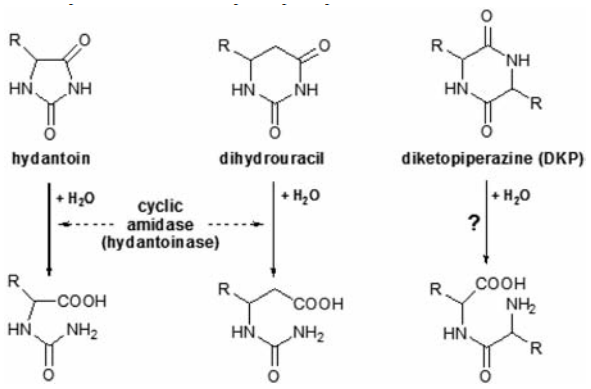

Fig. 1: Enzymatic hydrolysis of cyclic amides.
Hydantoins are cleaved to N-carbamoyl-α-amino acids, whereas dihydrouracil derivatives react to N-carbamoyl-β-amino acids. Depending on the substituent, both reactions can be performed by cyclic amidases (hydantoinases). An analogous hydrolysis of DKPs would lead to dipeptides and is investigated in this work.

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BTP34
Enzymatical peptide synthesis via reversed proteolysis

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Chemical peptide synthesis is dependent on protection group chemistry, organic solvents and enantiopure substrates. For certain applications, these major drawbacks can be overcome by enzymatical peptide synthesis using the reversed protease reaction. The most prominent example is the large-scale production of the sweetener Aspartame™ where educt coupling is achieved using the *Bacillus thermoproteolyticus* derived metalloprotease thermolysin [1]. However, a general method for enzymatic peptide synthesis is still missing, depending on the substrate specificity of the proteases used and their optimal reaction conditions concerning pH, temperature, solvent etc. as well as the solubility of the desired product and its components.
In general, the equilibrium of protease reactions can be shifted towards synthesis thermodynamically or kinetically, with the kinetic approach being limited to serine and cysteine proteases but giving much higher product yields and requiring a smaller amount of enzyme [2].
In this work, we are testing various enzymes for kinetically controlled dipeptide synthesis using different reaction strategies in non-conventional reaction media including frozen aqueous solutions and ionic liquids.

[1] K. Oyama: The Industrial Production of Aspartam. In: Chirality in Industry (Ed.: A. N. Collins, G. N. Sheldrake und J. Crosby), Wiley, 1992, S. 237-247
[2] F. Bordusa: Proteases in organic synthesis. Chem. Rev. 2002, 102, p. 4817-4867

BTP35
Optimization of an acetoin-controlled *Bacillus subtilis* expression system

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Bacillus subtilis is one of the workhorses of white biotechnology. We have developed an acetoin-controlled expression system for the heterologous expression of recombinant genes in this bacterium. In order to enhance the efficiency and applicability of the system a number of improvements was tested, such as additional copies of transcription factors, the modification of existing pathways, and the establishment of new pathways. Furthermore, the inducer was thoroughly analyzed and easily accessible plasmids were constructed.
The expression system is induced by use of the overflow metabolite acetoin, which guarantees a long lasting expression. In order to optimize the expression of the *acoA*-promotor we have created mutants with additional copies of both the activator *acoR* and the sigma factor L.
Moreover, pyrosequencing and annotation of the host strain *B. subtilis* ATCC 6051 allows us to directly target extracellular proteases, sporulation and lysis factors and to improve the inducer usage.
This comprehensive approach has already resulted in an increased yield and purity of heterologous model proteins overproduced in this alternative *B. subtilis* host vector system.

BTP36
Prospecting for novel thermophilic lipases in metagenomes

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Metagenomics is a powerful tool for the discovery of novel biocatalysts from non-cultivated microorganisms. Here we report on the identification of novel thermostable archaeal and bacterial lipases from three different microbial communities. Our metagenomic libraries were constructed from an enrichment using heating water as inoculum, a long term soil enrichment culture and a deep-sea hydrothermal vent enrichment. Cultures were maintained at 65-70°C and microbial communities were characterized on a phylogenetic level based on 16S rRNA genes. In the soil enrichment mainly thermophilc Firmicutes (*Bacillus* spp. and *Symbiobacterium* spp.) were identified after several months of incubation, while the heating water culture contained mostly novel *Thermus* species. The hydrothermal vent culture consisted predominantly of archaeal species that are closely related to *Thermococcales*. DNA was extracted from the designated enrichments and metagenomic libraries constructed comprising 800 to 8500 clones. Screening the libraries on *p*NP-substrates (C4 and C12) at temperatures between 50 °C and 70°C resulted in the identification of 15 lipolytically active clones. The enzymes currently characterized show a reproducible high activity after overnight incubation at 70°C and catalyze the hydrolysis of *p*NP-palmitate, -stearate and -oleate, indicating lipase activity. Further works concentrate on the efficient expression of the respective lipases and their biochemical characterization.

BTP37
Decolorization of reactive dyes by halotolerant fungi

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The ability of terrestrial fungi to decolorize industrial dyes has been widely investigated over the past years and documented for various fungal species, whereas halotolerant, facultative or obligate marine fungi have been hardly considered in such studies. On the other hand, halotolerant aquatic fungi may have unique properties concerning degradative biocatalysts and, with their adaptation to harsh conditions, they may be suitable for specific applications such as the biological treatment of effluents from textile industry with high salt concentrations, alkaline pH and residual reactive dyes. In this context, fungal strains were collected at the Cambodian coast line using "indirect" isolation procedures, i.e. samples of dead wood and leaf-litter were collected from beach and mangrove environments and placed on selective agar plates. After all isolation tests, 25 fungal strains were obtained and studied within a qualitative screening concerning the decolorization of agar plates containing recalcitrant model dyes. Some of the isolates showed decolorizing activities towards reactive dyes such as Reactive (R) Black 5, R Blue 172, R Green19, R Red 11, and R Yellow 22. Furthermore, they oxidized indicator substances for extracellular phenol oxidases and laccases such as ABTS (2,2´-azinobis[3-ethylbenzthiazolin-6-sulphonat]). These fungi were further investigated within a second screening test to decolorize respective dyes in liquid culture. The extent of decolorization was followed photometrically along with the activities of potentially involved ligninolytic enzymes such as laccase, manganese, and lignin peroxidases. Furthermore, the ITS-regions of the selected strains were sequenced and analyzed for their identification. Preliminary results indicate the isolation of typical wood-degraders (e.g. a strain of the basidiomycetous genus *Bjerkandera*) but also less well-known mangrove inhabiting fungi (e.g. a strain of the ascomycetous genus *Massarina*).

BTP38
Utilization of C2-metabolites and amino acids by *Bacillus licheniformis*

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In the environment microorganisms are typically confronted with a mixture of amino acids.
B. licheniformis DSM13 is able to use a variety of proteinogenic amino acids as carbon and nitrogen source. We determined the order of the depletion of amino acids from mixtures and characterized the products formed during growth on single amino acids via ion chromatography analysis. In our study we also used extensive transcription analysis with DNA microarrays to identifiy a set of genes characteristic for growth on single amino acids. Among the identified genes was a potential operon containing a paralog of *mmsA* together with several uncharacterized genes that was specifically expressed during growth on all amino acids and short chained acids tested. By means of the bioinformatic tool `BiBag` we found those genes to be missing from *B. subtilis* and close relatives but also to be present in the *B. cereus* group.
Using the method we developed for marker-less deletions in *B. licheniformis* we found a very small protein of only 45 AA lenght to be essential for growth on acetate. It is located upstream of the malate synthase and the isocitrate lyase forming a glyoxylate shunt in *B. licheniformis* DSM13. The small protein only shows high homology to an uncharacterized small protein upstream of the isocitrate lyase of *Bacillus clausii*. It posesses no helix-turn-helix motif and we hypothesize that it functions by a regulation of the glyoxylate shunt via protein-protein interaction with the isocitrate lyase. The deletion of those two genes resulted in a lack of growth on acetoine, 2.3-butanediol and acetate.
For further investigations of the metabolism of *B. licheniformis* DSM13 we establish a transposon mutagenesis based on the „*mariner*“ transposon.

BTP39
Proteomic characterisation of *Bacillus licheniformis* subpopulations in liquid media

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Gram positive Bacillus species are of industrial interest for homologous and heterologous gene expression. Recent studies on *Bacillus subtilis* [Lopez *et al*. 2008] lead to the thesis of a heterogeneity in bacterial cultures. It was shown that in genetically identical *B. subtilis* cultures different phenotypes can occur under the same environmental conditions and at the same time. These subpopulations show different metabolic strategies like motility, competence, cannibalism, spore formation and the production of extracellular matrixes for biofilm formation.
By means of density gradient centrifugation we were able to separate subpopulations from *Bacillus licheniformis* cultures grown on a complex medium. To characterise these subpopulations we analysed the proteomic pattern by 2-D-gel electrophoresis. Proteins which showed different expression patterns were analyzed by MALDI-TOF mass spectrometry.
The two major subpopulations revealed clear differences in their proteomic patterns. Further *B. licheniformis* subpopulations are currently being investigated by gradient centrifugation.

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BTP40
Metagenomic biocatalysts efficiently hydrolysing cellulose in Ionic Liquids

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Ionic liquids (ILs) are novel and chemically inert solvents for a wide range of reactions in organic synthesis and biocatalysis, and several of them have been described to dissolve cellulose and/or wood. ILs would provide novel options for cellulose degradation in homogenous catalysis if cellulases were sufficiently stable and active.
For the discovery of novel enzymes, metagenomic libraries from different highly hydrolytic communities were constructed. Among these are libraries originating from an enrichment culture with the shipworm Teredo navalis, feces of the asian elephant (Elephas maximus indicus) and a biogas plant. All these microbial communities were characterised via 16S rDNA analysis and exhibit a broad diversity of microorganisms. By screening the constructed metagenomic libraries 30 novel cellulase clones were identified and tested for their performance in the presence of ILs. Most of these enzymes showed only poor activities in the presence of the investigated ILs. But two cellulases, derived from a biogas plant, showed remarkable activity and stability in different ILs. These two enzymes are now overexpressed in E. coli, characterised and evaluated for their use in biotechnological applications.

BTP41
Improvement of H_2 production by the "bidirectional", NAD^+-reducing [NiFe]-hydrogenase of *Ralstonia eutropha* through modification of the subunit composition

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The NAD^+-reducing soluble [NiFe]-hydrogenase (SH) provides *R. eutropha* H16 with the ability to gain reducing equivalents in form of NADH directly from the oxidation of H_2. Under oxic conditions, most NADH is re-oxidized by complex 1 and, thus, serves as the main reductant of the respiratory chain [1]. A shift from aerobic to anaerobic conditions, however, induces a reversal in the direction of the SH resulting in the conversion of transiently accumulated NADH into H_2, which is finally released by the cells [2].
The SH is composed of a heterodimeric hydrogenase module and a heterodimeric NADH oxidoreductase module. Furthermore, two copies of the HoxI protein, which is proposed to provide a NADPH binding site, are primarily attached to the NADH oxidoreductase module [3].
Here we show that the removal of the HoxI proteins from the heterohexameric SH by genetic engineering results in a significant increase in the H_2 production capacity of the enzyme. Thus, the HoxI subunits seem to shift the enzyme's bias towards H_2 oxidation. This result is important for the assignment of the optimal gene configuration for the heterologous production of NAD^+-reducing [NiFe]-hydrogenase in cyanobacteria in order to meet the long-term goal of light-driven hydrogen production.

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[3] Burgdorf T., van der Linden E., Bernhard M., Yin Q.Y., Back J.W., Hartog A.F., Muijsers A.O., de Koster C.G., Albracht S.P., Friedrich B. (2005) J. Bacteriol., 187-9, p.3122-3132.

BTP42
Characterization of diauxic growth and reduced hydrogenase activity in *Clostridium acetobutylicum*

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Clostridium acetobutylicum is a strict anaerobic, fermentative bacterium that can be used for the industrial production of the biofuel butanol from various sugars as substrates. We are interested in catabolite repression by glucose because hydrolysates of hemicelluloses contain large amounts of glucose and xylose beside other sugars which could be converted into solvents by *C. acetobutylicum*. We studied cells grown on a defined minimal medium containing glucose and xylose as carbon source. The culture exhibited a classical diauxic growth in which glucose was utilized before xylose. DNA-microarray and real-time data pointed out, that a gene cluster including xylulose kinase (*xylB*, CAC1344), xylose-proton-symporter (*xylT*, CAC1345), L-arabinose isomerase (*araA*, CAC1346), transaldolase (CAC1347) and tranketolase (CAC1348), was strongly repressed in the presence of glucose and induced when glucose was depleted from the medium. In contrast to this, a paralog of xylulose kinase (*xylB*, CAC2612) was highly upregulated when glucose and xylose are present in the medium. In continuous culture with glucose but without xylose as carbon source, no transcription of both xylulose kinase genes (CAC1344 and CAC2612) was detected. In order to investigate possibilities for increasing solvent production, we studied the influence of the accumulation of reduction equivalents on solvent production by decreasing the activity of the major hydrogen forming hydrogenase. We constructed various antisense constructs with different length of the *hydA* gene. Northern blot experiments demonstrated the transcription of the antisense RNA and also a decrease of *hydA* mRNA. Measuring enzyme activities we quantified the hydrogenase activity from the wild type strain and all strains containing the antisense constructs of the *hydA* gene. To study the effect of reduced hydrogenase activity on solvent formation, we analyzed the fermentation products in batch culture and compared them to the wild type strain. In order to characterize differences in gene expression between the strain containing the most effective antisense construct and a the control, we perform transcriptional analysis using DNA-microarrays.

BTP43
Isolation and characterization of the unusual compatible solute NAGGN

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The compatible solute NAGGN (N-acetyl-glutaminyl-glutamine-1-amide), a dipeptide of two modified glutamine subunits, is very widespread among the pseudomonads [1]. It is commonly present in combination with other compatible solutes like trehalose and mannitol. *Pseudomonas putida* KT2440, a representative of the pseudomonads, is known for the synthesis of the solute NAGGN. The biosynthesis genes of the compatible solute NAGGN were first identified by Aspedon [2] via microarray analysis of *Pseudomonas aeruginosa* PAO1. Homologous biosynthesis genes found in the *Pseudomonas putida* strain KT2440 were integrated into the genome of *H. elongata* thereby replacing the ectoine biosynthesis genecluster. The generated strain *H. elongata* AKB enabled effective NAGGN production due to a high internal accumulation [3]. The major advantage of this approach lies with the ease of purification, because of the absence of uncharged co-solutes of similar molecular mass. The main by-products glutamate and salt as contaminating substances can be removed by simple chromatographic methods.
The application of heterologous production has, for the first time, enabled us to investigate the properties of NAGGN. It was demonstrated that this rare solute can be used by a *Halomonas elongata* mutant deficient in ectoine synthesis (strain WUB02) to promote salt tolerance. In addition, its potential application as a stress protectant against freeze-thaw denaturation of enzymes (lactate dehydrogenase as test system) has been established. These preliminary investigations provide fundamental insights into the characteristics and biotechnological potential of the unusual compatible solute NAGGN.

[1] Kets PW *et al.* (1996) Journal of Bacteriology 178 (23): 6665–6670
[2] Aspedon A *et al.* (2006) Journal of Bacteriology 188 (7): 2721-5
[3] Korsten A; Galinski EA (2009) VAAM Jahrestagung 2009; PX33

BTP44
Ectoine-Synthase: Substrate Spectrum and Reversibility

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The compatible solute ectoine is synthesized by a wide range of halotolerant and halophilic bacteria. Ectoine synthesis occurs in three enzymatic steps, concluded by the condensation of N-γ-acetyl diaminobutyric acid (ADABA) to ectoine by the condensing enzyme ectoine-synthase.

In *Halomonas elongata*, a moderate halophilic eubacterium of the family Halomonadaceae, the ectoine pathway and its three enzymes are well investigated [1; 2]. In this study we focussed on the heterologously expressed purified ectoine-synthase (EctC) of *H. elongata*. With the notable exception of a side reaction leading to 5-amino-3,4-dihydro-2H-pyrrol-2-carboxylate (ADPC) by cyclization of glutamine instead of ADABA [3], this enzyme has so far been considered to be very specific for ectoine synthesis and irreversible. A closer look into the substrate spectrum of EctC (including both options: condensation and hydrolytic reaction) has revealed that EctC is a reversibly acting enzyme which is able to catalyse both the intramolecular condensation reaction (via nucleophilic attack of the alpha amino nitrogen with a terminal carbonyl group) and the hydrolytic cleavage of circular amino acid derivatives. So far this reversibility has been demonstrated for ADPC, DHMICA (4,5-Dihydro-2-methylimidazole-4-carboxylate) and homoectoine. In the case of ectoine, however, the equilibrium appears to be largely on the side of the cyclic condensation product, which explains why irreversibility had erroneously been assumed.
One can conclude that further investigations into ectoine synthase actions and side reactions will provide interesting insights especially into the novel hydrolytic capabilities of this enzyme.

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BTP45
The role of DAHP-synthase and prephenate dehydrogenase (PDH) in the primary and secondary metabolism of the Balhimycin producer *Amycolatopsis balhimycina*

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Balhimycin is a glycopeptides antibiotic of vancomycin-type. Such antibiotics are used for the treatment of serious infections caused by multi-resistant gram-positive bacteria. To antagonize the consistently increasing number of the antibiotic resistance, it is important to understand the biosynthetic pathway of antibiotic production in details to optimize its production and advance its impact.
As glycopeptide balhimycin consists of a glycosylated heptapeptide backbone. Five of these seven amino acids derive from the shikimate pathway. The analysis of the gene cluster showed that in addition to the genes encoding the biosynthetic enzymes, the balhimycin gene cluster includes two genes (*dahp, pdh*) which encode the homologous key enzymes of the shikimate pathway. The previous research showed that the deletion and over expression of these additional genes in *A. balhimycina* affects the antibiotic production. The over expression of *dahp* from the antibiotic gene cluster causes increased production of balhimycin. The deletion of the same gene causes the decreased antibiotic production. In contrast the over expression of *pdh* from the balhimycin biosynthetic gene cluster leads to the lower antibiotic production and its deletion does not show any remarkable effects considering the antibiotic production.
From the actual state of knowledge the causal processes for such affects cannot be explained and need detailed research that in the future will help to optimize the balhimycin production.

BTP46
Strain development for the production of L-aspartate by *Corynebacterium glutamicum*

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Extensive studies on the central carbon metabolism of *C. glutamicum* revealed novel targets for improved synthesis of amino acids, like L-glutamate and L-lysine. Based on this knowledge, we are aiming at engineering a *C. glutamicum* strain for the industrial production of L-aspartate.
In strain development, improvement of precursor supply is a major goal. For aspartate synthesis supply of oxaloacetate may be achieved by the overexpression of the genes for the anaplerotic enzymes phosphoenolpyruvate carboxylase (*ppc*) and pyruvate carboxylase (*pyc*) [1]. Deletion of the gene for the gluconeogenetic phosphoenolpyruvate carboxykinase (*pckA*) [2] is circumventing a futile cycle between oxaloacetate and phosphoenolpyruvate.
The first step in L-lysine biosynthesis is catalyzed by aspartokinase (*lysC*) [3]. As deletion of *lysC* results in the requirement for amino acids belonging to the aspartate family, a *lysC* down-regulation via promoter exchange appears to be more promising.
In bacteria, aspartate is synthesized either by transamination e.g. by glutamate-aminotransferase (*aspB*) or by reductive amination e.g. by the aspartate ammonia-lyase (*aspA*) [4]. In first experiments recombinant aspartase genes from *E. coli* and *B. subtilis* were cloned and high specific activities were obtained. Supernatants of batch cultures, however, did not contain significant amounts of aspartate. In light of these results strategies for strain development will be discussed.

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BTP47
High-level expression of a recombinant fungal endo-β-1,4-xylanase in the methylotrophic yeast *Pichia pastoris* and characterization of the enzyme

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In recent years xylanases derived from fungi have attracted the attention of academic and industry due to their biotechnological potential in various industrial processes. Using PCR techniques a gene encoding an endo-β-1,4-xylanase was identified in the thermophilic fungus *Corynascus heterothallicus*. The deduced sequence of the 687 bp *xyn11a* cDNA encodes a 228-aa protein with an estimated molecular weight of 25 kDa, including a putative 19-aa residue signal peptide. Belonging to the glycoside hydrolase family 11, Xyn11a exhibits 81% identity to an endo-β-1,4-xylanase from *Humicola grisea* var. *thermoidea*.
In order to produce high levels of endo-β-1,4-xylanase *xyn11a* was integrated into the *Pichia* genome by homologous recombination using the pPIC9 vector. Fusion of the N-terminal α-factor secretion signal and a C-terminal HIS tag enabled efficient secretion and purification of the recombinant enzyme. Furthermore high level expression of recombinant Xyn11a was achieved by high-cell-density cultivation in a 2-L fermentor. Under optimal growth conditions 350 g/L wet cells were obtained with a protein yield up to 400 mg/L and xylanase activity of 165 U/mL. The optimum temperature and pH for Xyn11a activity was 60°C and pH 5.0, respectively.

BTP48
Improving Thermal Stability of a Cold-active and Metagenome-derived Lipase by Directed Evolution

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Lipases [EC 3.1.1.3] are one of the most prominent enzymes used for industrial applications such as food technology, chemical and pharmaceutical industries or as detergent additives. They often possess a wide substrate range, although they catalyse highly specific chemo-, regio- and enantioselective reactions without need of co-factors.
A metagenome derived cold-active lipase [1] was subjected to directed evolution for improving thermostability at elevated temperatures. The enzyme was introduced to two rounds of random mutagenesis using error-prone PCR with an average mutation rate of 3 bp per 1000. A diverse mutant library with 4230 active clones in *E. coli* was constructed in 96 well plates. The plates were heat-treated for 45 min at 50°C and residual lipolytic activity was assayed with *p*NP-laurate at room temperature. Enhanced thermostability was verified by measuring the residual activity by the formation of *para*-nitrophenole at 410 nm.

In the second round of mutagenic PCR of the fittest clone derived from the first generation, the mutant III B2/VII F6 was identified. The sequence analysis of the coding gene reveals 6 random point mutations leading to 4 amino acid substitutions. Two of the four amino acid substitutions have taken place in the proximity of two amino acids from the catalytic triade. A significant increase in thermal stability at 50°C was measured without loss in enzyme activity. The half life was raised from 3 min up to 20 min at 50°C. Furthermore, an elevated conversion of smaller *p*NP-acylesters (number of C-Atoms: C4, C5) as well as an improved conversion of *p*NP-myristate (C14), was observed.

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BTP49
Thermophilic bacterial cultures for biomass hydrolysis in biogas plants

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A rate limiting step in the chain of bacterial activities leading to biogas production from plant biomass is the hydrolysis of the polymers. Incomplete hydrolysis results in insufficient, suboptimal yield. Hydrolysis is therefore of great importance for the economy of the overall process. The project investigated the composition of hydrolytic bacterial cultures in biogas plants run with pure plant material. Optimally hydrolyzing bacterial cultures were selected under laboratory conditions at thermophilic temperatures (65°C). Their phylogenetic diversity was determined with molecular microbiological methods. Quantitative and semi-quantitative screening methods for the major hydrolytic bacteria were developed. One was an RFLP-based method to sort out groups of 16S rDNA clones from metagenomic libraries. In addition oligonucleotide primers were designed for a quantitative PCR method to determine selectively the major hydrolytic members in the bacterial community. With these methods, the bacterial community in hydrolytic enrichment cultures and of several biogas plants in Bavaria are analyzed. In another approach, the transcriptional level of cellulase genes will be investigated.

BTP50
Enantioselective production of α-hydroxy carbonyls by *Gluconobacter oxydans*

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α-hydroxy carbonyls are valuable intermediates and synthons for the production of pharmaceuticals, pheromones and foodstuff. Optically active α-hydroxy carbonyls have been successfully used as chiral auxiliaries and synthons for the asymmetric synthesis of natural products including antitumor agents, antibiotics, pheromones and sugars [1,2]. In classic organic chemistry the regiospecific reduction of a single keto group of an α-diketone is almost impossible. Even more problematic is the stereospecific reduction of one keto group. However, enzymes often catalyze regio- and stereospecific chemistries. The acetic acid bacterium *Gluconobacter oxydans* is used in large-scale industrial processes for the stereoselective conversion of organic molecules (e.g. vitamin C production). Two *G. oxydans* enzymes that catalyze the regio- and stereoselective reduction of α-keto carbonyls (e.g. α-diketones, α-ketoaldehydes and α-keto esters) to form their respective α-hydroxy carbonyl were overproduced and purified from *E. coli*. The reduction of 2,3-pentanedione to 2*R*-hydroxy-3-pentanone and 2*S*-hydroxy-3-pentanone by Gox0644 and Gox1615, respectively, was shown by NMR using the chiral shift reagent (*R,R*)- and (*S,S*)-bis-α-methylbenzylamine-*p*-methyl [3]. The enzymes are NADPH-dependent and a process for regenerating the cofactor was developed using a NADP-dependent aldehyde dehydrogenase to convert NADP to its reduced form, so cofactor is required only in catalytic amounts. These new biocatalysts are innovative tools in the preparation of optically pure key intermediates for pharmaceutical products in current and future industrial processes.

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BTP51
Characterisation of the NAD(H)-dependent D-arabitol dehydrogenase of the hyperthermophilic bacterium *Thermotoga maritima*

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Enzymatic bioconversions are increasingly used to produce industrially important products. But not all enzymes are suitable for industrial applications because of their poor stability and turnover under industrial conditions. One answer to this problem is the use of thermophilic enzymes to increase the stability.
Thermotoga maritima is a hyperthermophilic bacterium that contains a large proportion of genes dedicated to surgar/polyol metabolism. The production of expensive (rare) sugars and intermediates for food and pharmaceutical industries as enantiomerically pure intermediates are often necessary. Accordingly, the demand for environmentally friendly organic synthesis of optically pure compounds is of high interest[1]. So the thermostable and thermoactive enzymes produced by *T. maritima* have generated great interest from the biotechnology sector.
The thermophilic NAD-dependent D-arabitol dehydrogenase (Tm0297) from *T. maritima* was heterologously overproduced in *E. coli* and the recombinant protein was purified and characterised. The enzyme exhibited a single band on SDS-PAGE with a molecular mass of 27 kDa, and was active as a homohexamer. The enzyme oxidizes D-arabitol and xylitol and reduces D-ribulose and D-xylulose, and was specific for NAD(H). The temperature optimum of polyol oxidation and corresponding keto-sugar reduction was 80 °C, which corresponds to the optimal growth temperature. The kinetic constants of the enzyme were identified for all substrates. The maximum velocity for the reduction of D-ribulose was nearly 5-fold greater compared to D-arabitol oxidation, whereas the Km for D-ribulose was 2-fold greater than for D-arabitol. This enzyme has the potential to produce D-ribulose and other expensive rare sugar that are useful as industrial synthons from inexpensive starting material.

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BTP52
The Role of autophagy and its influence on the sexual development of *Sordaria macrospora*

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In filamentous ascomycetes, autophagy is involved in several developmental processes. Nevertheless, until now little is known about its role in fruiting-body development. We therefore isolated a gene of the homothallic ascomycete *Sordaria macrospora* with high sequence similarity to the *Saccharomyces cerevisiae* autophagy-related gene *ATG7*, encoding a core autophagy regulator. This is the first characterization of an *ATG7* homologue in filamentous ascomycetes. A *S. cerevisiae* complementation assay demonstrated that the *S. macrospora Smatg7* gene functionally replaces the yeast homologue. We were not able to generate a homokaryotic knockout mutant in *S. macrospora*, suggesting that *Smatg7* is required for viability. However, a heterokaryotic *SmΔatg7/Smatg7* strain and transformants by RNAi showed considerable morphological phenotypes during fruiting-body development. In a Y2H screen ATG8 and ATG12 were found as interaction partners of ATG7. Referring on this data we focused on *Smatg8* and could show that SmATG8, an autophagy-related ubiquitin-like modifier and structural component of the autophagosome, shares a high homology with ATG8 homologs of other ascomycetes. We were able to localize SmATG8 via N-terminal GFP fusion indicating that SmATG8-GFP is localized to the autophagosome and is degraded in the vacuole along with the autophagosome and its cargo. Construction of a homothallic *S. macrospora Δatg8* strain showed abolition of fruiting-body development.

BTP53

Screening of filamentous fungi for secretion of peptidolytic enzymes and partial purification and characterization of a Subtilisin-like peptidase from *Paecilomyces farinosus*

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Ten fungal strains comprising basidiomycetes and ascomycetes (*Agaricus bitorquis*, *Agrocybe aegerita, Hericium erinaceus, Heterobasidion annosum, Auricularia auricula-judae, Collybia tuberosa, Xylaria polymorpha, Cordyceps militaris, Paecilomyces farinosus, Tremella foliacea*) were screened for secreted peptidolytic activities in different complex media in agitated liquid cultures. *A. aegerita* and *P. farinosus* were found to be the most active producers of extracellular peptidases in the groups of basidiomycetes and ascomycetes, respectively, and a medium consisting of caseine and soy bean peptone turned out to be the most suitable inducer of peptidases. One enzyme from *P. farinosus* was purified by ion exchange chromatography using an ÄKTA FPLC system equipped with SP-Sepharose (1st step) and Q-Sepharose (2nd step) columns. Physical and biochemical characterization of this enzyme revealed following characteristics: a molecular mass of 55.4 kDa, temperature optimum at 45°C and a broad activity range concerning pH (5-11.5) with an optimum around pH 9. Inhibitor studies have indicated an affiliation of *P. farinosus* peptidase to the serine peptidases, most probably in family S1 or S8. Based on these findings, we propose a relationship of this new peptidase to a hypothetic protein of *P. farinosus* with a cDNA coding for a 56.9 kDa Subtilisin-like peptidase.

BTP54

An extracellular feruloyl esterase of the wood-rot ascomycete *Xylaria polymorpha*

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Molecular linkeages between plant cell-wall polysaccharides and lignin usually consist of hydroxycinnamic acid units (e.g ferulic or *p*-coumaric acid) forming ester bonds with hemicellulose sugars. This leads to a certain structural integrity of the lignin-carbohydrate complex and stabilizes the cell wall as a whole. Enzymes involved in the hydrolysis of these bonds are feruloyl esterases (FAE, EC 3.1.1.73), a specific group of carboxylic ester hydrolases (EC 3.1.1.x), which release phenolic acids and their dimers from lignocellulosic materials. Here we describe, for the first time, the production of an FAE by the soft-rot (type II) fungus *Xylaria polymorpha*, which is known to degrade hardwood substantially (though its lignocellulytic system is just poorly understood). FAE is produced during solid-state cultivation and in liquid culture in the presence of different lignocellulosic materials (sole carbon source) at levels up to 120 U L^{-1}. Purified FAE is a monomeric protein with a molecular mass of 81 kDa and a pI of 3.7. It converts both synthetic alkyl aryl esters and natural lignocelluloses (e.g. milled wheat and rape straw). Currently, peptide mapping of this FAE is under investigation, which will enable us to classify this new esterase within in the sequence-diverse family of carbohydrate esterases.

BTP55

Target specificity of the Efg1 regulator in *Candida albicans*

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The human fungal pathogen *Candida albicans* is responsible for a wide variety of mucosal and systemic infections. The transcription factor Efg1 is a bHLH-type transcriptional regulator of this pathogen known to regulate its cellular morphologies and carbon metabolism. To clarify the function of Efg1 recombinant Efg1 protein fused to a decahistidine tag (His-tag) was expressed and purified from both *E. coli* and *C. albicans* by metal affinity chromatography. Efg1 protein stability was enhanced by buffer optimization with a strategy based on gel filtration chromatography and the protein yield by the use of *E. coli* host strain Rosetta 2(DE3) pLysS. The purified protein was identified by peptide mass fingerprinting and used successfully to immunize rabbits to generate a polyclonal anti-Efg1 antibody. The native conformation of Efg1 was determined by gel filtration chromatography, in which mainly a signal equivalent to the dimeric form of the protein could be observed. In an electrophoretic mobility shift assay (EMSA) and also by one-hybrid analysis in *Saccharomyces cerevisiae* the binding of purified Efg1 protein to a 408-bp fragment of the *EFG1* promoter could be observed in agreement with negative autoregulation of *EFG1*. To define the DNA binding–motif for Efg1 a systematic deletion analysis of the *EFG1* promoter is in progress.

BTP56

Structure and function of the signaling mucin Msb2 in *Candida albicans*

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The Msb2 protein of *Candida albican*s functions as an environmental sensor regulating morphogenesis, cell wall biogenesis and stress resistance. Msb2 is inserted into the cytoplasmic membrane by a single transmembrane region that separates a large extracellular domain, which is highly glycosylated, and a short intracellular domain. We constructed a *C. albicans* strain synthesizing a variant Msb2 protein carrying a HA-tag in the extracellular domain and a V5-tag in its cytoplasmic portion. The functionality of the tagged Msb2 protein was verified by complementation of *msb2* mutant phenotypes. Results on deleted Msb2 variants demonstrated that the extracellular domain and the transmembrane region are minimally required for full functionality of Msb2. Immunblotting experiments revealed that *MSB2* is highly expressed from its native or the heterologous *ACT1* promoter and that large quantities of Msb2 are secreted into the growth medium, whereas secreted Msb2 only contains the HA- but not the V5-tag. Conversely, a V5-containing protein with a size consistent with the Msb2 cytoplasmic domain was exclusively detected in the cell extract. Therefore, Msb2 appears to represent a signaling mucin, which is cleaved proteolytically into extracellular and cytoplasmic portions. The secreted Msb2 portion showed a molecular mass of > 500 kDa suggesting that it is highly glycosylated. As shown by immunblotting experiments the protein *O*-mannosyl transferases Pmt1 and possibly Pmt2 appear mostly responsible for the glycosylation status of Msb2.

BTP57

Negative autoregulation of the *EFG1* gene in *Candida albicans*

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The human fungal pathogen *Candida albicans* is responsible for a wide variety of mucosal and systemic infections. Its ability to switch between different growth forms and to adjust its metabolism are important virulence traits. The transcription factor Efg1 is required for the yeast-hypha morphological transition but surprisingly, the encoding *EFG1* gene is strongly negatively autoregulated during hyphal differentiation. In the yeast form *EFG1* transcript levels are high, while rapid downregulation is observed even before maximal levels of hyphal transition have occurred. Deletion analyses revealed that extensive portions of the *EFG1* promoter and the 5′ UTR are not required for the negative autoregulation of *EFG1*. Furthermore, we analysed several morphogenesis-defective mutants to investigate their influence on the *EFG1* negative autoregulation. We could show that the regulation is largely lost in an *efg1* mutant as expected but also partially lost in strains lacking the Flo8 transcription factor and the Tpk2 isoform of protein kinase A. The results are consistent with a model, in which the signalling pathway leading to Efg1 activation is needed for hyphal gene expression but at the same time also for shutting down *EFG1* transcription as a prerequisite for morphogenesis.

BTV01
Oxidative ether scission by an extracellular fungal peroxygenase

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Several litter- and wood-decomposing fungi have recently been shown to secrete heme-thiolate peroxygenases that oxidize various organic chemicals, but little is known about the role or mechanism of these enzymes. We found that the extracellular peroxygenase of *Agrocybe aegerita* catalyzed the H_2O_2-dependent cleavage of environmentally significant ethers, including methyl *t*-butyl ether, tetrahydrofuran, and 1,4-dioxane. Experiments with tetrahydrofuran showed that the reaction was a two-electron oxidation that generated one aldehyde group and one alcohol group, yielding the ring-opened product 4-hydroxybutanal. Investigations with several model substrates provided information about the route for ether cleavage: *(i)* steady-state kinetics results with methyl 3,4-dimethoxybenzyl ether, which was oxidized to 3,4-dimethoxybenzaldehyde, gave parallel double reciprocal plots suggestive of a ping-pong mechanism ($K_{m(peroxide)}$, 1.99 ± 0.25 mM; $K_{m(ether)}$, 1.43 ± 0.23 mM; k_{cat}, 720 ± 87 sec^{-1}), *(ii)* the fission of methyl 4-nitrobenzyl ether in the presence of $H_2{}^{18}O_2$ resulted in incorporation of ^{18}O into the carbonyl group (-$CH^{18}O$) of the resulting 4-nitrobenzaldehyde, and *(iii)* the cleavage of 1-methoxy-4-trideuteromethoxybenzene gave an observed intramolecular deuterium isotope effect [(k_H/k_D)obs] of 11.9 ± 0.4. These results clearly point to a hydrogen abstraction and oxygen rebound mechanism (rather than the direct insertion of oxygen into a C-H bond), which oxidizes ethers to unstable hemiacetals that spontaneously hydrolyze. The peroxygenase appeared to lack activity on macromolecular ethers or on ethers that have no abstractable hydrogen, but otherwise exhibited a broad substrate range. It may accordingly have a role in the bioconversion of low molecular mass ethers (e.g. of phytoalexins or lignin-derived aromatic fragments) in wood, soils and plant litter.

BTV02
Characterization of the fungal population in biofilms – more than 80 new species identified

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It has been recently shown that the majority of microorganisms live in biofilms as an extremely successful way of life and only a few microorganims have a planktonic lifestyle. Biofilms are made of a matrix of organic molecules (extracellular polymeric substrates - EPS) in which microorganisms are embedded and which offers new habitats to other organisms, such as other bacteria or fungi. By taking samples of biofilms from a municipal sewage plant, we isolated several known species from the genus *Candida* and *Trichosporon*. The most common species was *Galactomyces geotrichum* but also *Saccharomyces cerevisiae* and *Candida tropicalis*. However, sequence analysis of the ITS-regions amplified directly from biofilms revealed the presence of more than 80 so far unknown fungi. Phylogenetic analyses revealed that most of them are closely related to other species from the genus *Candida* and *Trichoderma*. Investigations whether these fungi are biofilm-specific are under way.
A second aspect of the project is the prevention of biofilm formation on mineral surfaces. To this end, we are trying to use fungal hydrophobins, and derivatives there of, for coating surfaces. Hydrophobins are small proteins which self-assemble at any hydrophilic-hydrophobic interface into extremely stable amphipathic monolayers. As a result of covering hydrophillic surfaces, such as sanitary devices, these surfaces turn into hydrophobic which is supposed to inhibit biofilm formation. However, so far we did not find an inhibitory effect of hydrophobin coating on biofilm formation. Therefore, currently we are fusing different antimicrobial peptides as well as protease inhibitors to the hydrophobins.

BTV03
Unrelated clinical isolates of *Aspergillus fumigatus* are able to mate

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Sexual reproduction of the human pathogen *Aspergillus fumigatus* (teleomorph: *Neosartorya fumigata*) was assumed to be absent or cryptic until recently, when fertile crosses among geographically restricted environmental isolates were described. Here, we provide evidence for mating, fruiting body development, and ascosporogenesis accompanied by genetic recombination between unrelated, clinical isolates of *A. fumigatus*, which demonstrates the generality and reproducibility of this long undisclosed phase in the lifecycle of this heterothallic fungus. Successful mating requires the presence of both mating type idiomorphs *MAT1-1* and *MAT1-2*. Moreover, a conserved transcriptional regulator from *A. fumigatus*, the *nsdD* gene product, was confirmed to act as a functional, positively-acting factor for *A. nidulans* cleistothecia development. When expressing NsdD at high levels in the endogenous host *A. fumigatus*, the formation of coiled hyphae was triggered. Analysis of an *A. fumigatus* mutant deleted for the *nsdD* gene suggests a role of this conserved regulator of cleistothecia development in hyphal fusion and hence heterokaryon formation.

BTV04
Sulfate-esters are main sulfur source of wood-degrading basidiomycetes as revealed by S K-edge X-ray Absorption Near Edge Spectroscopy (XANES)

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White- and brown-rot fungi are the main wood degraders in aerobic terrestrial ecosystems, and their biomass can be created on wood decay only. However, total sulfur (S) concentration in wood is very low and only little is known about the different S fractions in wood today. We have incubated S-starved brown-rot fungi *Gloeophyllum trabeum* and *Oligoporus placenta* on sterilized pine wood blocks as well as *Lentinus cyathiformis* and the white-rot fungi *Trametes versicolor* on sterilized beech wood blocks. After 11 weeks of incubation, we analyzed the different S functional groups in wood, degraded wood and in biomass of wood degrading fungi by synchrotron based S K-edge XANES spectroscopy. In addition, total S was quantified by stepwise combustion and subsequent detection with a UV-fluorescence detector, and sulfate by ion chromatography. Total S and sulfate content in pine wood blocks was approx. 50 and 3 mg/kg, while in beech wood approx. 100 and 60 mg/kg was found, respectively. After incubation, the total S amount did not change significantly indicating no S loss but concentrations of sulfate increased in the incubated pine blocks. While the S functional groups of pine were mainly characterized as sulfate-esters, beech wood contained also significant amounts of sulfate. All selected fungi shifted significantly the S functional groups in wood from sulfate-ester compounds (+6) to sulfate (+6), sulfides (-2), thiol, cysteine (-1 and 0) and sulfoxides (+2). Growth experiments of these fungi on selective media showed that in particular sulfate-ester compounds enhanced total biomass production of *T. versicolor* and *G. trabeum*. We conclude that wood-degrading fungi are able to retrieve sulfate-ester from wood and transform them into reduced essential biomass structures and sulfate.

BTV05
Detailed analysis of the interaction between OdhA and OdhI in *Corynebacterium glutamicum*

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The Gram-positive soil bacterium *Corynebacterium glutamicum* is used for the annual production of about 1.5 million tons per year of the flavour enhancer L-glutamate. In this process a reduced activity of the 2-oxoglutarate dehydrogenase complex (ODHc) as part of the TCA cycle plays a decisive role, because ODHc competes with glutamate dehydrogenase for the common substrate 2-oxoglutarate, the precursor for L-glutamate synthesis. We previously identified OdhI (ODHc inhibitor), a soluble 15 kDa protein that inhibits ODHc activity by binding in its unphosphorylated state to the ODHc subunit OdhA [1]. In the absence of OdhI, glutamate production is strongly inhibited [2]. Binding of OdhI to ODHc is abrogated by phosphorylation of OdhI by the serine/threonine protein kinase PknG [1]. Besides PknG, also PknA, PknB and PknL are able to phosphorylate OdhI [3]. In this work we analysed the interaction between OdhI and OdhA in more detail. Using *in vivo* copurification and *in vitro* surface plasmon resonance experiments the domains of OdhI and OdhA required for the interaction were identified. Moreover, the significance of the OdhI phosphorylation status concerning this posttranslational regulation was underlined by the result that fully phosphorylated OdhI purified from a phosphatase deletion strain showed no interaction with OdhA and did not inhibit ODHc activity.

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BTV06
Pseudomonas sp. strain VLB120: a versatile bacterium

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In industrial biotechnology applications, a number of different microbial species with a high number of different strains are used as recombinant hosts for whole-cell biocatalysis. This is due to historic implications of different researchers and research groups, but also reflects the particular strengths of species and/or strains. Ideally, beneficial traits are combined in one strain simplifying the implementation of specific applications significantly. We present *Pseudomonas sp.* strain VLB120 as a candidate for such a versatile host. This microbe can grow on minimal medium with a variety of carbon and nitrogen sources, does hardly produce any by-products during aerobic growth [1], is solvent tolerant [2], self-immobilizing [3,4], does not foam under standard conditions, and has a large natural repertoire of enzymatic activities that can be easily exploited for biocatalytic applications [2,3].
We will present these different traits of *Pseudomonas sp.* strain VLB120 and correlate these to the genome encoded information, which we analyze in collaboration with CeBiTec (University Bielefeld). The results are discussed in the context of the applicability of *Pseudomonas sp.* strain VLB120 as host for industrial biotechnology.

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BTV07
Construction of a high performance plasmid for intra- and extracellular protein production in *Bacillus megaterium*

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The Gram-positive soil bacterium *Bacillus megaterium* is known for its high protein secretion capability. The most commonly used heterologous gene expression system for *B. megaterium* is still based on the native, homologue, xylose-inducible promoter P_{xylA}. In this work both, the transcriptional as well as translational efficiencies of this expression system were optimized by adapting the responsible genetic elements to the host *B. megaterium*. For determination of changes in heterologous protein production, the gene encoding green fluorescent protein (Gfp) from jellyfish *Aequorea victoria* was cloned under control of the optimized, xylose-inducible promoter P_{xylA} of the novel plasmid constructs. Expression analyses were performed in *B. megaterium* to quantify the amount of recombinantly produced GFP. Shaking flask cultivations of cells carrying the optimized, high performance plasmids yielded up to 10 times more Gfp (> 110 mg/l) compared to productions using the unoptimized plasmid (10.6 mg/l). Fed batch cultivations of *B. megaterium* employing the high performance plasmids, produced more than 1.3 g/l Gfp.
For secretory production of heterologous proteins, several new *B. megaterium* signal peptides (SPs) were tested. Therefore, the coding sequences of the SPs were cloned into both, the native and the new, high performance expression plasmid. The *tfh* gene, encoding a hydrolase originated from *Thermobifida fusca* (Tfh) was used as model to evaluate production and secretion. The amount of Tfh in the cell-free culture supernatant was determined by the hydrolytic enzyme activity. Comparing both expression systems, more Tfh was produced and secreted by the high performance plasmids (7200 U/l).

BTV08
Red/ET recombination: genetic engineering of *E. coli* hosts and plasmids

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Recombineering with the Red/ET system, which uses 50-base-long tails on the donor DNA for homologous recombination into target DNA circumvents the need for conventional *in vitro* cloning techniques. Red/ET is not limited by the disposition of restriction endonuclease cleavage sites and enables extensive DNA modifications of *E. coli* replicons comprising plasmids, BACs, and the bacterial chromosome. Applications such as transcriptional fine tuning, subcloning, and site-directed mutagenesis of multi-copy number plasmids demonstrate the broad scope of this *in vivo* technology. The power of this approach extends beyond *E. coli*, because cloned genes or regulatory sequences may be modified readily in *E. coli* and used to replace alleles in other species.

BTV09
Fluorescence-based *in vivo* reporter proteins for *Clostridium acetobutylicum*

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Clostridia represent a diverse group of strictly anaerobic Gram-positive bacteria, comprising toxin-producing strains like *Clostridium botulinum* and *C. tetani*, as well as terrestrial non-pathogenic species of biotechnological impact due to their unique ability to produce acetone and butanol. These solventogenic clostridia, with *C. acetobutylicum* being the model organism, regained much interest recently for biobutanol production and are currently subjected to major research studies to understand the complex life cycle-accompanied physiology.

In addition to the limited number of general molecular tools available for clostridia, these anaerobes lack suitable reporter proteins for *in vivo* promotor studies. Only a few *ex vivo* reporter systems based on either enzyme assays in cell extracts or oxygen-dependent methods have been described for clostridia. Recently, novel flavin mononucleotide (FMN)-based fluorescent proteins, FbFPs, were developed as non-invasive reporter proteins applicable for both aerobic and anaerobic conditions in *Escherichia coli* and *Rhodobacter capsulatus* (Drepper et al., Nat. Biotechnol. 25: 443-445). In this study, these FbFPs were further engineered to establish *in vivo* flurescent proteins for *C. acetobutylicum*. Respective shuttle plasmids for *E. coli* and *C. acetobutylicum* were constructed containing the FbFP reporter genes fused to a native constitutive promotor and the recombinant *C. acetobutylicum* strains were characterized according to their fluorescence. Interestingly, the engineered FbFP which originated from *Pseudomonas putida* revealed a much higher fluorescence than the modified FbFP from *Bacillus subtilis*. Thus, a new fluorescence-based *in vivo* reporter system for *C. acetobutylicum* and related clostrida is now available for detailed real-time gene expression analyses.

BTV10
Biotechnological production of succinic acid

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A noticeable number of bulk chemicals used for the production of plastics such as 1,4-butandiol or adipic acid are synthesized from succinic acid. To present, the industrial demand of succinic acid is covered by the petrochemical production route. With regard to the prospective depletion of fossil resources, prices and production costs are likely increasing. Therefore, the biotechnological production of succinic acid becomes an attractive alternative to the traditional processes. Furthermore, the biobased production process incorporates positive aspects related to the environment such as the usage of renewable resources and the fixation of carbon dioxide. An important factor for a successful development and installation of an alternative, biobased process is the cost effectiveness compared to standard petrochemical production. Hence, economically competitive biobased production processes focus on the usage of overproducing microorganisms. Recently, a few promising strains have been isolated from natural habitats that possess a broad spectrum of metabolic reactions for the production of succinic acid. Taking this as a starting point, it is often necessary to introduce a number of modifications into the organism to obtain an improvement and maximum production efficiency. Concerning this aspect, actual results from a collaborative research project between the University of Braunschweig and BASF SE (Ludwigshafen, Germany) are presented. Subject of research is the newly isolated strain *Basfia succiniciproducens* (DD1) that belongs to the Pasteurellaceae family and has the ability to grow on a number of different carbon sources (e.g. glucose, glycerol). Volumetric productivities for succinic acid of up to 1.5 g L^{-1} h^{-1} and a yield of 0.6 $g_{succinic\ acid}$ g^{-1} were reached on glucose as sole carbon source. By using glycerol, remarkably increased succinic acid yields of up to 1.2 $g_{succinic\ acid}$ $g^{-1}{}_{glycerol}$ were obtained [1]. To further elevate the yield and efficiency, a defined minimal medium was developed. In combination with metabolic flux analysis using 13C-labelled substrates this systems biotechnological approach led to a detailed insight into the metabolic network of *B. succiniciproducens*. Based on these findings key targets for genetic modifications were determined and successfully applied for a rational strain improvement.

Acknowledgements
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BTV11
Heterologous expression of a surface layer-like protein in *E. coli* causes a drastic morphological change of the cell

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Bacterial envelope proteins, so called surface layers (S-layer) are widely spread pararcrystalline surface structures which coat the cells of lots of bacterial strains and all archaea. They are mostly composed of protein monomers which form via self-assembling high regular two dimensional arrays. The S-layer proteins we investigate are from bacterial strains recovered from uranium mining waste pile Haberland in Saxony, Germany.
Their S-layer proteins selectively bind uranium and protect the cells from its toxicity. These special S-layer characteristics make them interesting for many technological applications such as filter materials, biosensors, as functional surfaces, or for example as drug containers.
In order to produce S-layer proteins in a high efficient way a heterologous expression in *Escherichia coli* is essential. In our study, the S-layer-like protein SllB of *Lysinibacillus sphaericus* JG-A12 was expressed in *E. coli* Bl21. Noteworthy, recombinant protein production resulted in a high stability of the cells against mechanical and chemical treatment. These unusual cells were analyzed by light microscopy, AFM and TEM. All methods demonstrated a total changed cell morphology with long filaments in the beginning of the exponential growth stage and 5-200 µm long tube like transparent structures at the end of the exponential growth stage containing *E. coli* single cells. Analyses by SDS-PAGE, N-terminal sequencing and IR-spectroscopy showed that the tube-like structures consist of outer membrane associated with recombinant surface layer proteins. These findings point to a disordered cell division. However, the underlying mechanism of these morphological changes are not known and will be analyzed in future. The long filaments, in combination with high expression level, good growth and high stability make these unusual *E. coli* cells interesting for biotechnological applications. In addition, these results cast a new light on one of the best studied microorganisms.

BTV12
Fast, Quantitative and Molecular System Analysis of the Microbial Community in Biogas Plants

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Anaerobic digestion of renewable biomass for energy, heat and natural gas generation has been drawing great attention in Germany. The influence of the microbial community on the anaerobic digestion process with biomass is still a not completely understood. Heterogeneity of environmental samples as well as its chemical components often affects an optical analytical method. E. g., organic matter from biogas plants fed with renewable energy crops offers extreme conditions. At present no procedure is available to quantify bacteria by fluorescence *in situ* hybridization (FISH) in such samples. In this work a quick molecular, practically orientated, quantitative analytical FISH method was developed for samples with high plant fibre content. For the quantitative FISH-technique a probe set of the major key players in the methanogenic anaerobic digestion was used. It should contribute to unseal the microbial "black box" of the biogasification process. With a dispersing device it was possible to homogenize the anaerobic reactor material in a smooth way. Interfering fibers and humic matter could be washed out. In combination with digital image analysis by self programmed counting software, the hybridization quantity could be increased to nearly 80%, in comparison with a reference procedure. With the developed new protocol it is now possible to create a fast, semi-automatic and quantitative screening of microbial populations in large scale biogas plants processing renewable biomass. The developed quantitative FISH Method could point out remarkable difference in the microbial population of industrial biogas plants, depending on the process parameters, seed bacteria and substrate.

BTV13
Structural and functional studies of a thermoactive lipase from *Thermoanaerobacter thermohydrosulfuricus* SOL1

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Recent studies have shown that extreme thermophilic anaerobic bacteria are a good source for unique lipases and esterases. The recombinant lipase from *Thermoanaerobacter thermohydrosulfuricus* is active over a broad temperature range (40-90°C) and is resistant against a large number of organic solvents (up to 99% solvent concentration) and detergents, and shows activity towards a broad range of substrates including triacylglycerols, monoacylglycerols and *p*-nitrophenyl esters.

After modification of the amino groups by reductive methylation large reproducible crystals (1.9 Å resolution) were obtained and the structure was determined through the anomalous signal of a selenomethionine-substituted lipase. In all crystal packing forms this thermostable lipase preserves a basic dimeric conformation of about 75x60x50 Å, which is the active form of the enzyme. The two monomers are rotated by 90deg to each other forming that way a large hydrophobic channel directing to the two catalytic centers. The dimer including the two smaller domains from the substrate hydrophobic channel, holds a very compact conformation that explains partially the increased stability of the structure. The lid is quite compact and smaller compared to similar structures. Large spatial deviations occur at the residues 148-160 between the lid alpha-helices a1 and a2. To investigate structure-function relationship a series of single-point mutations were designed. All designed mutants are active and up to 2-fold increase in the activity was observed. Furthermore, the effect of reductive methylation and delipidation on enzyme activity and thermostability was investigated.

BTV14
TatB and TatC tightly cooperate during substrate recognition by the *Escherichia coli* twin-arginine translocation (Tat) translocase

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The twin-arginine translocation (Tat) pathway transports folded proteins across the cytoplasmic membrane of bacteria. In *E. coli*, the Tat-translocase consists of the integral membrane proteins TatA, TatB and TatC. TatB and TatC are closely associated and form the TatBC receptor complex which recognizes and binds Tat-precursor proteins. Tat-substrates carry a characteristic SRRxFLK consensus motif within their signal peptides which is crucial for the binding to TatBC. So far, only little is known about the regions within TatBC that are involved in signal peptide binding. Our previous genetic approach identified several mutant Tat-translocases, that restore export of an previous export defective TorA[RR→KQ]-MalE reporter. In most cases, the suppressing activity was caused by a single suppressor mutation in TatC. In extension to these studies, we now used a variant of the TorA-MalE reporter (TorA[F14D]-MalE), which is export defect due to a replacement of the consensus phenylalanine residue by aspartate, to select Tat-coupled suppressor mutations of this reporter. Besides known suppressor mutations in TatC, we found several new suppressor mutations in a distinct region in TatB. Additionally, we found that some mutations in TatB can act synergistically together with some mutations in TatC, indicating that TatB and TatC tightly cooperate in Tat signal peptide binding during early stages of Tat-dependent protein translocation.

CCP01
Quorum sensing and inhibition of photosynthetic membrane production in high cell density cultures of *Rhodospirillum rubrum*

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The production of photosynthetic membranes (PM) in bioreactors is a major challenge when thinking about the industrial application of anoxygenic photosynthetic bacteria. *Rhodospirillum rubrum* is so far the only organism capable of producing maximal levels of PM in common stirred tank reactors microaerobically in the dark. Recently, *R. rubrum* has taken the second hurdle to biotechnological application by being cultivated to high cell density levels up to 60 g/l. Yet it has been proven impossible to induce anaerobic membrane production under these high cell density conditions. The observed inhibition of PM expression at high cell densities could not be attributed to obvious factors such as a general loss of viability, mutational events, limitation of substrates, or exceedingly high osmolarity.
A closer examination showed that it is sufficient to supply culture supernatants of high cell density cultures to *R. rubrum* cells to inhibit PM formation, with the extent of inhibition being directly correlated to the cell density of the original culture. This finding indicates that soluble factors were present in the culture broth, probably being excreted at high cell densities which interfere with gene expression and/or assembly of PM.
In the present work we report the identification of a N-(3-OH-octanoyl)-homoserine lactone in culture supernatants of aerobic and microaerobic high cell density cultivations by LCMS analysis. The compound represents the first autoinducer of the acylhomoserine lactone (AHL) type identified in *R. rubrum*. Further analysis of quorum sensing effects were done by growth inhibition zone assays using synthetic AHLs, the expression level of the luxI homologue and biosensor assays using *Pseudomonas fluorescens* as indicator organism. It is known that the close relative *Rhodobacter* sphaeroides possesses a quorum sensing regulatory system, which might have an impact on the photosynthetic membrane production. Blast searches showed homologies to LuxI and LuxR proteins in *R. rubrum*, which supports the idea that *R. rubrum* is capable of AHL synthesis and detection.
In addition to the presentation of experimental data, the possible biological functions of quorum-dependent regulation of PM expression will be discussed in the context of known light- and redox-sensing regulatory systems of anoxygenic photosynthetic bacteria.

CCP02
Structural and functional analyses of DNA translocator complexes of *Thermus thermophilus* HB27

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Bacterial DNA transfer is a normal and powerful means for single-cell organisms to communicate information about new ways to compete and survive in their shared chemical environment.
To get insights into the structure and function of DNA translocators we chose the thermophile *T. thermophilus* HB27 which exhibits highest natural transformation frequencies known to date. A genome-wide genetic screen followed by mutant studies led to the identification of 16 distinct proteins (1). One of the competence proteins, the secretin-like protein PilQ, was found to be essential for DNA binding and uptake in HB27 (2). Purification and single particle analyses of PilQ led to the identification of macromolecular PilQ complexes comprising of 12 subunits forming ring-like structures with a diameter of ~15 nm, a pore size of ~6 - 7 nm and a length of ~20 nm. These structures could serve as channels guiding the DNA and the DNA translocator through the outer membrane. The function and the quaternary structure of the PilQ multimers will be discussed.
Another competence protein, PilF, a member of the AAA-ATPase superfamily known to be involved in assembly/disassembly of type IV pili and in macromolecular transport systems was also purified and subjected to structural and functional analyses. PilF was found to form homohexamers catalyzing ATP hydrolysis in dependence of temperature, divalent cations and different nucleotides. Taken together PilF was identified as the motor of the dynamic DNA translocator of *T. thermophilus* and is suggested to power energy-dependent DNA translocation in *T. thermophilus* HB27.

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CCP03
Acidovorax sp. N35, an AHL-producing rhizosphere bacterium able to perform phase variation

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Acidovorax sp. N35 is a β-proteobacterium, isolated from surface sterilized wheat roots. This bacterium undergoes phase variation, with one phase type showing characteristic rough colony shapes on agar plates and forming flocks in liquid medium (N35e), while the other type grows in smooth colonies and without flocculation in liquid medium (N35r). A plant growth promoting effect was identified using both phase types in green house experiments on barley. However, the phase N35e showed a dominant colonization behavior on roots, when coinoculated with N35r.

AHL quorum sensing molecules with the structure 3-hydroxy-decanoyl homoserine lactone were detected as the dominant signaling substance with a fourier transformation ion cyclotron resonance mass spectrometer (FTICR/MS). In order to identify AHL synthesis genes, the bacterial genomes of both phase types of Acidovorax sp. N35 were sequenced with a 454 pyrosequencer (Roche). The same sequence stretch of 555 nucleotides encoding an AHL synthase protein was determined in both phase variants, which can be subjected to knock-out mutagenesis for a functional analysis of AHL signaling in this species. Interestingly, a 16 nucleotides deletion region was identified in the N35r genome compared to N35e. This region shows high sequence similarity to the mismatch repair protein MutL. This protein is truncated in N35r, thus, MutL might be directly or indirectly responsible for the differences in gene expression between the two variants.

CCP04
3D structure of Ignicoccus and Nanoarchaeum, as determined by serial sectioning and by electron tomography

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The hyperthermophilic Archaeon *Ignicoccus hospitalis* has a unique ultrastructure, including a periplasmic space with vesicles, and an outer membrane. *I. hospitalis* cells are the only known host for Nanoarchaeum equitans. These two Archaea form an intimate association; it is not clear yet to which extent *N. equitans* cells have damaging or beneficial effects to their host cells. *N. equitans* is not able to synthesize lipids, amino acids, cofactors, and nucleotides. Therefore, the interaction of these cells with *I. hospitalis* is of high interest, in order to understand the exchange of metabolites. Labeling showed that archaeal lipids and amino acids are transported from *I. hospitalis* to *N. equitans*.
Cells were cultivated in capillary tubes, immobilized by high-pressure freezing, freeze-substituted and resin embedded; serial sections were imaged by transmission electron microscopy, and data visualized as 3D stacks. 200 nm sections were analyzed by tomography. In all 3D data sets analyzed so far, the interpretation of the structures is facilitated, compared to images of single thin sections only. The *I. hospitalis* cytoplasmic membrane shows a high tendency to form membrane invaginations and vesicles, which are often found in contact with the cytoplasmic membrane and with other vesicles. The physiological role of this membrane vesicle system is unknown, yet. Often, the cytoplasm of *I. hospitalis* cells is involved in the interaction with *N. equitans*; rarely, periplasmic vesicles appear to be unloaded at the contact site, resembling the release of cargo to *N. equitans*. Immuno-localisation showed that the Ihomp1 protein, the sulfur reductase and the ATP synthase are found in the outer membrane of *I. hospitalis*. In addition, the S-layer of *N. equitans* is present at and involved in the contact site. Biochemical studies helped to identify further proteins which might be relevant for cell-cell interaction and / or metabolite transport, like components of ABC transporters.

CCP05
Communication in biofilms between different species: *Candida albicans* and *Pseudomonas aeruginosa*

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Candida albicans, a polymorphic fungus, and *Pseudomonas aeruginosa*, a Gram-negative bacterium, are two opportunistic pathogens that can cause serious infections in different sites within the human host. Growing in a particular lifestyle called biofilm they are well protected against the human immune system compared to conspecific in planctonic growth. Therefore these organisms are used as model systems for fungal and bacterial biofilm research. Biofilms are structured communities of microorganisms enclosed in a self-produced polymeric matrix and adherent to an inert or living surface. One of the hallmarks required for the formation of biofilms is the so-called quorum sensing modulated by specific molecules which regulate this developmental process via defined signal cascades. Farnesol is a quorum sensing molecule used by *C. albicans* which inhibits fungal biofilm formation. It is mediated through a two-component signal transduction histidin kinase Chk1p. In *P. aeruginosa* *N*-3-oxo-dodecanoyl-L-homoserine lactone (3OC12HSL) represents a quorum sensing molecule. It acts as a positive regulator via LasR. Both molecules, which contain twelve-carbon backbones, repress *C. albicans* filamentation without altering its growth rate.
To see how the organisms communicate with each other in biofilms a method to quantify the influence of quorum sensing molecules on *C. albicans* and *P. aeruginosa* biofilms was used. To visualize the interaction between both organisms mixed biofilms have been studied. Reporter strains have been constructed to analyse the influence of quorum sensing molecules during biofilm formation.
Our results indicate that 3OC12HSL has impact on *C. albicans* biofilm formation and biofilms of *P. aeruginosa* are manipulated by farnesol.

CCP06
Rhomboid intramembrane proteases in *Corynebacterium glutamicum*

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Intercellular communication is an important process in cell populations. Thereby organisms are enabled to observe and react on population sizes, environmental settings and stress conditions. To permit a fast regulation to changing conditions the cell signaling needs to be precisely regulated. By the "Regulated Intramembrane Proteolysis" (RIP) proteins are selectively cleaved within their membrane spanning segments to become a new functional state. So far 4 classes of intramembrane proteases are known: presenilins, site-2 proteases, signal-peptide-peptidases and rhomboid proteases. Rhomboid proteases belong to the group of serine proteases. While the other intramembrane proteases release factors into the cytosol, rhomboids release cleavage products to the periplasm. Although in eukaryotes much is known about the function of these proteases, such as their role in EGFR signal transduction, their function in prokaryotes remains elusive. Our major aim is to shed light on the physiological role of rhomboid proteases and to identify their natural substrates in prokaryotes using the model organism *Corynebacterium glutamicum*.
For *C. glutamicum* two rhomboids named Cg0049 and Cg2767 are predicted. We suppose that these proteases are involved in cell wall biosynthesis, stress response and cell communication. To clarify the regulation we use molecular biological methods like northern and western blot analyses. Putative substrates should be found by a bioinformatic approach, a proteomic approach with LC-ESI-MS/ MS and a site-directed mutagenesis of Cg2767. So far it seems that the two rhomboid proteases are differently regulated. Heat stress leads to an obviously increase of Cg2767 in western blot analyses whereas Cg0049 could not be detected. Both rhomboids are not effected after osmotic stress. Furthermore a deletion of *cg2767* causes resistance against penicillin in plate diffusion assays.

CCP07
Cell division protein AmiC is necessary for filament formation, cell differentiation and intercellular communication in the multi cellular cyanobacterium *Nostoc punctiforme* PCC 73102

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Nostoc is a complex filamentous cyanobacterium, which is able to form differentiated cells: heterocysts for N_2 fixation, stress resistant akinetes and small motile filaments, called hormogonia. It can be assumed that during cell differentiation massive changes in the cell wall structure take place. AmiC, encoding N-acetylmuramoyl-L-alanine amidase, which functions in murein remodelling, is part of the cell division machinery in bacteria. *E. coli* mutants, lacking the AmiAC amidases grow in chains of connected cells. To reveal the role of cell wall amidases in filament formation in *Nostoc*, amidase knockouts were created. Knock out of NpF1846, an *amiC* ortholog of *Nostoc*, results in a massive "filament dystrophy". In cells of the AmiC mutant, the division planes are irregularly arranged. As a result, the mutant does not form regular filaments, but clumps of irregular aggregates. Single cells of the mutant are bigger than single cells of the wild type and are surrounded by a thick mucosa. Furthermore the mutant is unable to perform any differentiation processes.

To find out, why the mutant lacks cell differentiation, the exchange of molecules between the cells of the filament was directly visualized, using the small hydrophobic molecule calcein (623-Da). By fluorescence recovery after photo-bleaching (FRAP), we observed that the diffusion between single cells is completely impaired in the mutant. How mutation of this murein amidase results in such a severe phenotype is currently under investigation.

CCP08
Biochemical characterisation of two novel N-acyl homoserine lactone hydrolases DlhR and QsdR from *Rhizobium* sp. NGR234

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The complete genome analysis of *Rhizobium* sp. NGR234 [1] revealed the presence of eight potential lactone hydrolases involved in the quenching of the autoinducer I signalling molecule. To verify these findings, a cosmid library of NGR234 was constructed and screened for the presence of clones conferring N-AHL degradation ability. Using the biosensor strain *Agrobacterium tumefaciens* NTL4 at least six loci that interfere with bacterial quorum sensing (QS) were confirmed. Here we report on isolation and biochemical characterisation of two novel hydrolases, designated as DlhR and QsdR, involved in the degradation of N-AHLs. The deduced AA sequence of DlhR is similar to the esterase-lipase superfamily of proteins with weak similarities to a dienelactone hydrolase. Sequence alignment of QsdR indicated that it contains a conserved domain, a HxHxDH motif, which is known to be involved in Zn^{2+} binding in other known lactonases. In different bioassays the exogenous addition of purified DlhR and QsdR extracts reproducibly inhibited QS-regulated motility, biofilm formation, reduced pyocyanine production in *Pseudomonas aeruginosa* PAO1 and violaceine production in the reporter strain *Chromobacterium violaceum* CV026. In complementation tests the altered swarming phenotype of PAO1 caused by DlhR and QsdR could be restored by excessive addition of AHL. The AHL degradation and the cleaving mechanism of both proteins could be verified by HPLC-MS analysis. Construction of Δ*dlhR* and Δ*qsdR* mutants of NGR234 as well as determination of gene expression profiles are in progress.

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CCP09
Metagenome-derived quorum-quenching clones interfering with *P. aeruginosa* biofilm formation

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Many opportunistic pathogenic bacteria regulate their virulence expression by quorum sensing. In *Pseudomonas aeruginosa*, quorum sensing-regulated gene expression contributes to the formation and maintenance of biofilms and their tolerance to conventional antimicrobials. Thus quorum sensing is a consequential target for new antimicrobial drugs which could block quorum sensing signal reception. In this context we intended to find new autoinducer-degrading ORFs. Metagenomics offers the possibility to scan the uncultivated bacteria for new biocatalysts, among those also quorum sensing inhibiting biomolecules. We found two novel quorum-quenching ORFs, designated *bpiB05* and *bpiB09*, by screening a genome bank with an *Agrobacterium tumefaciens* reporter strain. The ORFs were cloned into the broad-host-range vector pBBR1MCS-5 and transferred to *Pseudomonas aeruginosa* PAO1, where they caused a decreased motility and biofilm formation. A homoserine-lactone (HSL) degradation assay together with HPLC-MS analyses proofs decomposition of these autoinducers. The Bpi amino acid sequences are different from already known HSL-degrading enzymes. Current work focuses on a detailed biochemical characterisation of the Bpi proteins and their impact on *P. aeruginosa* biofilm formation.

CCV01
Signal transduction of c-di-GMP in *Pseudomonas aeruginosa*

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Living in matrix enclosed biofilms is a common lifestyle of many bacteria. One key component controlling the transition between a motile, single-cell way of life and community based biofilm formation is the second messenger bis-(3'-5') cyclic di-GMP (c-di-GMP). Low intracellular levels of c-di-GMP promote a planktonic lifestyle, where bacteria are motile and virulence factors are expressed, and high c-di-GMP levels stimulate multicellular behavior, adherence and biofilm development. The synthesis of c-di-GMP is mediated by diguanylate cyclases containing a conserved GGDEF domain whereas the degradation is controlled by phosphodiesterases containing a conserved EAL domain. GGDEF and EAL domains are often fused to transmembrane and/or signal input domains. Thereby, c-di-GMP links environmental stimuli to an adaptive answer of the bacterial cell. However, little is known about the molecular details of the c-di-GMP signaling network. Up to date, only few types of c-di-GMP effector molecules are known. Among them are PilZ domain proteins and proteins containing a RXXD motif, which was first identified as an allosteric site of product feedback inhibition (I-site) in many diguanylate cyclases.
In this work we used a c-di-GMP coupled sepharose for the isolation of c-di-GMP binding proteins of Pseudomonas aeruginosa PA01 which were subsequently identified by mass spectrometry. This approach yielded in seven putative c-di-GMP binding candidates, among others one protein with a PilZ domain and one containing a degenerated GGDEF-motif and the I-site motif RXXD. We report here on one further identified c-di-GMP binding protein that interferes with flagella driven motility in a c-di-GMP depending manner.

CCV02
Opportunistic growth of *Pseudomonas aeruginosa* in co-culture with the chitinolytic bacterium *Aeromonas hydrophila* is dependent on quorum sensing

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Polymer degradation in the environment evokes multiple interspecies interactions between bacteria. Bacteria producing extracellular enzymes for polymer hydrolysis can be exploited by opportunistic bacteria that utilize the resulting degradation products without the energy expenditure of enzyme production. This scenario was investigated with a co-culture of the chitinolytic bacterium *Aeromonas hydrophila* and *Pseudomonas aeruginosa* during growth with chitin. Chitin is an abundant polymer of N-acetyl glucosamine (GlcNAc). In single cultures, *A. hydrophila* could grow with colloidal chitin as a source of carbon, nitrogen and energy, while *P. aeruginosa* could not. In co-culture, *P. aeruginosa* could grow along with *A. hydrophila*. As *P. aeruginosa* could not utilize dimers and trimers of GlcNAc, this growth could not be based on the consumption of the primary chitin degradation products generated by chitinolytic enzymes of *A. hydrophila*. HPLC-analysis of supernatants from co-cultures revealed that high concentrations of acetate were released, which could serve as a growth substrate for *P. aeruginosa*. After chitin depletion, cells of *A. hydrophila* were inactivated as detected by viable counts. This inactivation coincided with the production of pyocyanin and other secondary metabolites by *P. aeruginosa*. After inactivating *A. hydrophila*, *P. aeruginosa* continued growth. Mutants of *P. aeruginosa* with defects in quorum sensing (*lasIrhlI*, *pqsA*) did not cause this inactivation and reached lower cell numbers in co-cultures. These mutants could be complemented by addition of the respective N-acylhomoserine lactones and the Pseudomonas quinolone signal (PQS). These results show that *P. aeruginosa* uses quorum sensing-regulated properties to exploit chitinolytic activities of *A. hydrophila*, thereby profiting from a nutrient source that otherwise would not be bioavailable. The molecular and biochemical details of this opportunistic growth strategy are currently under investigation.

DVP01
Novel probe-based real-time PCR for rapid diagnosis of bovine tuberculosis in tissue samples with suspect lesions

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Bovine tuberculosis (bTB), caused by *Mycobacterium (M.) bovis* or *M. caprae*, is considered one of the most important neglected zoonoses worldwide. After successful eradication of the agent from > 99.9 % of the cattle herds, Germany was declared free of bTB in 1997. Ongoing bTB surveillance is principally based on meat inspection at abattoirs. Suspicious organ lesions shall be confirmed by bacteriological culture. This results in a substantial delay between sampling and confirmation and impairs monitoring of this zoonotic disease in livestock. Hence, a novel probe based two-target real-time PCR system was developed for the detection of DNA of members of the *M. tuberculosis* complex (MTC), including *M. bovis* and *M. caprae*, in tissue samples from cattle.
Seven primer/probe combinations (PPC) for three different target regions (IS*6110*, IS*1081*, putative helicase [HELI]) were tested. Analytical sensitivities were determined using DNA from different MTC strains diluted in buffer and diluted in DNA extracted from bovine lymph node tissue. DNA from 35 mycobacterial species and from 9 other bovine bacterial pathogens was tested for nonspecific amplification.
Detection of IS*6110*, the most widely used PCR target in human TB samples, was not suitable for bovine tissue samples because it inhered a low analytic sensitivity. Usage of PPC for IS*1081* and HELI, respectively, resulted in high analytical sensitivities and specificities. Based on these two PPC and β-actin as internal control, two duplex real-time PCR assays were established and combined with a modified commercial tissue DNA extraction method. The diagnostic specificity and sensitivity of the final system was 100% and 73%, respectively. Performance of this novel assay was validated in cooperation with federal state veterinary diagnostic laboratories.
By shortening the time for confirmation of bTB in organ samples with suspect lesions to a few days, the method is expected to improve abattoir-based bTB surveillance in Germany.

DVP02
MALDI-TOF mass spectrometry in a routine microbiology department of a laboratory in Germany: two years of experience

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Introduction of MALDI –TOF mass spectrometry is changing the general workflow in a clinical microbiology laboratory, with the expectation of increased speed and accuracy of microbiological results. We report about our experiences during two years after introduction of the MALDI Biotyper (MBT) system in our routine laboratory.
As a fundamental change, after validation of the MBT system, we cancelled all biochemical identifications except for mycoplasma. Basic tests such, as oxidase, catalase and *Staphyloccous* coagulase were disposed as not needed prior to mass spectrometry. Coagulation tests for subtyping of *Shigella*, *Salmonella*, *Yersinia*, *Haemophilus influenzae*, pneumococci and meningococci were continued. First step to identification always is MALDI-TOF profiling. We also stopped to rely on chromogenic media, as are considered not very reliable.
Introduction of MALDI-TOF MS significantly reduced the usage of consumables. It led to far better results especially for rare microbes, where the biochemical approaches are limited. For instance, Helicobacter pullorum now readily can be distinguished from *Campylobacter sp.*. Workload has been reduced considerably for *Salmonella*. The Kligler test prior to sero-subtyping is only needed after an isolate has been identified as *Salmonella sp.*. In addition, we save about 30 % of petri dishes as in most cases identification and AST testing may be done from a single colony eliminating the need for subculture. Identification of anaerobic bacteria is speeded up for several days since from appearance of colonies the bacterium now readily can be identified. Success rate here is at 95%, a 50% increase over biochemical methods. Yeasts are quickly and reliably identified after extracting the cells prior to mass spectrometry (success rate > 95%).
Introduction of MALDI-TOF MS considerably changed laboratory workflow, it shorted turn around time and improved quality of results. Workload and consumption of consumables have been reduced.

DVP03
Direct analysis of positive blood cultures using MALDI-TOF MS profiling and a fundamentally improved sample preparation technique

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Blood culture is an important routine clinical analysis. Fast and accurate identification of microorganisms after signalling of growth is of fundamental interest. Currently, identification takes up to two days because of sub-cultivation and biochemical tests. The presented facile protocol has the potential to shorten identification time to minutes.
Blood cultures spiked with bacteria or yeasts were used for method establishment, inoculated blood cultures and routine samples to optimize and validate the protocol. 1ml of a blood culture was mixed with 200µl of lysis solution, followed by vortexing and centrifugation. Supernatant was removed and the pellet carefully suspended in wash solution. After centrifugation, supernatant was removed and a part of the pellet were transferred to a MALDI target, directly or after a short extraction procedure. Species ID was achieved by MALDI-TOF profiling and analysis of spectra using the MALDI Biotyper 2.0 software. The method was optimized by variation of volumes and ratios of blood culture liquid and lysis solution.
Positive blood cultures in average showed cell density of about 10^7 cfu/ml and a high number of blood cells. Blood cells have been removed without significant loss of microorganisms resulting in mass spectra of high quality. Different bacterial species could be identified, reliably. Also yeasts which could not be detected with previously published protocols were identified. In some cases identification was possible even 1-2 h before blood cultures were positive. Mixed cultures in most cases lead to non-identification or observation of only one species which has to be resolved by bioinformatics.
The new protocol was found to be very fast and accurate. Identification time is shortened from days to minutes for the majority of samples, thereby enabling quicker adoption of antibiotic therapy.

DVP04
High-resolution in-situ analysis of Legionella pneumophila populations in drinking water by MLVA – genotyping of environmental DNA

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Central to the understanding of infections by *Legionella pneumophila* is the detection of this waterborne pathogen at a clonal level. Currently, Multi-Locus VNTR Analysis (MLVA) of *L. pneumophila* isolates is providing such a high resolution genotyping method. However, *L. pneumophila* is difficult to isolate and isolation of the outbreak strain often fails due to a Viable But Non-Culturable (VBNC) state of the respective environmental population. Therefore, we developed a cultivation independent approach to detect single clones in drinking water. This approach is based on the extraction of DNA from 3 litres of drinking water followed by PCR using a set of 8 primer pairs for all 8 Variable Number of Tandem Repeats (VNTR) loci necessary for MLVA genotyping of L. pneumophila. The PCR amplicons were analyzed by Single Strand Conformation Polymorphism (SSCP) and capillary electrophoresis to obtain the respective MLVA fingerprints. Parallel to the high resolution analysis, we used the same environmental DNA to quantify the number of *L. pneumophila* cells in the drinking water using real-time PCR with 16SrRNA targeted primers. We used a set of drinking water samples from a small scale drinking water network to test our approach. With these samples we could demonstrate that the developed approach was directly applicable to drinking water. We were able to detect different *L. pneumophila* MLVA genotypes in drinking water which could not all be detected by isolation. We think that the developed approach could help identifying outbreak strains long after the outbreak has occurred if the DNA or the water samples have be preserved for later analysis. In addition, this approach could also be applied to clinical samples, such as sputum and nasal aspirates, without cultivation of the infective strains and contributes thereby to an improved surveillance of Legionnaires' disease.

DVP05
Identification of *Streptococcus bovis/equinus* isolates by AXIMA@SARAMIS MALDI-TOF Mass Spectrometry compared to NAT-methods

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The taxonomy of the *S. bovis/equinus group* (belonging to group D Streptoccoci) undergoes several changes in the last ten years. Simultaneously the clinical relevance of this group becomes increasingly interesting because of findings which suggest a high importance within infective endocarditis, colon carcinoma and systemic bacteremia. Furthermore the identification of members of the *S. bovis/equinus* complex by standard methods is still difficult. The API-20 STREP System (bioMérieux) can only distinguish *S. bovis* type II or I and *S. equinus* but is not capable to identify different species or subspecies. The interpretation of 16S rDNA sequence data may be complicated by the fact that closely related species may have nearly identical sequences. In the present study we compared the identification of 100 different isolates from the *S. bovis/equinus* complex (human isolates, animal isolates, reference strains) by NAT methods and AXIMA@SARAMIS MALDI-TOF MS. Identification by NAT methods were performed by amplification of a part of the manganese-dependent superoxide dismutase A (*sodA*) and gyrase subunit B (*gyrB*) gene followed by sequencing analysis. For MALDI-TOF analysis bacterial samples were prepared directly from agar plates applying a standardized protocol and analyzed automatically. Mass spectra of samples were matched against the database of SARAMIS (Spectral Archiving and Microbial Identification System) containing a high number of reference strains. Mass spectrometry analysis revealed a specific and distinct pattern for each species and subspecies of the *S. bovis/equinus* complex. Comparison of MALDI-TOF dendrogram based on mass spectral similarities revealed a high degree of similarity to the *sodA* gene sequence tree. In conclusion AXIMA@SARAMIS MALDI-TOF MS is a reliable, cost efficient and time saving alternative for exact identification and differentiation of members of the *S. bovis/equinus* complex compared to standard NAT methods.

DVP06
Manual versus automated plate streaking of stool samples: a comparative evaluation using PREVI Isola®

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We performed this study on automated plate streaking of stool samples using the PREVI Isola® (Biomerieux) to evaluate the system in terms of quality of streaking , time saving and positivity rate compared to the manual method used for years in our laboratory.
An initial sensitivity test was performed using serial dilutions of four main gastrointestinal pathogens using both methods. To compare the quality 56 clinical samples were inoculated in parallel. The quality of the automated method was evaluated. For routine evaluation a total of 2811stool samples was analysed within six months. 1358 faecal specimens were plated manually in 3 month (05-07/2009) and 1453 specimen were inoculated by an automated plate streaker (08-10/2009). The number of detected bacterial pathogens was analysed in retrospect. For manual streaking the stool was directly plated using a loop, for automated streaking faeces was diluted in saline. The time measurement for manual and automated performance was recorded for all steps.
Serial dilutions of Salmonella, Shigella, Yersinia and Campylobacter strains in inhibitor free stool of healthy adults resulted in an at least comparable plating efficiency of the automated stool inoculation.
The quality of the automated streaking was rated as higher in 48% and as equivalent in 48% of all samples, only 4% of the plates were ranked as lower in quality.
Within 1358 stool samples plated manually 12 Salmonella species were detected (0.9%) and 3 Campylobacter strains (0.2%) between May and July 2009. In 1453 faecal samples processed on the PREVI Isola® (August-October 2009) 24 Salmonella species (1.7%) and 6 Campylobacter species (0.4%) were found. The PREVI Isola® allowed a faster processing of the faeces than the manual method.
The PREVI Isola® system is suitable for streaking of stool samples. It produces higher quality plates in less time. In two consecutive 3-month periods detection rate for gastrointestinal pathogens was higher with the automatically streaked plates.

DVP07
A novel high volume DNA extraction method for the sensitive detection of bacterial and fungal pathogens in whole blood

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Identification of the causative microorganism is still a challenge in the diagnosis of sepsis and infective endocarditis (IE). The continuity of antibiotic treatment, the low-level bacteraemia and growth characteristics of fastidious pathogens reduces the sensitivity of blood cultures. In the present study a novel high-volume DNA extraction protocol followed by broad-range real-time PCR was developed for the sensitive detection of bacterial and fungal pathogens in whole blood. The novel DNA extraction method affords the extraction of 3-7 mL EDTA-anticoagulated blood. The analytical sensitivity was determined to be <50 CFU/mL. To assess the clinical applicability, DNA was extracted from 61 blood samples from IE patients and 18 control patients and analyzed with broad-range bacterial and fungal real-time PCR. Molecular genetic analysis of patient samples showed concordant results to cultivation in seven cases, in two cases bacterial DNA could be detected only by PCR. Blood cultures were positive in 11 cases, while the detection of bacterial DNA was negative. In conclusion, our novel DNA extraction method followed by broad-range PCR assay offers a rapid, sensitive and cost-effective screening method for the detection of pathogens in blood of IE patients. However, the sensitivity is not just yet competitive to culture-based methods due to the low-level bacteraemia during IE. Certainly, we recommend the usage of our novel molecular genetic detection method in IE patients with multiple negative blood cultures or previous antibiotic treatment without a proven pathogen. At present, we improve the sensitivity by the use of a commercially available pathogen enrichment technology (Looxster, SIRS-Lab GmbH, Jena) and compared the applicability of our method with regards to detection limit, implementation and performance with commercially available detection systems.

DVP08
Chlamydia pneumoniae serology with a MIF-calibrated threshold value

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Background: The microimmunofluoresence (MIF) assay is considered the 'gold standard' in *Chlamydia pneumoniae* (Cp)-serology but ELISAs are more commonly used. A MIF-IgG-titre of 512 represents the cut-off between acute and former infection. Here we provide a technical validation to establish a cut-off for a novel Cp-IgG-ELISA that corresponds to the well-established MIF titre.
Methods: A multicentre study (4 centres) compared the technical performance of the new quantitative Cp-IgG/IgA-ELISAs (medac, Wedel) and two MIF assays (Focus/MRL, Labsystems) using 44 sera. Subsequent ROC analysis (144 sera) was used to correlate the MIF titre 512 with the respective IgG-ELISA value expressed in arbitrary units (AU). Two patient populations (21-60 years), 247 with respiratory tract infections (RTIP) and 89 blood donors (BD), were used for plausibility testing.
Results: Correlation of the ELISA-AU for the assay series from 4 centres was between r = 0.9583 and 0.9829 (IgG) and r = 0.9765 and 0.9880 (IgA). AU values for sera with identical reactivity did never differ significantly (CV <15%). The qualitative correlation between MIF and ELISA results for IgG and IgA was between 95.5% and 100%. The correlation between MIF titres (same titre level) for identical sera was 53% (IgG) and 25% (IgA). In the IgG-ELISA a value of 270 AU/ml corresponded to a titre of 512 (sensitivity 100%, specificity 80%). When this value was used, 9% of the RTIP but none of the BD had a pathological result.
Conclusions: ELISA results showed a more stable reproducibility than MIF-titres. Consequently, the identification of abnormal results and the comparability of ELISA results are more reliable using a MIF-calibrated threshold. The initial results indicating a prevalence of 9% for community-acquired Cp respiratory infection are plausible. Studies with clinically defined cases are indispensable to supplement this technical validation.

DVP09
Elaborate MALDI-TOF MS based identification of microorganisms: the SARAMIS concept

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The reliability MALDI-TOF MS for microbial identification depends largely on the reference database and the algorithm for spectral comparison. Most simply, a sample´s spectrum is directly compared to spectra of reference strains and a rated list of matches is provided. A more elaborate approach is realized in the Spectral Archive and Microbial Identification System. SARAMIS uses two types of spectral data in the database: SuperSpectra for fully automated first-line identification and Reference Spectra for semi-automated second-line identification. The concept of SuperSpectra has been developed in consideration of the intra-specific diversity encountered in all microbial species, which is observed in mass spectral fingerprints of individual isolates as variations in peak patterns. To compute SuperSpectra, spectral fingerprints of a number of 10-20 isolates of a species are searched for conserved mass signals that are summarized in a consensus spectrum. In a next step, each mass in the consensus spectrum is compared to the reference database to establish its specificity at different taxonomic levels. By excluding masses that are specific only at a higher taxonomic level, the pattern of the remaining masses is highly specific. This makes random matches highly improbable, practically excluding false identifications. The SARAMIS database contains at present 2700 SuperSpectra, representing 900 microbial species. Since SuperSpectra by definition represent typical isolates of a species (not necessarily type strains), a certain percentage of isolates of a given species will not be captured in automated routine analysis. In this case, the second-line identification applies a direct comparison to all 35,000+ Reference Spectra in the database (representing 1500 species). By this two-step approach basically all clinically, most veterinary and, increasingly, environmental isolates can be reliably identified by a largely automated procedure.

DVP10
Detection of extended-spectrum beta-lactamases in *Escherichia coli* and *Klebsiella spp.* by ESBL test of the VITEK 2 AST-N111 card

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Extended-spectrum beta-lactamase (ESBL)-production is the main cause of 3rd gen. cephalosporin resistance in nosocomial, gram negative pathogens. Outbreaks caused by ESBL-producing Enterobacteriaceae have been reported worldwide. Therefore, a rapid and reliable ESBL detection is indispensable for an appropriate antibiotic treatment and infection control.
The objective of this study was to evaluate the suitability of the VITEK 2 AST-N111 card for identification of ESBL producing *E. coli* and *Klebsiella spp.*.
A total of 379 non-duplicate clinical isolates of *E. coli* and *Klebsiella spp*. were investigated for ESBL production. Using the VITEK 2 AST-N111 card, 33 ESBL-producing organisms were identified correctly. False-positive results occurred in five isolates. Based on these results, the ESBL test of the VITEK 2 AST-N111 card showed a sensitivity of 100% and a specificity of 98.5%. The positive predictive value (PPV) was 86.8%, and negative predictive value (NPV) 100%.
The ESBL test of the VITEK 2 AST-N111 card seems to be a reliable tool for detection of ESBL *in E. coli* and *Klebsiella spp.*

DVP11
Development of a qualitative multiplex real-time PCR assay for detection of *Clostridium chauvoei* and *Clostridium septicum*

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Clostridium chauvoei is a Gram-positive, rod-shaped and endospore-forming bacterium that causes blackleg in cattle, sheep and other ruminants. This severe disease is very similar to malignant edema caused by *C. septicum*, other *Clostridia* species belonging to the gas edema complex, and *Bacillus anthracis*. *C. chauvoei* and *C. septicum* are often found together in animals. Thus, there is a need for a fast and reliable identification method that allows the specific detection of both species in clinical samples.
The multiplex real-time PCR-assay presented here is based on the detection of *spo0A* and enables the simultaneous identification of *C. chauvoei* and *C. septicum* using species specific HEX- and FAM-labeled hydrolysis probes. The assay design includes an amplification control DNA-template for the recognition of PCR-inhibitors. Assay validation was performed using a collection of 29 *C. chauvoei* strains, 38 *C. septicum* strains and 27 other *Clostridium* species. All samples were additionally analyzed using three published conventional PCR tests specific for *C. chauvoei* or *C. chauvoei* and *C. septicum*. Furthermore, the real-time PCR-assay was clinically evaluated on tissue samples from 14 clinical blackleg cases.

DVP12
Quantitative molecular detection of putative periodontal pathogens from clinically healthy subjects and periodontitis patients

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The chronic inflammatory disease periodontitis is characterized by microbial induced progressive destruction of the alveolar bone around the teeth which might lead to early tooth loss and possibly also to systemic diseases such as cardiovascular disorders. The pathogenesis of periodontitis is multifactorial depending on host factors and the composition of the oral microbiom. Up to date, approximately 800 different microorganisms and phylotypes have been identified from the human oral cavity, whereas the majority of them is still uncultivable by currently available methods. Knowledge of quantitative differences in the oral microbial composition between healthy individuals and periodontitis patients is scarce. Therefore, the objective of the present study is to describe the microbial population structure within the human oral cavity of individuals with and without periodontal disease and to identify putative periodontal pathogenic microorganisms by applying culture-independent techniques such as microarray and multiplex quantitative PCR (qPCR) analyses. Samples were obtained from individuals participating in an epidemiological investigation of the north-eastern German region of Pomerania called Study of Health in Pomerania (SHIP). For qPCR experiments the following microorganisms were selected: *Streptococcus sanguinis* as a representative for a healthy oral flora; the Gram-negative putatative peridodontal pathogens *Fusobacterium nucleatum*, *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Treponema denticola*; and the archaeon *Methanobrevibacter oralis* which has recently been associated with periodontitis. Future studies will correlate quantitative microbiological data with available information from the SHIP cohort on environmental and genetic risk factors for periodontal disease.

DVP13
Identification of human and veterinary clinical isolates with MALDI-TOF mass spectrometry

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Daily routine in human and veterinary bacteriology deals with a plethora of different pathogenic, opportunistic bacteria and harmless commensals. A fast and reliable identification of the relevant ill-causing microbe is essential. Usually species identification is done with classical biochemical tests e.g. API tests (BioMérieux) or the Phoenix system (BD). They all need 1-2 days until the result is available. An alternative is the whole cell MALDI-TOF mass spectrometry. The characteristic mass spectral fingerprint of each isolate is compared with a database of reference spectra with a score based pattern matching algorithm, resulting in a list of hits sorted by score values. One sample can be identified in less than 15 minutes including sample preparation. In our study we compared the classical biochemical identification of more than 400 routine isolates of the LGL bacteriology lab with the MALDI-TOF MS (Biotyper, Bruker Daltonics). Among them were 40 different genera including *Enterobacteriaceae*, *Bacillus* spp., *Acinetobacter* spp., *Pasteurella* spp., *Staphylococcus* spp. or *Lactobacillus* spp.. Currently isolates with discrepant results are further analyzed by sequencing of the 16S RNA gene or other suitable genes. The results suggest that MALDI-TOF MS is a fast and reliable method to identify bacterial isolates but the reference database has to be further extended.

DVP14
Biomarker discovery in pneumonia

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Pneumonia is an acute disease which is reflected by lung inflammation and congestion of the lung. It can be caused by bacterial, viral, or by noninfectious agents and affects individuals of all ages. For older people and children, pneumonia is the most common cause of death worldwide.
Currently pneumonia can be defined clinically in three different severity stages: (i) uncomplicated community acquired pneumonia (uCAP), (ii) severe CAP (sCAP) and (iii) with septic shock (ssCAP). However, clinical diagnosis is limited in the possibility to recognize risk patients showing non-expectable progression to more severe CAP stages. Thus new biomarkers, in terms of small molecules or peptides, can improve diagnoses, drug development and the success rate of therapy. The aim of this study is to determine diagnostic and prognostic biomarker in blood plasma from CAP patients.
In a pilot study we have analyzed human urine of CAP patients by using the gold standard CE-MS for biomarker detection and accurate mass spectrometry (UPLC-Orbitrap MS) for their detailed characterization (peptide sequence & post-translational modification). We identified 8 modified peptides with representatively altered levels in acute pneumonia in comparison with the uncomplicated controls. Thus, we have identified first potential biomarkers for diagnosis that might also contribute to our knowledge about the pathophysiology of pneumonia.

DVP15
Development of a stable isotope dilution analysis for the quantification of *Bacillus cereus* toxin cereulide in food

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The pathogenicity of the emetic (vomiting provoking) *Bacillus cereus* arises from the small dodecadepsipeptide cereulide [1]. This cyclic toxin is resistant to heat, proteolysis as well as to acid and basic conditions. Because of these properties cereulide preformed in food will not be destroyed in the intestinal tract or by reheating of food. Due to its lipophilic character it could rapidly be absorbed from the gut and may have neurotoxic and immuno-modulating effects.
In recent years three detection methods of cereulide have been described: LC/MS analysis; cytotoxicity assay and a boar sperm-based motility assay [2]. Hitherto all assays were performed using the antibiotic valinomycin as surrogate standard. However, it can not be ruled out that valinomycin behaves differently than cereulide in complex matrices and/or in biochemical assays. Due to changing lifestyles and eating habitats, emetic *B. cereus* is gaining increasing prominence as an emerging food borne pathogen and an appropriate standard for a conclusive detection and quantification of the emetic toxin cereulide is urgently needed.
Therefore, a protocol for *in vivo* production of cereulide and a ^{13}C-labeled cereulide standard has been established and a HPLC based purification system was developed. Structures and purity of toxin preparations were confirmed by means of LC-MS/MS and 1D/2D-NMR spectroscopy. The ^{13}C-labeled cereulide was used to establish a highly sensitive, accurate, and robust stable isotope dilution analysis (SIDA) for the quantitative analysis of cereulide in foods using LC-MS/MS detection [3]. The SIDA developed was cross-validated with valinomycin as the internal standard. The MS response of valinomycin was found to be significantly influenced by the food matrix, thus leading to rather low recovery rates from model foods.

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DVP16
Comparison of commercially available selective media for the isolation and identification of Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP)

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Numerous cases caused by Methicillin-resistant *Staphylococcus* (*S.*) *aureus* (MRSA) have been reported in human medicine over the last decades. In veterinary medicine, Methicillin-resistant staphylococci have become a cause for concern only recently. However, not only MRSA but also Methicillin-resistant *S. pseudintermedius* (MRSP) may be a potential source for infections of humans. A number of selective media for MRSA isolation and identification have been developed in the past. For MRSP, specific selective media are not available. Therefore, selective media have been used only rarely in former studies on MRSP. The aim of our study was the comparison of commercially available MRSA media for isolation and identification of MRSP as a basis for future studies on the distribution of MRSP.
Four media were included in the study [ChromID MRSA agar, BioMérieux, Nürtingen, Germany (1); Oxacillin resistance screening agar base, Oxoid, Wesel, Germany (2); Chrom MRSA II agar, BD Diagnostics, Heidelberg, Germany (3); Brilliance MRSA agar, Oxoid (4)]. *MecA*-positive *S. aureus*, *S. pseudintermedius*, *S. epidermidis* (MRSE), *S. haemolyticus* (MRSH) and *S. sciuri* were used as well as sensible control strains for staphylococcal species and other bacteria frequently isolated from the skin of dogs. In addition to single inoculation assays, mixtures of MRSA and MRSP or with sensible staphylococci and other bacteria were tested.
MRSP grew on each medium, but on agar 3 and, with exceptions, on agar 1 with very small colonies. On agars 2 and 4, MRSP could be differentiated from MRSA, on agar 2 even in mixed assays, whereas MRSP was of the same colour as MRSA on the other two media. On agars 3 and 4, some sensible strains were detected with very small colonies at day 2. MRSE and MRSH grew on all four media, and MRSH was similar to MRSP on agar 2. Nevertheless, agar 2 seemed to be most applicable for the isolation and identification of MRSP. Details of the agars concerned will be presented.

DVP17
Development of a chip-based pen-side analysis system for the fast and specific detection of the causative agent of contagious bovine pleuropneumonia (CBPP)

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Contagious bovine pleuropneumonia caused by *Mycoplasma mycoides* supspecies mycoides SC is a devastating disease of cattle which today is confined mainly to the African continent. As a result of rigid control measures and a consequent stamping out policy, Europe has been CBPP free since the late 1990ies. Even so, the risk of introducing CPBB is still high and rising mainly due to an increase in international trade of live cattle. In case of a sudden CBPP outbreak in Europe caused by clinically inconspicuous animals or by intentional introduction, rapid action such as setting up exclusion zones and culling of affected live stocks is essential to efficiently prevent transmission and spread of the infection.
Laboratory-based diagnostic methods can provide reliable results within a few hours. However, the transport of suspicious samples to central laboratories is often very time-consuming. Therefore, we are developing a portable PCR-chip-based diagnostic device designed to assist first responders and authorities in rapid decision making on scene.
The system incorporates a miniaturized PCR-chip which is microfluidically coupled to a DNA-chip for identification of the PCR products by probe hybridization with subsequent electrical detection. In conjunction with an automatic nucleic acid extraction handheld, the device could also be operated by untrained staff and should provide reliable results as fast as 1.5 hours after sampling. The test combines two independent PCR-hybridization assays with primers and probes targeting a variable (lipoprotein) and a conserved target (lepA) and includes internal controls. The data of experiments using MmmSC and strains of the Mycoplasma mycoides cluster as well as clinical samples are presented and discussed in comparison to alternative diagnosis of the infectious agent like real time PCR and culture.

DVP18
Biotyper 2.0 based identification of Candida species

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Objectives: In daily routine the identification of Candida species is important but time-consuming.
Beside chromogenic test methods the biochemical identification is widely used. The Matrix-assisted-laser-desorption-ionisation time-of-flight mass spectrometry (MALDI-TOF MS) might be an inexpensive method for rapid identification of Candida species.
Methods: The aim of this study was to compare the Biotyper 2.0 based identification in comparison to the Vitek2 (bioMerieux) YST identification card. 39 clinical isolates of 8 different Candida species were included.
Results: Biotyper 2.0 based identification was identical to the YST card-based identification in 36 of 39 isolates. In 29 of 39 Biotyper-based identifications a score between >=2 and 2.29 was achieved, indicating probable species identification. In 8 isolates a score of >=2.3 was achieved indicating excellent species identification. Only in two identifications a score below 2.0 was achieved. Two of 39 isolates remained unidentified using the YST-identification card, in one isolate the YST-identification card could not discriminate between 2 species.
Conclusion: Biotyper 2.0 based identification was equivalent to biochemical identification using Vitek2 YST (bioMerieux) identification card. In contrast to the latter the matrix-assisted-laser-desorption-ionisation time-of-flight mass spectrometry and Biotyper 2.0 based identification was significantly faster.

DVP19
Automated detection of mixed cultures of microorganisms from positive blood cultures using MALDI-TOF mass spectrometry

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MALDI-TOF MS fingerprinting for identification of microorganisms recently has emerged as a powerful tool in clinical microbiology diagnostics. Profile spectra are acquired for an unknown microbe and identification is performed by bioinformatic comparison with a dedicated database. A current drawback of the technology is that in the case of mixed cultures, e.g. in case of contaminations, generally only one of the microorganisms is detected in automated analyses. Sometimes even any identification is hampered by the mix of profiles in the mass spectra. We recently presented an approach to check spectra for the probable existence of such contaminations. Here we report the improvement of the respective algorithm and its application to positive blood culture.
Blood cultures were spiked with two bacteria and incubated for several hours at 35°C. Next, they were harvested under standard conditions, purified and prepared for MALDI-TOF MS analysis according to a protocol developed in our lab. Bacteria were analysed using a microflex mass spectrometer according to standard procedures. Resulting spectra were automatically analysed using the MALDI Biotyper 2.0 software package and, in parallel, with the novel algorithm which is calculating the possibility of a mixed-culture spectrum.
Several two compound-mixed cultures were unambiguously recognised as mixed-cultures using the novel algorithm. For some mixes still only one species was detected or analysis failed at all. No false mixture alarm appeared from blood cultures, also no wrong identification was observed. Two compound mixed cultures spiking experiments using other body fluids (e.g. human urine) are under current investigation.
The principle applicability of automated mixed culture identification for positively flagged blood cultures could be demonstrated. The potential of further improvement of sensitivity of the method and algorithm is being investigated.

DVP20
Evaluation of MALDI-TOF MS based identification of clinically important bacteria in a routine laboratory

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Matrix-Assisted Laser Desorption Ionisation/ Time-of-Flight Mass Spectrometry (MALDI-TOF MS) offers a promising method for rapid identification of microorganisms. This new approach was successfully implemented and accreditated in our clinical routine laboratory. In a previous study we showed the high validity of MALDI-TOF MS in identification of Enterobacteriaceae. The aim of the present study was to evaluate its reliability in identification of other bacterial species isolated in our laboratory, including Staphylococcaceae, Enterococcaceae, Neisseria spp. and *Haemophilus spp*.
Results of MALDI-TOF identification of 241 bacterial strains belonging to 31 different species and 16 genera were verified by biochemical re-identification. Mass spectra were acquired by using AXIMA-LNR and AXIMA-Assurance mass spectrometers (Shimadzu) and analysed by SARAMIS software (AnagnosTec). Samples were prepared by directly applying colonies on target plates and mixing with matrix solution. Biochemical identification was performed by conventional routine methods. In case of divergent results, biochemical and MS identification were repeated. Continuing discrepancies were analysed by 16S rRNA sequencing. At least 5 up to 10 strains of each species were tested, and if MALDI-TOF identification showed no false results, it was accepted as reliable for laboratory routine.
Concordant results were obtained with 215 strains (89.2%), whereas 26 strains (10.8%) had divergent results of MALDI-TOF and biochemical identification. Further experiments showed false biochemical identification of 23 strains in the first analysis and still 11 in the second one, but no false results of MALDI-TOF MS. Three strains could not be identified definitely even by sequencing.
In conclusion, the results of the MALDI-TOF MS/ SARAMIS system are highly reliable for all bacterial species included in this study. Thus, routine identification of these microorganisms in our laboratory is now performed by MALDI-TOF MS.

DVP21
Volatile organic compounds (VOCs) emitted by *Pseudomonas aeruginosa* and Candida albicans

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Volatile organic compounds are released by all kind of microorganisms. Many of them are familiar to us as special odors of microbial strains and aromas of food products, e.g. in cheese and wine. Some of these compounds may play a role in interspecies communication. Therefore, VOCs profiles are species-specific and should allow differentiation of microorganisms offering the possibility for improved diagnostics for mircobial infections. *C. albicans*, a dimorphic yeast, and *P. aeruginosa*, a gram-negative bacterium, are opportunistic pathogens, known to grow in interspecies biofilms. While they are part of the normal microbial flora of many humans, their pathogenicity in immunocompromised individuals is high. Both colonize frequently the lung of patients with cystic fibrosis and cause serious pneumonia. To detect microbial volatile compounds from these pathogens solid phase micro-extraction (SPME) was performed by exposing a fiber, coated with absorbance material, to the headspace of *P. aeruginosa* and *C. albicans.* The cultures were grown in gas-impermeable closed vials for about 24 hours and the headspace was afterwards sampled by SPME. Separation and identification of the absorbed volatiles was done by gas-chromatography/mass-spectrometry (GC-MS). Comparison of different profiles led to the characterisation of species-specific VOCs. Furthermore, headspace SPME was also done in cell culture infection models. To account more realistic conditions human carcinoma lung epithelial cells (A-549) were infected with *P. aeruginosa* and *C. albicans.* SPME was performed regularly during the course of infection. Hereby, pathogen-specific volatile compounds were determined and compared with the profiles of pure cultures. SPME/GC-MS offers new opportunities for the differentiation of microorganisms growing on surfaces. By identification of microbial VOCs their bioactive functions can be clarified and used for new applications, for example to improve diagnostics of lung infections.

DVP22
Two years of experience with T-Spot – a critical retrospective review

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Background: The correct identification of latent TB is crucial before the initiation of any immunosuppressive management not only in patients in rheumatology and dermatology departments. The classical skin test is gradually replaced by faster and more specific *in vitro* detection test of TB-specific T-cells like the interferon-gamma-test T-Spot (Oxford Immunotec).
Methods: We retrospectively analysed all samples sent to our lab for interferon-gamma-testing (IFG) in respect to test results, performing laboratory technician, department and co-diagnosis of mycobacteria in other samples of the same patient.
Results: Since July 2007 to September 2009 we received 1367 samples for IFG-testing in total. 853 (63,5%) test were tested negative, 160 (11,3%) were not interpretable, 180 (13,1%) were tested positive and 153 (12,1%) samples reached the laboratory too late to meet the necessary preanalytic conditions. We received samples from 67 different departments of our university hospital (dermatology: n=457, rheumatology: n=214, pulmonology: n=189, nephrology: n=78, infectiology: n=61, gastroenterology: n=54, ICU medical: n=19). The percentage of not interpretable results was lowest in samples from dermatology outpatient department (8,4%; total: n=298) and highest in samples from medical ICU (31,6%; total: n=19). In general the monthly average rate of not interpretable results was below 10% over the study period except two intervals of 6 respectively 8 weeks in winter 2008 and summer 2009, when the tests were not performed by the designated technician. Otherwise there was no cluster observed in time considering positivity and rate of negative results. There seems to be a correlation of the number of detected spots with either a positive result in TB culture or PCR.
Conclusion: The IFG-test performed with skilled and experienced hands serves as a useful tool in *in vitro* diagnosis of latent TB in patients expected to undergo immuno-compromising procedures. The success of test performance depends on proper pre-analytic conditions, e.g. quick transportation to the laboratory and hence priming of clinicians is essential.

DVP23
Discrimination of *Enterococcus* spp. by MALDI-TOF MS Profiling

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Enterococci are opportunistic human pathogens. The two most important species, *Enterococcus faecalis* and *Enterococcus faecium* are one of the most common cause of hospital-acquired infections, which include those of bloodstream, central nervous system, urinary tract and heart. The differentiation of Enterococci have been a difficult problem for diagnostic laboratories for many years. The aim of this study is to demonstrate the applicability of MALDI-TOF MS profiling for species identification and, as presence and degree of antibiotic resistance are strain-specific, strain differentiation of Enterococci. Sets of MALDI-TOF spectral data of reference strains including 17 different enterococcal species were created using different sample preparation protocols.
Our data showed that treatment with mutanolysin for 30 min. prior to ethanol precipitation considerably improved the correlation coefficient compared to treatment with lysozyme, glass beads and only ethanol precipitation. While other sample preparation methods failed to differentiate between certain species, e.g. between *Enterococcus casseliflavus* and *Enterococcus phoenicoelicola*, all species were identified accurately using mutanolysin treatment.
After optimization of the sample preparation protocol, 80 clinical isolates of the genus *Enterococcus* were investigated by MALDI-TOF MS using the mutanolysin treatment. All isolates were identified correctly.

DVP24
Multiplex-PCR based sepsis diagnostic in patients from the surgical ICU with a new onset of SIRS

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Background: Previously we have shown that direct molecular analysis of blood samples for microbial pathogens using multiplex-PCR (LightCycler® SeptiFast Test MGRADE, ROCHE) could successfully support Effective treatment for sepsis. However, screening all severe ill patients on an ICU in a surgery department specialized on peritonitis patients on a routinely basis was not applicable for economic reasons.Therefore this study focused on patients with upcoming SIRS parameters and prospectively analyzed their benefit from the additional molecular analysis of blood samples without precultivation.
Methods: Patients were screened within 24h after appearance of SIRS parameters for microorganisms directly from blood samples by multiplex-PCR as well as by blood culture. Samples were taken from other body sites as well for diagnostics including procalcitonin- and CrP- analysis. The impact of fast (within 4 hours) results obtained by multiplex-PCR on antibiotic therapy was monitored.
Results: Over a time period of 20 months 120 patients were enrolled into the study. From a total of 148 blood samples analyzed 59 samples got positive by multiplex-PCR and 77 different microorganisms could be detected. Blood culture detected only 30 microorganisms. Positive PCR results and positive blood culture results went along with elevated procalcitonin but not with elevated CRP values. In 25 cases (25.3% of positive PCR results, 16.9% of total analysis) the PCR results led to a change in antimicrobial therapy.
Conclusion: In conclusion, the multiplex-PCR approach facilitates rapid detection (within 4-6h) of sepsis-relevant microorganisms in blood samples of patients with SIRS parameters. The results of this study clearly show that positive PCR results enable earlier antimicrobial therapy and/or optimized therapy for patients especially with regard to microorganisms with a high intrinsic resistance against antimicrobials used in calculated therapies

DVP25

Will not be presented and published

DVP26
Potential of styryl dyes for clinical microbiology

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In clinical microbiology, examination of the vast majority of samples is traditionally performed by preparing smears, which are heat-fixed and stained according to Gram. Sometimes, fluorescent dyes like acridine orange are employed, in particular if normally sterile samples have to be analyzed, and sensitivity is limiting. Despite the established usefulness of these standard procedures they suffer from certain limitations: the need for fixation of samples precludes application to the analysis of live organisms, and accordingly dynamic observations are not possible. Moreover, liquid toxic waste is produced during removal of unbound dyes, and staining requires several working steps. Styryl dyes may help to overcome these limitations, as they can be used to stain microorganisms by simple addition to liquid samples. Styryl dyes partition into the outer leaflet of membrane bilayers of intact cells, and into the extracellular fluid. When incorporated into membranes, fluorescence upon excitation with UV-light increases >100-fold over levels in culture media. Curiously, despite their common use in cell biology, styryl dyes are not routinely employed in clinical microbiology. Therefore, we have started to explore their potential in this context and found that they are promising tools.

DVP27

Quality of Field Safety Notices released by manufacturers in cases of failure of tests and reagents for infection diagnostics – Analysis of cases reported to the BfArM between 2005 and 2007

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The European Directive 98/79/EC for *in-vitro* diagnostic medical devices (IVD) regulates marketing and post marketing surveillance of IVD in the European Economic Area. Manufacturers have to inform the responsible Competent Authorities (CA) about incidents and field safety corrective actions (FSCA) related to IVD. In Germany, the Federal Institute for Drugs and Medical Devices (BfArM) is the responsible CA for most IVD (only few IVD (no analysers), specified in Annex II of the Directive are under the responsibility of the Paul-Ehrlich-Institute (PEI)). In case of FSCA manufacturers have to inform customers by means of Field Safety Notices (FSN) which shall be sent to the BfArM prior to release and are also published on the BfArM homepage. Between begin of 2005 and end of 2007 the BfArM received a total of 1025 reports regarding IVD. From these 38 related to tests and reagents for infection diagnostics. FSCA were performed in Germany in 32 (84.2 %) of these cases. Written FSN were received in German and English language in 31 / 27 cases delayed up to 56 / 42 days after notification to the BfArM, respectively. Product failures were sufficiently described in the text in 29 / 25 cases and the required measures were sufficiently described in 29 / 25 cases, respectively. However, there were cases with differences in the information quality between the German and the English versions. A customer confirmation form was part of the customer information in 24 / 17 cases only. Our results suggest that for IVD for infection diagnostics FSCA and FSN are frequently performed. A number of relevant deficiencies regarding the quality of the FSN are demonstrated. In detail, manufacturers should shorten the time until release and improve the contents of FSN to ensure the safety of IVD in cases of product failure.

DVP28

Distinct lineages of *S. aureus* prevalent in Bochum area cause false negative *S. aureus* identification test results in the Slidex Staph Plus agglutination test

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The sensitivity of commercial agglutination kits for fast identification of *S. aureus* is well below 98%. In this study we reevaluated the sensitivity of the Slidex Staph Plus (bioMérieux) agglutination kit with three sets of molecular characterized *S. aureus* strains. In this study 363 molecularly defined clinical non-copy strains of methillin-susceptible *S. aureus* (MSSA) and 240 molecularly defined clinical non-copy strains of methillin-resistant *S. aureus* (MRSA) were tested. Additionally, a collection of 104 pulsed field gel electrophoresis (PFGE) divergent MRSA strains were included, to compare the sensitivity of agglutination based identification to the reference method.
The sensitivity of matrix assisted laser desorption / ionisation time of flight mass spectrometry based *S. aureus* identification using the Biotyper 2.0 database was 100%. Sensitivity of Slidex Staph Plus (bioMérieux) kit was higher in MSSA strains (356 of 363 strains = 98%) than in MRSA strains (227 of 240 strains = 94.5%, p=0.0035). Two of seven MSSA agglutination negative MSSA strains were spa-type t001(28.5%). Ten of thirteen agglutination negative MRSA were spa-type t001(76.9%).
In the collection of PFGE divergent MRSA-strains, the sensitivity of the Slidex Staph Plus (bioMérieux) agglutination kit was unexpectedly low (70.1%). Interestingly, strains tested false negative by the Slidex Staph Plus agglutination method in this collection were spa-type t001(24 of 32) and t001 related (7 of 32).
The sensitivity of *S. aureus* identification by MALDI-TOF MS was superior (100%) to the sensitivity of the Slidex Staph Plus (bioMérieux) agglutination test. The sensitivity of the Slidex Staph Plus was significantly higher in MSSA compared to MRSA strains. The MALDI-TOF MS based identification could be a substitution in agglutination test negative strains, especially if the local prevalence of t001 and t001 related strains is high.

DVV01

Improvement of molecular genetic pathogen identification by 23S rDNA real-time PCR in heart valves of infectious endocarditis patients

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Identification of the etiologic agent is the deciding factor in diagnosis and effective therapy of infectious endocarditis (IE). Recently, we demonstrated that broad-range 23S rDNA real-time PCR improved the molecular genetic detection of bacteria in heart valves (HV) of IE patients with a high sensitivity of 96.4%. Unfortunately, the real-time PCR product length of 121 bp only allows identification of the pathogen to genus and the species identification had to be done subsequently with 16S rDNA conventional PCR. In the present study, we extended the length of the PCR product to improve pathogen identification to the species level by subsequent sequencing analysis directly after real-time amplification. We investigated the limit of species identification for the most important pathogens causing IE including *Streptococcus*, *Staphylococcus*, *Enterococcoccus*, as well as infrequent or rare pathogens such as members of the HACEK-group, *Coxiella* or *Bartonella*, revealing a sufficient intra- and interspecies differentiation. Furthermore, a non-competitive internal control was applied to prevent false-negative results due to PCR inhibition for further improvement of the assay´s diagnostic reliability. To assess the clinical applicability and to determine the balance between PCR sensitivity/efficiency and species identification based on the length of the amplification product, 405 surgically removed HV of patients with proven IE or suspicious intra-operative morphological findings were analyzed in parallel by 23S rDNA real-time PCR using both, the 121 bp and the 306 bp PCR product. The combination of the expanded real-time PCR assay facilitates the simultaneous detection and discrimination of almost all relevant pathogens causing IE in HV tissue of IE patients. Considering the utility of this real-time PCR assay for routine sample processing currently performed in the microbiological laboratory, our method offers the possibility of a significantly faster pathogen detection and identification.

DVV02

Fine tuning of *P. aeruginosa* virulom and metabolom during chronic cystic fibrosis lung disease

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Träger des Becton Dickinson Forschungspreises

The long-term persistance of *P. aeruginosa* in the cystic fibrosis (CF) lung is characterized by the selection of a variety of genotypes and phenotypes that typically descend from one infecting *P. aeruginosa* clone, a process described as adaptive radiation. To provide a detailed survey of these adaptations of *P. aeruginosa* during chronic infection of the CF lung, we performed a comparative proteome and transcriptome analysis of isogenic co-existing non-mutator and *mutS* mutator isolates from selected CF patients. These data showed that during CF lung persistence *P. aeruginosa* mutators converge to a virulence-attenuated phenotype due to the down-regulation of several established virulence factors. Further, we demonstrated that the adaptation process of *P. aeruginosa* predominantly comprises metabolic pathways. In end-stage mutator strains, several transcripts of genes or proteins involved in metabolism of fatty acids, nucleotides, amino acids and the generation of energy were increased. Of particular interest is the increased expression level of genes involved in (i) the anaerobic arginine-deiminase pathway, (ii) the anaerobic respiration (iii) the tricarboxylic acid cycle (TCA), glyoxylate shunt and the uptake of dicarboxylates. These changes indicate an adaptive shift towards constitutive expression of genes of metabolic pathways obviously required for growth under micro-aerobic and nutritional conditions of suppurative CF lung tissue. Finally, these data will give us new insights into the chronic virulence pattern of *P. aeruginosa* in order to determine possible novel antimicrobial drug candidates to combat chronic CF lung disease.

DVV03
Development and evaluation of a real-time PCR protocol for rapid identification of mutations in the DHPS gene of *Pneumocystis jirovecii*

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Pneumocystis jirovecii is an important cause of severe respiratory infections in immunocompromised patients. The combination of trimethoprim-sulfamethoxazole (TMP/STX) is widely used for the prophylaxis and treatment of *Pneumocystis* pneumonia (PCP).
Because culture of *P. jirovecii* is not possible, conventional antimicrobial testing is no option to predict TMP/STX susceptibility or to investigate cases of presumptive therapy failure. Molecular "susceptibility testing" is the only possibility to identify *P. jirovecii* strains that are most likely resistant to TMP/STX. Many studies have shown that mutations in the drug target, the dihydropteroate synthase (DHPS) gene, are presumably involved with the failure of prophylaxis and treatment.
PCR amplification of the DHPS gene and subsequent sequencing of the amplicons, techniques which are not suitable as a routine workup in micorbiological laboratories, are necessary to identify such mutants. Here we present a rapid method for identifying mutations in the DHPS gene using real-time LightCycler PCR. Melting curve analysis of the hybridization probes allows distinguishing between the wildtype sequence and different mutants described in the literature. Applying this novel approach to clinical specimens, typical mutations can be detected and even heteroresistant populations can reliably be identified.
Using real-time PCR for the rapid identification of *Pneumocystis jirovecii* isolates presumably resistant to TMP/SXT allows for the early adjustment of therapy in the treatment of PCP.

DVV04
Concept of a cooperation project for the development of a chip-based on site detection system for animal disease

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Motivation: Agriculture forms part of critical supply-related infrastructures which are particularly affected by biological hazards. Animal diseases can have severe economic consequences and may considerably upset the general public. Efficient protective and remedial measures as well as damage control depend on the rapid identification of potential biological agents. However, the detection of pathogens on the spot is only possible to a limited extent so far.
Project description and goals: For this reason, a new system is to be developed which enables the on-site detection of different animal diseases e.g. FMD, blackleg and CBPP. The approach includes three main modules: The first module is the on-site sample processing. The second comprises a PCR chip which allows the highly sensitive amplification of target DNA without requiring much space or energy. The third module is a DNA chip which enables the highly specific identification and differentiation of pathogens.
Innovations and applications: Such an innovative miniaturized lab-on-a-chip system will ensure a quick and flexible response to potential hazards caused by epizootic pathogens. To make the diagnostic data available to an immediate analysis and crisis management a web based online data management tool is developed. Accompanying network analysis of trade connections between farms is performed to develop models for the possible spread of pathogenic agents.
Project title
- Autonomous animal disease lab-on-a-chip system (ATLAS)
Project partners
- Friedrich Loeffler Institute (FLI), Federal Research Institute for Animal Health, Jena
- National Reference Laboratory for Foot and Mouth-Disease
- National Reference Laboratory for Contagious Bovine Pleuropneumonia
- National Reference Laboratory of Blackleg
- Institut of Epidemiology
• Friedrich Schiller University of Jena, Jenaer BioChip Initiative, Jena
• Institute of Photonic Technology, Microfluidics Department, Jena
• Analytik Jena AG, Jena
• Tecart GmbH, Erfurt
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DVV05
Quality assured species identification of pathogenic Herpotrichiellaceae achieved by using an ITS2 based barcode approach

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Members of the *Herpotrichiellaceae* (black yeasts - BY) are well-known for their pathogenic potential including biosafety level 3 organisms like *Cladophialophora bantiana* and *Rhinocladiella mackenziei*. Due to their pleoanamorphic life cycle classical species identification based upon morphology is cumbersome and often impossible to achieve. Physiological identification is also time consuming and not always reliable due to variable test results within a given species. Therefore, a genetic based identification seems to be a straightforward alternative, e. g. sequence analyses of rDNA. This approach using the BLAST algorithm is hampered by the poor quality of the entries (species designation and sequence) in public accessible databases.
Here we describe an approach by using a quality-checked barcode database to ensure a highly reliable species identification in case of BY.
The sequences of the ITS1-5.8S-ITS2 region of 61 pathogenic type strains of BY were compared to fungal sequences in GenBank by a BLAST search using (A) the complete sequence, (B) the ITS2 region only, and (C) a substring of the ITS2 region (length 35 - 55 bp with conserved flanking sequences) with an assumed interspecies variability only.
Hits in terms of fungal species names were sorted according to their maximal identity score (query coverage 100%) and were compared for the three searches performed. Multiplicity of species names recovered was highest in search A and lowest in C.
Search C revealed 94 unique barcodes. Sequences with barcodes not identical with those of the type strains had been assigned tentatively to sequevars on base of sequence similarity to established types. Thereby 20 of the 61 species studied turned out to consist of 2 to 5 sequevars.
With very few exceptions, namely *Exophiala dermatitidis* and *E. phaeomuriformis* as well as 3 other species all sequences studied could be unequivocally assigned to a species name using the barcode.
False identifications resulting from e.g. sequences of bad quality deposited at GenBank occurred when longer query sequences were used. This could be avoided efficiently by using the barcode.
In conclusion we could prove that a barcode based approach provides an easy-to-perform quality measure for fast and cost-efficient species identification in case of pathogenic *Herpotrichiellaceae*.

DVV06
Malaria Rapid Diagnostic Tests: is external quality testing necessary?

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Malaria rapid diagnostic tests (RDTs) which detect parasite-specific antigens are increasingly applied when expert microscopy is not available. The number and range of commercially available products have rapidly increased. At present, assays utilize different target antigens like histidine-rich protein 2 (HRP-2), Plasmodium aldolase, or parasite lactate dehydrogenase (pLDH). The "detection rate" is defined as 100% positivity of RDTs against samples with 200 parasites/µl and 2000-5000 parasites/µl, respectively (WHO, 2008).

We here report the results of a comparative study on travellers (n= 159) returning with fever from different malaria-endemic areas. Malaria diagnosis was based on light microscopy (gold standard). In addition, two pan-malaria RDTs applying either HRP-2/aldolase (Binax Now® Malaria Test, Binax, USA) or HRP-2/pLDH (NADAL Malaria 4 species, nal von minden GmbH, Germany) as target antigens were used.
There was no false positive reaction with the RDTs against the Plasmodium spp. negative samples from 111 patients. The *Plasmodium spp*. positive samples were taken before (n=30) or after (n=18) initiation of treatment and were classified by light microscopy as *P. falciparum* (n=43) or *P. vivax* (n=5). The samples showed a wide variety of parasite densities (32/µlup to >50.000/µl). Both RDTs performed very well in our specific clinical setting (no false negative reaction). HRP-2-antigen persistence in microscopically negative samples did confirm the anamnesis of recently treated Malaria tropica (n=7). The HRP-2 band occurred in combination with the pan-malaria antigens (aldolase or pLDH) in 11 samples but double-infection was suspected in only two patients. Reactivity of a single pan-malaria antigen was restricted to patients with *P. vivax*-infection.
The results will be discussed on the background of the WHO RDTs testing report (2008). It is concluded that even after approval by the WHO, malaria RDTs may require a further external quality control to test the application and interpretation of the user.
WHO: Malaria Rapid Diagnostic Test Performance: Results of WHO product testing of malaria RDTs: Round 1 (2008)

DVV07
Comparison of MALDI-TOF MS based Microbial Species Identification Systems

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For management of infectious diseases, rapid identification of the causative agent is an important factor as correct choice and timely application of the antimicrobial regime is a crucial component of successful treatment. However, traditional differentiation procedures can be labor-some and time-consuming processes. Here, the recent arrival of biophysical methods such as MALDI-TOF MS in routine diagnostic laboratories holds the promise of significantly speeding up these processes for the majority of microbial pathogens.
To evaluate and compare the two commercially available MALDI-TOF systems (AnagnosTec Saramis and Bruker Biotyper2) for use in diagnostic procedures, we have assembled a set of 1200 fungal and 800 bacterial clinical isolates, either collected prospectively at different locations or taken from culture collections to reflect a broad species distribution. All isolates were identified with standard diagnostic procedures (Walkaway, API, standard manual biochemical tests, rDNA sequencing) and with both MALDI-TOF systems.
All three systems had an over all success rate and agreement of >95%, but both MALDI-TOF systems displayed a superior resolution of closely related species and easier recognition of species unknown to the system.
Taken together, both MALDI-TOF systems allowed microbial species identification at a higher resolution and in a fraction of the time needed with conventional biochemical tests. This will clearly improve response times for pathogens with known intrinsic resistances. However, at the moment additional resistance testing is still needed in many cases.

DVV08
Identification of clinical fungi by MALDI-TOF MS: How to deal with growth-dependent variability in peak patterns

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The identification of microorganisms by MALDI-TOF MS is about to replace biochemical identification procedures for routine diagnostics. While the mass spectral identification of most bacteria is straightforward, the identification of fungi with whole cell MALDI-TOF MS is more challenging for several reasons. Most importantly, the peak pattern of an individual isolate can change dramatically in dependence of incubation time and medium composition. Especially the transition from non-sporulating to sporulating mycelium generally results in marked differences in mass fingerprints.
One option to overcome this difficulty is to strictly standardize the cultivation conditions of reference and sample isolates. This can, however, be rather impractical due to differences in growth behaviour, particular medium demands, and handling requirements of individual isolates. Another strategy is to obtain reference data from well characterized isolates for different growth conditions. The latter strategy is followed for the Spectral Archive and Microbial Identification System (SARAMIS) by the acquisition of whole cell mass spectra of reference isolates grown on a variety of solid media and at different incubation times. Generally, reference isolates are incubated on three different media and mycelium samples are taken after three different incubation times. By this 3 x 3 approach the variability of mass fingerprints of individual isolates is largely captured and the mass spectra are deposited in the reference database. When multiple isolates of a species are contained in the database, the corresponding data were used to compute SuperSpectra for fully automated identification.
SARAMIS allows the rapid, automated identification of most clinically relevant fungi by direct on-target smear preparation of fresh mycelium taken from agar plates. Examples will be presented for the identification of dermatophytes, yeasts, *Aspergillus* sp., and *Fusarium* sp.

DVV09
Identification of bacteria with MALDI-TOF-MS that are difficult to identify by standard methods

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Background: The standard methods of microbiological identification are based on the detection of distinct metabolic activities (API™, VITEK™) or specific fatty acids by gas chromatography.
Bacteria that have been exposed to certain chemicals (e.g. antibiotics or chemotherapy drugs) or other influences (e.g. the viscous bronchial mucus in the bronchia of patients with cystic fibrosis) often change their caracteristic properties and therefore elude the detection.
In some of these cases the standard methods are not capable of delivering any identification result which is essential for the further clinical treatment of the patient.
We examine whether the analysis of ribosomal protein patterns by MALDI-TOF-MS (Matrix Assisted LASER-Desorption Ionisation Time of Flight Mass Spectrometer) results in a definite identification.
Methods: Samples from the clinical routine that could neither be identified by VITEK™ nor by gas chromatography are measured by MALDI-TOF-MS.
A small amount of bacterial cells from the culture plate is directly deposited onto a sample plate. Bacteria are inactivated and intracellular, mostly ribosomal, proteins are extracted by the use of 0,3 µl 2,5-dihydroxy benzoic acid as organic matrix.
The spectra of these proteins are compared with the entries of a database (SARAMIS™, AnagnosTec). The whole process of sample preparation, measurement and database search takes only a few minutes.
Results: With MALDI-TOF-MS we could identify more than 60 % of all isolates which had no result using the standard methods. In most cases the samples came from patients with cystic fibrosis and were identified as *Pseudomonas aeruginosa.*

DVV10
Speeding up Identification of Bacteria from Urine by the use of an ALIFAX HB&L Uroquattro incubator followed by MALDI Biotyper identifaction

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Direct identification of bacteria from infected urine by the MALDI Biotyper (MBT) has been shown previously. To enable quantification as well as identification, both necessary for routine diagnostic, we have tested the combination of an automated quantification system, the Uroquattro (ALIFAX HB&L), with the MALDI-TOF workflow.

161 urine samples from routine were analysed in duplicate. For the novel combined workflow, a 500 µl aliquot was transferred into a 2ml HB&L tube with nutrient broth and stirrer, placed into the Uroquattro for 3h at 37°C and CFU value was read. Then, 500 µl aliquots from the positive vials were pipetted onto a 100 µl Ficoll-Paque Plus cushion and centrifuged (5 min at 15000 g in a 1.5 ml Eppendorf cup). Supernatant was discarded and part of the pellet transferred onto a MALDI target, air dried, overlayed with matrix solution (HCCA) and subjected to identification by the MBT. The approach was compared to our standard workflow: plating the urine on 2 Petri dishes in a quantitative way, MBT identification of colonies after over night incubation at 37°C.
Bacterial count above 10E4 CFU was obtained for 77 of the 161 samples. These patients where considered to have a bacterial infection. Bacterial count was identical to a large extend with both methods. However, MBT identification was only possible in 63 of the samples incubated in the HB&L. Here, results matched those of the standard protocol. Non-identifications were due to the presence of more than one pathogen (shown by the standard workup) which the current MBT software cannot resolve.
The protocol enables reliable bacterial identification together with a bacterial count in few more than 3 hours in cases of mono-bacterial infection. Ongoing improvement of the MBT identification algorithms will resolve the problem of poly-bacterial infections. Extension of the approach to usually sterile body fluids, tracheal secretions and pleural aspirates may also be possible.

DVV11
Chip based point-of-care analysis for the detection of epizootics - Autonomous epizootic lab-on-a-chip system

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The proceeding global trade of live stock increases the risk of spreading epizootic and other pathogens, so diagnostic methods and tools are needed that enable a fast point-of-care analysis. These novel methods would be a substantial improvement, because the testing and diagnosis can be done on-site without the time consuming transport and analysis of these samples in highly specialized laboratories. Our aim is the development and realization of a device for a point-of-care analysis of epizootic diseases and selected plant pathogens. The device combines two chip-based platforms which is a first step on the way to an integrated system for bimolecular analysis: a chip-based PCR and a chip-based hybridization. The latter carried out on the DNA-chip with electrical detection. The PCR-chip represents a miniaturized form of classical thermocyclers and enables a fast and sensitive amplification and labeling of specific DNA sequences with minimal space and energy requirements. The detection and identification of the PCR products is performed on the DNA-chip with an electrical detection scheme. For the electrical readout scheme specifically modified chips with electrode gap structures are used. If target DNA binds, silver can be deposited and the metal deposition bridges a gap between the electrodes. This silver deposition can be read out by a simple conductivity measurement. The electrical readout of biochips is a very robust and cost efficient technique and it enables the construction of small portable analysis devices. The combination of the two technologies enables a very fast and locally highly specific sequence-based detection and differentiation of pathogens. Further it combines the accuracy of sequence analysis with the speed of arrays. In addition the detection of various strains in a single experiment is possible. The DNA-chip-analysis system is automated and provides a novel, robust and compact technology for the identification of epizootic and plant pathogens for the use in point-of-care in the future.

DVV12
Direct Identification of Uropathogens in Urine Samples with MALDI-TOF-MS

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Introduction: Urinary tract infections (UTI) are the most common nosocomial infections worldwide and a major health concern due to complications and frequent recurrence. Standard methods for identification of uropathogens depend on the incubation of agar plates and take often more than one day. The initial non specific antibiotic treatment is one reason responsible for increasing rates of antibiotic resistance and complications. Our intention is to test the viability of MALDI-TOF-MS as a time saving method for the direct identification of uropathogens in urine.
Methods: We use urine samples from patients after bone marrow transplantation or surgical intervention of the bladder from the clinical routine lab that undergo standardized identification methods. The bacterial count is controlled microscopically to be in the range of 10ε-10ζ/ml. The urinary sediment consists not only of bacteria but very often also of erythrocytes, leukocytes, cell detritus and protein casts as contaminants. For separation of the uropathogens we use centrifugation followed by filtration of the supernatant. The filter residue is then eluated and transferred onto the spot of a metal plate. After inactivation with 2,5-dihydroxybenzoic acid the protein spectra are aquired with a MALDI-TOF-MS (Voyager-DE^{TM} STR, AppliedBiosystems). For identification the spectra are compared with a database (SARAMIS^{TM}, AnagnosTec). The whole process is finished within 30 minutes.
Results: After identification with MALDI-TOF-MS we compare our results with those obtained with VITEK^{TM} or gaschromatography. In more than 80 % both results are identical. The best scores of identification are achieved when the bacterial count is high with very few contamination. In many samples the identification score is below 90 %. This is the case when the patient was treated with antibiotics or chemotherapy.

ECP01
Geomicrobiology of High Saline Mine Tailings in the Desert Atacama, Chile

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A marine shore copper mine waste tailings deposit in arid climate at Chañaral, Chile was studied to understand the influence of high salinity on the microbial community composition and biogeochemical processes in this extreme environment. Samples were taken from the oxidized zone at five sites up to a depth of about 1 m and had a paste pH in the range of 2-8.
The microbial community of the mine tailings deposit was quantitatively analyzed using different methods: 1) total cell numbers by SYBR Green II direct counting, 2) quantitative, real time PCR, 3) most probable number cultivation of acidophilic Fe(II)-oxidizers. In parallel, novel, acidophilic halotolerant Fe(II)-oxidizing microorganisms were enriched at salt concentrations of up to 1 M. The results showed that *Bacteria* dominated over *Archaea* in the mine tailings site. The acidophilic Fe(II)- and/or sulfur-oxidizing *Acidithiobacillus spp.* dominated over the acidophilic Fe(II)-oxidizing *Leptospirillum spp.* among the Gram-negative *Bacteria* . The acidophilic Fe(II)- and sulfur-oxidizing Gram-positive *Sulfobacillus acidophilus* was generally less abundant. Total cell numbers were in the range of 10^4 - 10^8 cells g^{-1} tailings. Cultivable acidophilic Fe(II)-oxidizing microorganisms were present in numbers of up to 10^4 cells g^{-1} tailings. The average potential pyrite oxidation rate measured by microcalorimetry was -0.32 µg pyrite Kg^{-1} tailings s^{-1}. Currently, the biomining potential of the enrichment cultures is tested by carrying out bioleaching experiments. An efficient chalcopyrite oxidation at high salt concentration would allow copper production by biomining with seawater.

ECP02
Trichoderma metagenomics and kin interactions

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In this study the *in situ* molecular ecology of a mycoparasitic fungus *Trichoderma* (teleomorph *Hypocrea*, Hypocreales, Ascomycota, Dykaria) in a vertical profile of the Danube floodplain soils was approached. Using metagenomic methods we found that up to a dozen *Hypocrea/Trichoderma* species can coexist in one gram of soil. The *in vitro* modeling of possible infrageneric interactions showed that some species compete with one another while others behave synergistically. Our data suggest that the synergistic behavior of some mycoparasitic species should be considered in a screening for *Hypocrea/Trichoderma* agents of biological control of pests.

ECP03

Active microbial communities in gas reservoirs in the North German Plain and the effects of high CO_2 concentrations

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From the IPCC report on global warming, it is clear that large-scale solutions are needed to reduce emissions of greenhouse gases. The CO_2 capture and storage offers one option for reducing the greenhouse gas emissions. Favourable CO_2 storage sites are depleted gas and oil fields and thus, are currently investigated by the BMBF-Geotechnologien RECOBIO 2-project. Our study is focusing on the direct influence of high CO_2 concentrations on the autochthonous microbial population and environmental parameters (e.g. nutrients).
The gas field "Schneeren" in the North German Plain is operated by Gaz de France SUEZ E&V Deutschland GmbH. The conditions in the reservoir formation waters of two bore wells differ in various geochemical parameters (pH, salinity and temperature). In previos studies the community of this gas field was described by Ehinger et al. 2009. Based on these results our study included cultivation and molecular-biological approaches.
The microbial activity of two wells differed clearly in the inducible activity after substrate addition. The fluids of well A showed a high methane production rate after the addition of methanol or acetate. Well B showed a high sulphide production after the addition of sulphate and hydrogen. The molecular biological analysis of the original fluids supports the activity profile for both sites. The community analysis via real-time PCR showed for the production well A higher abundances for Archaea than for B. The community at site B in contrast was dominated by Bacteria.
Fluids of both wells were also incubated with high CO_2 concentrations. These enrichments showed a significant decrease of methane and sulphide production with increasing CO_2 levels. Currently, the community composition is analysed to identify changes connected to increased CO_2 concentrations. This will provide information about possible biogeochemical and microbiological changes during and after the storage of CO_2, and their effects on the reservoir formation.

ECP04

Seasonal and vertical dynamics of ammonia-oxidizing microbial communities in a eutrophic and in a neighbouring oligotrophic freshwater lake

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Ammonia oxidation, the first and rate-limiting step of nitrification, is a key process of nitrogen cycling and is carried out by ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA). Here, we compared early spring and mid-summer distribution patterns of AOB and AOA in the water column of a eutrophic and a neighbouring oligotrophic lake. The objectives of this study were (i) to investigate the effect of lake trophic status on the abundance of AOA and AOB and on their community composition and (ii) to analyze spatio-temporal patterns of ammonia oxidizer communities including their association with suspended particles. Vertical gradients of oxygen and nitrogen compounds suggested differences in nitrogen cycling between the eutrophic and the oligotrophic lake. Quantitative PCR targeting bacterial and archaeal *amoA* genes revealed distinct depth-dependent distribution patterns for the two groups of ammonia oxidizers in the water column with AOB dominating the ammonia-oxidizing community in the deeper water layers at decreasing oxygen and increasing ammonium concentrations. A large fraction of the AOA population was associated with particles larger than 13 µm. Community composition of AOA and AOB showed clear differences between the oligotrophic and the eutrophic lake. Our results suggest distinct distribution patterns of AOA and AOB in the water column of stratified lakes that could be linked to an ecological niche differentiation of the two groups. The contribution of each group to *in situ* ammonia oxidation remains to be investigated.

ECP05

Nickel sulfide precipitation of a sulfate-reducing consortium originated from a metal contaminated creek soil in the former uranium-mining district Ronneburg

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Uranium mining activity at the former district Ronneburg (Thuringia, Germany) led to acidic, sulfate and heavy metal enriched leakage water, which contaminates the surrounding aquifers. Sulfate reducing prokaryotes (SRP) can affect the mobility of metals directly via reduction/oxidation reactions or indirectly via immobilization as metal sulfides. Consequently, the activity of SRP may decrease the input of contaminants into adjacent ecosystems. The objectives of this study were (1) to characterize heavy metal tolerant SRP in the contaminated creek soil and (2) to investigate the influence of SRP on metal precipitation. Sulfate-reducing activity that was measured by the $^{35}S\text{-}SO_4^{-2}$ tracer technique was only observed in water saturated reduced horizons. The highest rate was 142 nmol cm^{-3} day^{-1} at *in situ* sulfate concentrations. The horizon with the highest total sulfur (2%) and heavy metal concentrations was used for investigating the diversity of the sulfate-reducing community by amplifying, cloning and sequencing the functional gene marker gene *dsrAB* (dissimilatory bisulfite reductase). *dsrAB* clones grouped within the families *Desulfobacterales* (66% of total clones), *Desulfovibrionales* (2% of total clones) and *Syntrophobacterales* (17% of total clones) within the *Deltaproteobacteria* and the *Clostridiales* (15% of total clones) within the *Firmicutes*. Resident SRP growing in a modified Postgate medium were resistant to several heavy metals, such as Ni, Zn, and Co, and a stable enrichment culture selected on 22 mM $NiCl_2$ could be obtained. Analysis of consortial 16S rRNA sequences indicated the presence of *Desulfosporosinus* sp. and potentially *Citrobacter* sp. as sulfate-reducing bacteria. The experimental formation of amorphous and nanocrystalline nickel sulfides by the consortium was confirmed via high resolution TEM and EDX analysis. Surprisingly, α-NiS with a grain size of about 5 nm could be identified that is thermodynamically stable only at temperatures higher than 280°C. Although the Ni-enriched microcosms represent an artificial ecosystem these experiments demonstrate the effect of SRP on biomineralization. Our results indicate that an active and diverse community of SRP is present in contaminated creek soils, which may influence metal mobility through sulfide formation.

ECP06

Exploring the role of sulfate-reducing bacteria associated with methanotrophs of the ANME-2 clade in the anaerobic oxidation of methane

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The anaerobic oxidation of methane (AOM) with sulfate as terminal electron acceptor is mediated by consortia of methanotrophic archaea (ANME) and sulfate-reducing bacteria (SRB). ANME are not monophyletic and form three clades (ANME-1, ANME-2 or ANME-3) within the methanogenic archaea. Depending on the ANME clade, the associated SRB have been identified to be closely related to the deltaproteobacterial *Desulfosarcina*/*Desulfococcus* group or the genus *Desulfobulbus*. In contrast to the ANME clades which have been repeatedly studied with respect to phylogeny, key genes, and genomic capabilities, little is known about their sulfate-reducing partner. Thus, their exact role in the AOM process still remains to be determined.
In our study we identified SRB associated with the ANME-2 clade to belong to the *Desufosarcina*-related SEEP1a group. Bacteria from the SEEP1a group were detected as the dominant partner of ANME-2 at distinctly different AOM sites. They seem to be highly adapted to their methanotrophic partner as they are mainly detected in ANME-2/SEEP1a consortia and rarely as single cells. Ongoing molecular studies on sediment-free AOM enrichment cultures will reveal the genomic potential of these ANME-2 associated SEEP1a bacteria and will provide some insight into their role in the AOM process.

ECP07
Biofilm formation in halophilic Archaea

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Biofilms and other forms of surface adherence are important life styles of microorganisms. Biofilm formation is not well characterized among Archaea and especially not in the Haloarchaea. For the screening and quantification of adherence a fluorescence-based adhesion assay was developed. Seven out of fourteen halophilic strains showed a firm adhesion to glass and plastic surfaces, including three extremely halophilic *Halobacterium salinarum* strains, three moderately halophilic *Haloferax volcanii* strains and one new halopsychrophilic strain. *Halorubrum* and other *Halobacterium* strains generated a swimming biofilm at the water/air surface due to the large formation of gas vesicles.
One gas vesicle-producing *Halobacterium salinarum* isolate, that showed a strong adherence, was further analysed. The fluorescence-based quantification of adherence showed an at least two fold higher signal compared to other halophilic strains. This isolate is closely related but at the genomic level distinguishable from the well-characterized *Halobacterium salinarum* strains NRC-1, R1 and PHH1. After incubating a glass slide in platonic culture for a day first adherent cells were detected on the surface. Afterwards patches of closed cell layers and additional three-dimensional microcolonies are formed, growing from the bottom to the top. These microcolonies are generated by an accumulation of irregularly arranged single cells and comprise an undefined matrix. Further analyses demonstrated that the cells were surrounded by a large amount of free nucleic acids. In addition a yet undefined glycoconjugate structure was exclusively found within the microcolonies but not with surface adherent cells.

ECP08
Detection of pathogenic *Clostridium* species in fermenter samples fed with energy crops

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Renewable energy projects are granted by governmental aid in many European countries, due to its relevancy for environment and climate protection. In this context, anaerobic digestion of energy crops like maize silage in biogas plants has gained increasing importance in the last decade. During hydrolysis of the substrate representatives of the genus *Clostridium* play an important role in the effective degradation of cellulose-rich fibres. However the genus *Clostridium* represents a diverse group of bacteria, which harbours beside beneficial bacteria also species with a highly pathogenic potential for humans. This might pose a hygienic problem, as after fermentation of the highly cellulosic substrates the nutrient-rich digestate can be applied to fields as fertilizer or as a soil conditioner, to close the nutrient cycle.
The objective of this research was to detect pathogenic Clostridia in fermenter samples originating from two-phase and single-phase plants, fed with maize and grass silage applying molecular methods. Several primer systems targeting 16S rRNA genes of either *C. perfringens* or *C. difficile* published previously to detect respective species in clinical samples were tested for their suitability to specifically detect low cell copy numbers in fermenter samples. To allow efficient amplification conditions for PCR in a first step optimization of DNA extraction from different fermenter samples was performed to obtain high quality DNA. A nested PCR approach was established for the specific amplification of *C. perfringens* and *C. difficile* rRNA genes. Results of the approach applied to different fermenter types and loads are presented here and were verified by sequence determination of the retrieved amplification product.

ECP09
Characterization of the microbial communitiy in a biogas production plant

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The resident microbiota was analyzed in a mesophilic, continuously operating agricultural biogas plant supplied with food leftovers, maize silage and pig liquid manure. The dominating archaeal and bacterial community members were characterized by two different 16S rRNA culture-independet approaches. First, prokaryotic 16S rRNA clone libraries containing 62 bacterial and 38 archaeal clones were constructed from genomic DNA preparations. The 16S rRNA molecules were compared by restriction fragment length polymorphism (RFLP) and dominant 16S rRNA (1500 bps) molecules were sequenced in full length. Phylogenetic tree estimation was done using the software MAFFT (Multiple Alignment using Fast Fourier Transform) for multiple sequence alignment and MrBayes 3.1.2 for Bayesian inference. The bacterial microbiota in the biogas plant was dominated by the phylum *Firmicutes* with representatives from the families *Lactobacillaceae*, *Erysipelotrichaceae*, *Thermoanaerobacteraceae* and *Ruminococcaceae*. The phylas *Chloroflexi* and *Bacteroidetes* were also present represented by the families *Anaerolinaceae* and *Porphyromonadaceae*. The diversity of archaea was quite low, with *Methanoculleus* and *Methanosarcina* as dominating methanogenic genera.
Second, the microbial community in the biogas plant was analyzed using denaturating gradient gel electrophoresis (DGGE). Therefore, genomic DNA was prepared from samples drawn within over a period of one year. With the identification of the dominating archaeal and bacterial community members based on RFLP and sequencing data an internal classification standard was developed for DGGE analysis. A mixture of classified 16S rDNA molecules obtained from the archaeal and bacterial clone libraries was selected to run in DGGE as reference for different genomic DNA preparations from the biogas plant microbiota.

ECP10
Seasonal and vertical dynamics of microbial communities in two contrasting freshwater lakes in Northwest Germany

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In this study, we follow vertical profiles of key physicochemical parameters and vertical patterns of microbial communities during mixis and stratification phases in two freshwater lakes. Comparative investigations are carried out in a eutrophic and a neighbouring oligotrophic, slightly humic lake situated in the nature reserve "Heiliges Meer" in Northwest Germany, addressing three hypotheses:
(1) Differences in trophic status and pH result in different vertical patterns of microbial community composition of the two lakes.
(2) Seasonal dynamics of physico-chemical parameters strongly affect the vertical distribution of microbial communities.
(3) Lake trophic status has a special effect on the composition and activity of microbial communities involved in the transformation of nitrogen, a key parameter of lake trophy.
Total cell counts using SYBR Green II revealed higher dynamics of microbial abundances in the eutrophic compared to the oligotrophic lake. Denaturing gradient gel electrophoresis indicated differences in the microbial community composition between the two lakes. Moreover, vertical patterns of the microbial communities showed a clear correspondence to profiles of key physico-chemical parameters. DGGE-based analysis of microbial communities will be complemented by clone library analysis. Spatio-temporal patterns of the abundance of identified key phylogenetic groups will be analyzed using fluorescence *in situ* hybridization.

ECP11
Direct molecular detection of environmental *Burkholderia pseudomallei* in soil from endemic areas

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Burkholderia pseudomallei, the causative agent of melioidosis, is a soil-dwelling Gram-negative bacterium. Although the bacterium has been known for a century, its worldwide distribution as well as its natural lifestyle still remains uncertain. The environmental surveillance of *B. pseudomallei* mostly relies on culture-based methods. However, these approaches lack sensitivity and, therefore, low numbers of *B. pseudomallei* will likely remain undetected. To overcome this limitation, we established DNA isolation and purification protocols for soil samples allowing direct quantification of *B. pseudomallei* cells by quantitative PCR (qPCR) without need of an initial cultural enrichment step. The problem of PCR inhibitors co-extracted with soil DNA was solved by purifying DNA embedded in agarose and adding non-acetylated bovine serum albumin (BSA) to qPCR. Quantification of *B. pseudomallei* genomic copies in the soil DNA extracts was carried out by a qPCR assay targeting for the type three secretion systems 1 (TTSS 1) gene. An internal amplification control was constructed to monitor for possible PCR inhibition and to exclude false-negative PCR results and inaccurate quantifications. Specificity of the assay was tested on 29 different *B. pseudomallei* isolates (all positive) and, as a control, on 70 isolates phylogenetically closely related to *B. pseudomallei* (all negative). The detection limit was as low as 10 *B. pseudomallei* cells per gram soil. Culture-negative soil samples from northern Vietnam displayed amplification in our qPCR indicating the presence of *B. pseudomallei* at concentrations below the detection limit of culturing methods. The presented assay provides both a highly specific and highly sensitive tool for environmental monitoring of *B. pseudomallei*.

ECP12
Disentangling chemical antagonisms in microbial biofilms associated with the marine macroalga *Ulva australis*

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Marine microorganisms are versatile producers of secondary metabolites. Although the sea has yielded thousands of bioactive metabolites over the past two decades, we are only beginning to explore the natural functions of these molecules. Many microorganisms exist in the environment as multicellular communities, so-called biofilms. Many plants and animals are defended from their consumers by inhibitory secondary metabolites, which in the marine environment are very common among sessile organisms. An emerging hypothesis is that sessile bacterial communities organized as biofilms are chemically defended against predation by bacterivorous protists.
We use the biofilm community associated with the marine macroalga *Ulva australis* to unravel the complex web of chemical crosstalk in biofilms mediating microbial symbiosis, competition and defense against predators and pathogens. By testing feeding selectvity, growth and survival of bacterivorous protists on a diverse set of bacterial isolates, we find evidence that chemically mediated resistance against predators is common among biofilm bacteria. Using bioassay-guided chemical and genetic analysis, we have identified antiprotozoal compounds and their producers that effectively interfere with phagocytosis and redox homeostasis of the protist cell. Such chemical defenses are found to contribute to the successful persistence of biofilm bacteria and to determine the underlying population dynamics.
Taken together, chemical communication is an essential part of the way in which biofilm populations coordinate their behavior and respond to environmental challenges. Understanding the molecular basis of biofilm interactions in their ecological context bears the potential of refining natural product discovery and the development of biofilm-derived biotechnologies.

ECP13
Analysis of microbial communities around gas wells and in the reservoir in a long-term used gas field

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As part of a joint research project microbial communities in the area of the second largest natural gas field in Europe in the Altmark, Germany are analyzed. The Altmark gas field operated by GDF SUEZ E&P Germany GmbH is located at the southern edge of the Northeast German Basin. The reservoir horizons belong to the Permian Rotliegend formation (Saxon) and have an average depth of about 3300m. CO_2 will be injected to enhance the recovery of gas in this with conventional extraction methods nearly depleted gas field (Enhanced Gas Recovery–EGR, BMBF project CLEAN).
Microbiological analyses are used to supplement a continuous gas monitoring program at the soil surface above the EGR-site. Microbial production and consumption of CH_4 and CO_2 are determined together with the carbon isotopic compositions to separate these indigenous biological activities from possibly upward migrating reservoir gases including CO_2. The $\delta^{13}C$ of CO_2 collected *in situ* was similar to those in incubations, confirming a biological origin. Archaeal cell numbers were approximately one magnitude lower than bacterial cell numbers. In all samples the total number of detectable microorganisms was high in contrast to a generally low activity for CO_2 and CH_4 production and oxidation.
For monitoring of the deep reservoir microbiological and isotopic analyses are used to investigate the microbial community before and after injection of CO_2. The $\delta^{13}C$ of CO_2 and CH_4 collected *in situ* in production waters indicate a thermogenic origin. High cell numbers for bacteria and archaea were detected in production waters from different wells. In contrast microbial activities for CO_2 and CH_4 production and oxidation were relatively low. So far microbial activities in reservoir fluids collected with *in situ* samplers at 3512m depth could not be determined in this hypersaline (salinity of 400 per mill) and hot (around 120°C) environment.

ECP14
Microbial enrichment populations with manganese(II) originating from the South Pacific Gyre sediment

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The deep-sea sediment in the South Pacific Gyre is the most oligotrophic habitat on earth. The sea floor is covered with manganese nodules, a product of aerobic manganese oxidizing bacteria. During the Knox02RR cruise through the South Pacific Gyre, sediment samples were taken from the first five centimetres of the deep sea sediment with an estimated age of up to 6.1 million years. Oxic slurries were inoculated into artificial sea water containing manganese and acetate as electron donor and were incubated for 21 months at 4°C. Microbial communities in the enrichment cultures were characterized by a combined approach using T-RFLP and clone libraries. The T-RFLP analysis revealed a large community change with acetate as electron donor, but not with manganese(II). Analyses of bacterial 16S rRNA gene sequence clone libraries revealed population affiliated within the genera *Sulfitobacter*, *Stenotrophomonas*, *Salegentibacter*, *Marinobacter*, *Halomonas*, *Pseudomonas*, *Idiomarina*, *Alcanivorax*, *Rhizobium* and *Methylophaga*. The closest relatives had previously been reported from cold marine waters and deep-sea sediments, including some close relatives with the ability to oxidize manganese. The clone library from the manganese enrichment culture was in comparison to a control culture with only endogeneous electron donors enriched in phylotypes affiliated with the genera *Flavobacteriaceae*, *Rhizobialis*, *Alcanivorax*, *Marinobacter* and *Methylophaga*.
In silico predicted terminal restriction fragments of clone library sequences were related to operational taxonomic units (OTU) to calculate the relative abundance of phylogenetic groups. The terminal restriction fragments enriched in the manganese(II) culture coincided in size with the *in silico* predicted fragments of the aforementioned phylotypes.

ECP15
Anaerobic oxidation of arsenite by the phototroph *Chloroflexus aurantiacus*

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On early earth, a lack of electron acceptors was a major hurdle for evolving life. Since free oxygen was absent on the young Earth and Mars, light may have served as an oxidant. To demonstrate the anaerobic oxidation of arsenite under light, *Chloroflexus aurantiacus* was incubated under photoheterotrophic conditions. *C. aurantiacus* possesses genes encoding arsenite oxidase and was able to oxidise arsenite exclusively under anaerobic conditions in the light. Arsenite was also oxidised under aerobic conditions abiotically, but at significantly lower rates. Furthermore, arsenite oxidase activity was detected in cell free extracts using DCIP as the electron acceptor. Although green filamentous bacteria (e.g., *Chloroflexus*) and green sulfur bacteria both utilise the same light harvesting structure, the chlorosome, their photosystems are significantly different. *Chloroflexus* has a photosystem more reminiscent of that found in purple bacteria (e.g., Photosystem II) with Bacteriochlorophyll a (870 nm) in the reaction centre. Whether this impacts the ability to couple arsenite oxidation especially in the green sulfur bacteria needs further investigation. However, the results obtained here suggest that *Chloroflexus* is capable of using As(III) as an electron donor in anoxygenic photosynthesis. Thus, it is quite conceivable that microbial mat communities in the Archean on Earth used As(III) as an electron donor for photosynthesis, providing a local source of As(V) prior to the advent of atmospheric oxygen. That this also occurred on early Mars is quite possible as well.

ECP16
SIP-tracking of protein synthesis in anaerobic methanotrophic communities

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Black Sea microbial mats, capable of anaerobic oxidation of methane (AOM), were investigated to identify functional proteins, to shed light on the metabolic diversity in this consortium. We used ^{13}C and ^{15}N labelled substrates to follow their incorporation into functional proteins such as the mcrA-reductase (MCR) and the APS-reductase (APR). Both enzymes are believed to be key enzymes in anaerobic methanotrophy coupled to sulfate reduction. While the MCR is assumed to catalyse the initial methane attacking step, the APR is involved in the second step of sulfate reduction. For this, homogenates of ANME-1 or -2 dominated mats were fed with ^{15}N labelled ammonia, nitrate or dinitrogen. All substrates were incorporated in the bulk biomass in amounts of up to several atom percent during the incubation time of six months. Dinitrogen fixation could be observed only at lower levels. MCR subunits were the most prominent proteins in all incubations. In contrast, APR subunits were completely absent in incubations with nitrate as electron acceptor instead of sulfate. Using FPLC followed by elemental analysis, nitrogen incorporation into selected MCR-dominated fractions reached $\delta^{15}N$ values of 1758‰ for ANME-2 dominated black mats and 9‰ for ANME-1 dominated pink mats. The more active ANME-2 mats incorporated nitrogen substrates faster than the pink mats, where almost no incorporation was observed. RNA-based molecular analyses confirmed the presence of active nitrogen cycling microorganisms. The combination of different stable isotope labelled substrates thus allows us to identify key microbial processes and players in the complex mat systems, and to track the flow of carbon and nitrogen throughout the AOM community.

ECP17
Enhanced methanogenesis from hexadecane and ethylbenzene under non-methanogenic conditions

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Methane formation upon hydrocarbon degradation may provide access to remaining, but yet inaccessible petroleum reservoirs. Moreover, methanogenesis could remove toxic hydrocarbons from contaminated aquifers and sediments. Therefore, sediment samples from a contaminated sea port basin in Zeebrugge (Belgium) were investigated to assess the *in situ* potential for methanogenic hydrocarbon degradation. Since this process is believed to be a sequential syntrophic procedure, non-methanogenic conditions were created in sediment microcosms to facilitate the first hydrocarbon attacking step. To achieve this, a high electron potential was created by the addition of ferrihydrite, manganese oxide, nitrate or sulfate as electron acceptors. Hexadecane, ethylbenzene or naphthalene were used as model substrates. Methanogenesis evolved rapidly from set ups treated with iron and manganese, but not nitrate, reflecting the *in situ* conditions at the site. On sulfate, methanogenesis was neither inhibited nor supported. Methane formation rates were the highest with hexadecane as substrate, followed by ethylbenzene and naphthalene. Methane was removed in high rates at the same time by anaerobic methanotrophs. The microbial community *in situ* and *in vitro* was dominated by members of the Geobacteraceae. Their methanogenic partners were quantified, targeting the genes encoding for the methyl coenzyme M reductase (*mcrA*). Methane consumption in the microcosms and the presence of methanotrophic anaerobes belonging to the ANME-1 and ANME-2 clusters suggest anaerobic methanotrophy as an accompanying process. *mcrA* genes belonging to the ANME-1 & -2 clusters were detected in lower copy numbers than the methanogenic *mcrA*, which is in good agreement with the activity measurements. In summary, the addition of alternative electron acceptors enhanced synthrophic, hydrocarbon removal by methanogens.

ECP18
New insights in herbicide-toxicity in Cyanobacteria

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Ammonia is the preferred nitrogen source in Cyanobacteria. It is assimilated by the cell via the GS/GOGAT-cycle, in which ammonia is transferred on the carbonskelleton of 2-oxoglutarate, resulting in the formation of glutamate, the precursor of all other nitrogen containing compounds within the cell. However, in high concentrations, ammonia can be toxic. Inhibiting the activity of the ammonia-assimilating enzyme glutamine synthetase (GS) with an analogue of glutamate, methionine-S-sulfoximine (MSX), leads to excretion of ammonium, when the cells are utilizing nitrate. GS-inhibitors are widely used class of herbicides. Their toxic effect has been ascribed to intoxication with ammonia.
In our experiments we used the unicellular strain *Synechococcus elongatus* PCC 7942 and the filamentous strain *Anabaena variabilis* 7120. We treated the cells with different concentrations of MSX and determined the parameters ammonia excretion, GS-activity, viability and growth. According to our data, the assumption, photosynthetic organisms die of intoxication with ammonia, when treated with a GS-inhibiting herbicide, is apparently invalid: The excreted amounts of ammonia cannot be responsible for the loss of viability, because they are lower than the amounts that are commonly used to feed the cells with ammonia. Furthermore, the data did neither support a direct correlation between ammonia excretion and loss of viability nor a correlation between the loss of GS-activity and loss of viability. The drop of GS-activity occurs at low concentrations of MSX, whereas the toxic effects become evident only at much higher concentrations: Therefore, the drop of viability is independent of the inhibition of the GS. These results strongly imply, that there must be another target for MSX, which is ultimately responsible for lethality of this compound. Further investigations shall reveal the true mechanism of MSX-toxicity.

ECP19
Carbon fluxes in anaerobically methane-oxidising microbial mats revealed by Stable Isotope Probing – SIP

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The anaerobic oxidation of methane (AOM) is an important process within the global methane cycle. AOM is conducted by consortia of anaerobic methanotrophic (ANME) archaea and sulphate-reducing bacteria (SRB) although our recent metagenomic investigations demonstrate a high microbial diversity in AOM-settings. Little is known about the metabolic process in AOM performing consortia since none of the involved organisms has been isolated in pure culture so far.

Several molecular biomarkers have been identified, which are diagnostic for different members of such a microbial community and some also hint to the metabolic strategy of individual members.
Consequently, one promising strategy to get information on the functioning of such microbial systems is to use ^{13}C-labelled substrates (stable isotope probing: SIP) and follow the uptake into cell biomass and individual lipids. Previous *in vitro* studies showed uptake of methane carbon into archaeal and bacterial lipids and indicated autotrophic growth of the sulphate-reducing bacterial partner. However, many aspects of AOM performing consortia are still insufficiently understood such as individual responses of distinct ANME-community members, additional metabolic activities as well as the role of recently discovered bacteria in AOM associations.
We therefore performed stable isotope probing experiments with ^{13}C-labelled methane, acetate and bicarbonate with AOM communities from the Black Sea, dominated by ANME-1 or ANME-2 consortia.
First results of our ^{13}C-methane experiments demonstrate different ^{13}C-changes for individual lipids. Moreover, methane-oxidising activities and ^{13}C-uptake rates between ANME-1 and ANME-2 dominated communities were different, with highest rates and ^{13}C-uptakes into biomass and lipids of the latter. We also observed ^{13}C-uptakes into biomarkers of bacteria so far not known to be involved in AOM (e.g. Chloroflexi).

ECP20
Acid shock triggers heavy metal detoxification in *Listeria monocytogenes*

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L. monocytogenes is a food borne pathogen ubiquitous in the environment. The bacterium is exposed to low pH in various ecological niches, such as the stomach, macrophages or acidic soil. Acidic conditions are also found in ecological niches associated with food production.
In this work, the acid shock response at pH 5 for 15 min, 30 min, 45 min, 60 min and 120 min and the acid adaptation at pH 5,2 (growth to mid log-phase) was studied at the transcriptional level using microarrays. Besides transcriptional induction of well characterized genes which are involved in acid tolerance, an up regulation was also found for genes whose products are potentially participating in heavy metal detoxification, such as ion export proteins (heavy metal exporting ATPase: *lmo0641*, *lmo1852-1854*; cation efflux protein: *lmo2231*, *lmo2575*) or heavy metal reduction proteins (arsenate reductase: *lmo2230*). Furthermore, it was demonstrated that, after induction of the acid tolerance response (60 min, pH 6) *L. monocytogenes* exhibits a higher resistance against various heavy metal ions, e.g. against Cd^{II}, Hg^{II}, and Ni^{II}; and especially against Cu^{II} and As^{V}.
Acidic pH appears to serve as a trigger not only for the induction of genes involved in acid tolerance itself, but also for the induction of genes involved in heavy metal resistance. Since metals become more bioaccessible at low pH, especially in soil, acid solubilized heavy metals might reach noxious doses in certain environments and, therefore, detoxification genes may be up regulated. Ongoing analysis of deletion mutants indicates that the upregulated genes indeed are connected to heavy metal resistance.

ECP21
Changes in the bacterial community during the iron fertilization experiment LOHAFEX

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Changes in the bacterioplankton community during the iron fertilization experiment LOHAFEX in the South Atlantic were investigated using Catalyzed Amplified Reporter Deposition Fluorescence *in situ* Hybridization (CARD FISH) and automatic cell counting. In response to the iron fertilization a *Phaeocystis* sp. dominated algal bloom covering an area of approximately 300 km^2 was induced and closely monitored over the course of 38 days after iron addition. In order to cover the three main groups of marine bacteria *Alphaproteobacteria*, *Gammaproteobacteria* and *Bacteroidetes*, 9 CARD FISH probes were used in nested approaches. Furthermore the automatic cell counting method developed by Pernthaler (2003) was used for the investigations. A review of this method will be reported in this study. The total cell numbers increased only slightly after the iron fertilization, but increased similarly outside the fertilized patch. Within the *Bacteroidetes* a minor decrease was found during the experiment. The genus *Polaribacter* within the *Bacteroidetes* showed no response to the iron fertilization. Similar findings were obtained for the *Gammaproteobacteria*, whose abundance did not change significantly inside or outside the patch. *Roseobacter*, the second most abundant alphaproteobacterial group, showed only minor changes but these changes were not mirrored by changes in roseobacterial subclades, the RCA or the NAC11-6 clade. However the alphaproteobacterial clade SAR11 increased during the first days of the experiment and decreased after day 21. Our findings point at a grazer controlled system, in which the small cell sized SAR11 increased, while all other groups were controlled by top-down specific or unspecific grazing.

ECP22
Microbial communities of marine methane seeps at Hikurangi Margin (New Zealand)

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In recent decades as research has shed ever more light on the deep-sea a hitherto sleeping giant has been awoken. Deep-sea sediments have not only turned out to be far more diverse and complex than previously assumed, but also more significant in terms of biomass and turnover rates. Microbial activity in marine sediments greatly influences global carbon cycles and therefore plays a role in future efforts to estimate the impact of marine ecosystems on climate change. The microbially mediated anaerobic oxidation of methane (AOM) is a significant process in the global carbon cycle and a major sink for the greenhouse gas methane on earth. Cold seeps where methane leaves the seafloor and enters the water column are main sites of AOM.
In this study, we characterized the microbial community structure of different methane seep sites from recently discovered cold seeps at the Hikurangi Margin, northeast of New Zealand. The sites varied by their seep-associated faunal communities (siboglinid tubeworms or polychaetes), presence of filamentous mat-forming sulfide-oxidizing bacteria on top of the sediments and spatial scale. The site inhabited by polychaete worms is of special interest because polychaetes have not been discovered yet to be associated with methane anywhere else. Using fluorescence in-situ hybridization (FISH), automated ribosomal intergenic spacer analysis (ARISA), and 16S rRNA clone libraries we investigated the influence of spatial scaling, methane flux, methane oxidation rates, and surface population on the microbial diversity at these sites.
Preliminary results indicated marked differences between the sampling sites, e.g. in AOM rates or abundance of methanotrophic communities. Data will be correlated to previously generated biogeochemical, geographical and physical data.

ECP23
Towards an improved understanding of trophic connectivities in belowground microbial food webs

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The flow of carbon and energy through natural systems is largely controlled by organisms engaged in complex trophic interactions. Although such food webs have been intensively studied for higher organisms, involved microbes are mostly treated as a black box. Thus, the quantitative flux of carbon and nutrients from primary resources into different functional groups within microbial communities, between them, and from them to other trophic levels (fungi, protists, microfauna) is poorly understood.

Here, we aim to identify important trophic connectivities of microbes in a model belowground food web, and to quantify the fluxes of carbon through them. Stable isotope tracers are used at both natural abundance and under ^{13}C labelling. An agricultural soil depending on plant derived carbon inputs was chosen as model system. Identification of key microbial groups and connectivities in the belowground food web is done by stable isotope probing (SIP) after pulse labelling of plants with ^{13}C-CO_2 in mesocosm. To unravel the importance of identified microbial populations in the field, spatial and temporal distribution of bacterial communities dependent on season, depth and plant influence are examined via T-RFLP fingerprinting, sequencing, as well as quantitative community analyses. In the soil, bacterial communities displayed surprising diversity and stability, but significant distinctions regarding depth and season were identified using multivariate statistics. This was also true for rRNA-based community analyses. Next, microbes specifically active in channelling of fresh C-inputs into the belowground food web will be identified by SIP, and the turnover of the respective trophic connectivities will be deducted via population-based food web models. Thus, a quantitative understanding of belowground food web functioning will be provided, which will be extremely valuable to predict carbon fluxes and storage in terrestrial ecosystems also under changing environmental conditions.

ECP24
Plant diversity increases soil suppressiveness by fostering biocontrol pseudomonads

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Some soils are naturally disease suppressive which is due to the presence of specific bacteria producing exometabolites antagonistic to soil-borne pathogens. Therefore these bacteria present a great potential as biocontrol agents. To turn this to account the environmental factors affecting their abundance and activity need to be understood.
We investigate the effect of increasing plant diversity on soil suppressiveness. Increased plant diversity results in a higher microbial biomass and activity in soil, current agricultural practices where crop plants are grown in monocultures therefore may prevent exploiting the full potential of microorganisms as biocontrol agents. We hypothesized that plant polycultures promote the antifungal activity of soil bacteria and therefore contribute to higher soil suppressiveness. We determined this effect by testing the infection of sugar beet seedlings with the fungal pathogen *Rhizoctonia solani* when growing the plants in soil from sites differing in plant diversity. Furthermore, we explored the mechanisms underlying the suppressive effects by analyzing the biocontrol potential of the bacterial community by quantitative PCR targeting the genes responsible for the production of 2,4-DAPG and pyrrolnitrin, two of the most common and potent biocontrol toxins in temperate agricultural soils.
We found out that plant diversity improves protection against soil-borne pathogens by fostering biocontrol bacterial populations. Therefore we propose that plant polycultures play an important role in natural plant protection and that the use of pesticides could be strongly reduced by increasing local plant diversity.

ECP25
Tracking culturability: Detection of genes responsible for starvation resistance in Legionella and Salmonella

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L. pneumophila is the causative agent of legionnaires` disease, a potentially fatal pneumonia and *S. typhimurium* represents one of the most common causes of bacterial foodborne illnesses worldwide. Prolonged starvation of the pathogens under nutrient limitation induces entry into a dormant form, called the VBNC (viable but non-culturable) state. Once entered, bacteria exhibit enhanced robustness towards human decontamination strategies and can not be grown on standard culture media, but maintain their virulence properties. Consequently, VBNC bacteria need to be taken into consideration for culture-based monitoring of bacterial contamination and most importantly for sufficiently antagonizing the threat of infections. Although reversion of VBNC bacteria into a culturable state is successfully achieved for *Salmonella spp*., *Vibrio spp*., *Campylobacter spp*. and others by using supplemented media or altered culture conditions, there is no general concept concerning the genetic basis of the VBNC phenomenon. In an attempt to identify genes important for entering the VBNC state due to starvation stress, we employed EZ-Tn5 mutagenesis to generate randomly mutagenized knock-out clone libraries of L. pneumophila and *S. typhimurium*. Each clone bank was then inoculated to sterilized tap water microcosms and routinely sampled for CFU over one year (Legionella) and 6 months (Salmonella), respectively. EZ-Tn5 mutants showing extended culturability compared to the wildtype were isolated. Transposon insertions in several mutants were mapped to genes involved in bacterial stress defence mechanisms. These include heavy metal- and multidrug efflux transporters, as well as a polyketide synthase gene, potentially involved in antimicrobial compound synthesis in *L. pneumophila.* Here the question how the detected genes contribute to the entry into the VBNC state and therefore to long-term starvation resistance is of major importance. Analysis of the molecular mechanisms necessary for long-time survival of bacteria will be beneficial for understanding and importantly prevention of bacterial persistence in the environment and in infectious diseases. Manipulation of starvation resistance by application of selective, non toxic compounds that resuscitate dormant bacteria into a culturable state may help us to improve culture-based detection of pathogenic bacteria and antibiotic therapies.

ECP26
Fine scale depth-distribution of microbial lineages and aerobic toluene degraders at an oxic/anoxic transition zone in a contaminated aquifer

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Monoaromatic hydrocarbons (BTEX) are widespread, toxic groundwater pollutants originating from petroleum derivatives. Microorganisms are capable to degrade BTEX in order to utilize them as carbon and energy source under different hydrogeological and chemical settings. In fact, biodegradation is the most important process for natural attenuation (NA).
We have previously shown that the distribution of anaerobic toluene degraders as traced via functional marker genes and that of typical iron- and sulphate-reducing lineages is highly correlated to zones of increased anaerobic degradation activity, i.e. the reactive plume fringe underneath an actual contaminant plume. The same can be hypothesised for aerobic degraders, but this still needs to be established. At present, it is not known whether the respective aerobic populations are also established at corresponding biogeochemical intersections, i.e. the oxic/anoxic capillary fringe above the contaminant plume, and how the contribution of aerobic degraders to net contaminant removal compares to that of anaerobes.
Therefore, we explored the fine-scale (2-3 cm intervals) depth distribution of microbial lineages as well as that of aerobic toluene degraders over the capillary fringe of a tar oil contaminated aquifer in Duisseldorf-Flingern. Genetic monitoring strategies based on the fingerprinting and sequencing of ribosomal as well as catabolic gene amplicons were applied. We discovered surprisingly well-defined small-scale distinction in lineage specific distribution patterns of typically aerobic and anaerobic phylogenetic lineages, as well as catabolic genes related to aerobic toluene degradation. Our ongoing research will underline the concept of specific, local, distribution patterns of physiologically distinct degrader populations in contaminated aquifers, and help to understand the controls of ongoing degradation processes in this "special" ecosystem.

ECV01
Methanotroph community structure across a Chinese paddy soil chronosequence

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Given that rice paddies are anthropogenic methane sources and the inevitable need to increase rice production to sustain human population growth, it is pertinent to identify the effects of long term agriculture on the selection of methanotrophs. Methanotrophs play a crucial role in mitigating methane emission from rice paddies. Therefore, we analyzed the methanotroph community along a chronosequence of paddy soils from China covering recently reclaimed sites to paddies under permanent culture since 2000 years.

We observed a steady increase in maximum potential methane oxidation rate (PMOR) with the age of the soil. This mirrors the higher potential for paddy soil methanotrophs to oxidize methane after long term agriculture. Our results also suggest that long term agriculture impose a selection pressure on different groups of methanotrophs. In ancient soil, type Ib methanotrophs were observed to multiply in correspondence with increasing PMOR, while other groups showed a relatively stable community composition as revealed by *pmoA*-based T-RFLP and qPCR analyses in a time course experiment. Besides the cultured methanotrophs; *Methylococcus* and *Methylocaldum*, type Ib also consists of various environmental clusters retrieved from paddy soils. Through cloning and sequence analysis of *pmoA* marker gene, our soil was found to harbour extant methanotrophs, ammonium-oxidizing bacteria and interestingly, sequences affiliated to *Crenothrix polyspora*, a methane oxidizer in possession of an unusual *pmoA*.

ECV02
Stability of the methanogenic community in an acidic fen to experimental drought

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Acidic wetlands are sources of the greenhouse gas methane. A temperature rise as a result of global warming might lead to more extreme weather phenomenons. Thus the methanogenic community of an acidic fen and its reaction to enhanced drought were investigated. Translated amino acid sequences of the structural gene marker *mcrA* (coding for the α-subunit of the methyl coenzyme M reductase) grouped into 21 species-level operational taxonomic units (OTUs), displaying a high diversity of fen methanogens. Some OTUs were related to known *Methanomicrobiales*, *Methanobacteriaceae*, *Methanosarcinaceae*, and *Methanocellaceae*, while 10 OTUs were not closely related to known methanogens, indicating phylogenetic novelty of fen methanogens. An artificial watertable drawdown of up to 60 cm resulted in reduced methanogenic diversity based on the Shannon diversity index while rewetting restored diversity to the original level. Based on qPCR analysis of *mcrA*, methanogens were most abundant in the 20-30 cm soil layer while highest *mcrA* expression was found in the 0-20 cm soil layers. Relative *mcrA* copy numbers were not changed by the artificial drought, but the rewetting event increased relative *mcrA* copy numbers. The level of *mcrA* expression was not affected by artificial drought or rewetting. TFLP analyses revealed high similarity in the methanogenic community structure during the course of the experiment, even though minor changes in community composition due to artificial drought were observed. The TRFLP profiles of expressed *mcrA* differed from those of the DNA profiles. Artificial drought had only small effects on the active methanogenic community.
The collective data indicate that (i) a novel diversity of methanogens occurs in the acidic fen, (ii) the abundance of methanogens in the acidic fen is rather stable under extreme weather conditions, and (iii) artificial drought does not alter *mcrA* expression.

ECV03
New life in old reservoire - the microbial conversion of oil to methane

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Since almost 20 years it is known from stable isotope studies that large amounts of biogenic methane are formed in oil reservoirs. The investigation of this degradation process and of the underlying biogeochemical controls are of economical and social importance, since even under optimal conditions, not more than 30-40 % of the oil in a reservoir is actually recovered. The conversion of parts of this non-recoverable oil via an appropriate biotechnological treatment into easily recoverable methane would provide an extensive and ecologically sound energy resource. Laboratory mesocosm as well as high pressure autoclave experiments with samples from different geosystems showed high methane production rates after the addition of oils, single hydrocarbons or coals. The variation of parameters, like temperature, pressure or salinity, showed a broad tolerance to environmental conditions. The fingerprinting of the microbial enrichments with DGGE showed a large bacterial diversity while that of Archaea was limited to three to four dominant species. The Q-PCR results showed the presence of high numbers of Archaea and Bacteria. To analyse their function, we measured the abundances of genes indicative of metal reduction (16S rRNA gene for *Geobacter spp.*), sulphate reduction (sulphate reductase, *dsr*), and methanogenesis (methyl coenzyme M-reductase, *mcrA*). The methanogenic consortia will be further characterised to determine enzymatic pathways and the individual role of each partner. Degradation pathways for different compounds will be studied using 13C-labelled substrates and molecular techniques. Our stable isotope data from both, methane produced in our incubations with samples from various ecosystems and field studies, implies a common methanogenic biodegradation mechanism, resulting in consistent patterns of hydrocarbon alteration.

ECV04
Role of *Bacteria*, *Archaea* and *fungi* involved in methane release in abandoned coal mines

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Worldwide, abandoned coal mines release substantial amounts of methane which is largely biogenic. Our investigations revealed a complex network of metabolic steps enabling the conversion of recalcitrant materials like timber and even hard coal to methane. A diverse community of *fungi*, *Bacteria* and *Archaea* is involved, inhabiting very distinct ecological niches. The first steps of weathering of coal and timber in our case proceeded in a suboxic atmosphere initiated by *Bacteria* and wood-degrading fungi with a large pool of biomass on timber. Oxygen depleting processes create anoxic conditions where *fungi* might perform incomplete oxidation and release reduced substrates which can be channeled into methanogenesis. A broad spectrum of facultative anaerobic bacteria and acetogens belonging to all five groups (α - ε) of the *Proteobacteria*, as well as the *Bacteroidetes*, *Tenericutes*, *Actinobacteria*, *Chlorobi* and *Chloroflexi* fuel the methaogenesis. Obviously, acetate is the main precursor of methane released by acetoclastic *Methanosarcinales* that constituted about half of the *Archaea*. These observations confirm the detection of high methane formation rates in acetate amended enrichments, and also the accumulation of acetate in incubations after inhibition of methanogens by 2-bromoethanesulfonate(Krüger *et. al*, 2008). Stable Isotope Probing (SIP) analyses of DNA based on ^{13}C acetate and ^{13}C bicarbonate incorporation confirmed that methanogenesis is driven by acetoclastic *Methanosarcinales*.

[1] Krüger *et al.* (2008) Microbial methane formation from coal and mine timber in abandoned coal mines. Geomicrobiology Journal 25, 315-321.

ECV05
Importance of soil spatial heterogeneity for sustaining microbial communities and their capacity to degrade phenol

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Interactions between organic chemicals and soil microorganisms can best be understood if the heterogeneity of soil is considered. In this context we want to elucidate how soil organic matter affects the distribution of organic pollutants and how it sustains specific microbial communities. Our studies are conducted with three soil variants from a long-term fertilization experiment which differ in their soil organic carbon contents. Soil particle size fractionation revealed that most organic matter was associated with the smallest fraction (clay). Total soil DNA, extracted from each particle size fraction separately was utilized for quantitative PCR of SSU rRNA genes to determine the population sizes of *Bacteria*, *Archaea*, and *Fungi*, respectively. The largest microbial population sizes were associated with the clay and the coarse sand fraction. The proportion of *Fungi* within the microbial community was higher with coarse sand than clay. Terminal restriction fragment length polymorphism (TRFLP) profiles indicated particle size specific differences in the diversity of the *Bacteria* and *Fungi*. An effect of soil organic matter content was also found. Phenol was chosen as an organic pollutant, and its partitioning to different particle size fractions was investigated using the ^{14}C-labeled compound. The release of $^{14}CO_2$ allowed, in a comparable experiment with ^{13}C-phenol, to estimate the incorporation kinetics of this isotope into the DNA by stable isotope probing.

Phenol utilizing bacteria were detected after density ultracentrifugation and genetic profiling of PCR amplified SSU rRNA gene sequences by single strand conformation polymorphism (SSCP). Different intensities of SSCP bands in the "heavy" C-fractions suggested mainly quantitative differences of the phenol utilizing bacteria in response to the level of soil organic carbon. The results demonstrate the importance of soil organic matter to sustain microbial diversity and activity in soils.

ECV06
Multispecies community as an alternative to degrade PAHs

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Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous pollutants that persist in the environment for a long time. These compounds are very stable, highly hydrophobic and, consequently, very difficult for bacteria to degrade. However, bacteria can cooperate within a community making the degradation process viable. Therefore, the aim of this work is to find a community able to degrade 16 different PAHs crystals listed as priority pollutants, to characterize it and to try to understand how the community works. The microbial communities were obtained from soil samples collected at 3 different sites. Microcosms were constructed with the soil samples, sterile water and slides with the 16 PAHs crystals were exposed in it. Samples were taken periodically to characterize the community by single strand conformation polymorphism (SSCP), and PAH degradation measured by HPLC. Samples were also analysed by confocal and scanning microscopy. Bacteria were both isolated from the soil and the biofilm formed on the crystals for comparison with the taxonomic units found in the SSCP gel and to construct a new community. The SSCP gel and the microscopy revealed multispecies biofilms, which was different for each sample site. The population from a polluted creek could degrade both higher amounts of PAH and more PAHs, including complex compounds, than the others. This community was diverse and included sulphur-oxidizing bacteria. Completely different bacteria were isolated directly from the soil and from the biofilm of the same sample. From these isolates new communities were constructed, however, the artificial communities were much less efficient as the biofilm communities from soil and could not degrade complex PAHs. This results show that communities from soil, especially those already from polluted sites, are usually superior to artificial ones for the control of PAH pollution.

ECV07
Repeated cycles of drying and rewetting select for members of the family *Sphingomonadaceae* in semiarid savanna soils

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Soils in semiarid savanna ecosystems are often subjected to wetting by sparse rain events followed by rapid drying and prolonged periods of drought. In order to investigate the impact of such rapid changes in water availability on the soil bacterial community, soils collected in the Kavango region (Northern Namibia) were subjected to repeated cycles of drying and rewetting (DRW). Potential changes in numbers, culturability and composition of the microbial community were observed by total cell counts, most probable number (MPN) techniques, isolation and identification of abundant members of the culturable fraction, and cultivation-independent techniques as quantitative PCR and group-specific PCR-DGGE. In most cases, total cell numbers started to increase already from the first cycle of drying and rewetting, reached maxima in the second cycle, and often decreased again in the third cycle. In contrast, MPN counts declined after first drying, but recovered in the second and third DRW-cycle. Comparison of isolates gained from untreated soil and from soil of the third DRW-cycle showed a decrease in the percentage of *Actinobacteria* and *Gammaproteobacteria*, a slight increase in the percentage of *Firmicutes*, and a clear increase in the fractions of *Beta-* and *Alphaproteobacteria*. Within the later, especially members of the family *Sphingomonadaceae* showed a raise from 15 to 37% of all isolates. Similarly, DGGE-patterns showed clear shifts in distinct bands over the consecutive treatment cycles. These results indicate that extensive DRW leads to consistent and pronounced shifts in soil bacterial community composition.

ECV08
"Death in soil" or how microbes contribute to the genesis of soil organic matter

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Soil microorganisms do not only catalyze the transformation of plant residues to soil organic matter, but also serve as considerable carbon source for the formation of refractory soil organic matter by providing cell fragments as structural interfacial surfaces in soil systems.
After incubation of 13C-labeled Gram negative bacteria in soil for 224 days, we could show that 44% of the bulk carbon remained in soil. 30 - 35 % of the remaining bulk C from Gram negative microbial biomass was stabilized in non-living soil organic matter (SOM). Surprisingly, the added labeled biomass proteins remained in soil almost completely which clearly indicates the stabilization of proteins in cell aggregations being more resistant to biodegra¬dation than free proteins and amino acids. Scanning electron micrographs of the soil showed very rarely intact cells but highly abundant patchy organic cover material of 20 to 50 nm2 size on the mineral surfaces.
A possible mechanism for this stabilization and the observed material could be found by analyses of microbial communities and biofilms developing on Biosep® beads within *in situ* microcosms exposed to contaminated aquifers. Scanning electron micrographs of the developing biofilms on the beads showed the formation of such patchy material found in the soil by fragmentation of empty bacterial cell envelopes (cell walls) and all stages of decay. The fragmentation of these cell walls provided a mechanistic explanation for the observed stabilisation, the genesis of SOM derived from dead bacterial cells, and the enzyme activity always found associated to SOM.

ECV09
Predation resistant bacteria create a protective niche for non-toxic cheaters

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Bacterial populations are in most ecosystems top-down controlled by protozoan predators, and various strains evolved resistance strategies such as toxicity. Fluorescent pseudomonads are an ubiquitous group of soil bacteria which presents a great potential as antagonists of plant pathogens. These bacteria produce extracellular toxins that protect them against predation by protozoa and nematodes. However, despite this beneficial trait is unstable, and natural populations are often loaded with spontaneous mutants lacking toxicity. These mutants probably function as cheaters, profiting from the toxin of the wild type strain without contributing to it.
In this study we investigated if toxic populations provide a predator-free niche for non-toxic cheaters. We measured the advantage of toxicity for the biocontrol bacteria Pseudomonas fluorescens CHA0 under predation by the ciliated protozoa Tetrahymena pyriformis, and the predators feeding behavior on a mixed population of toxic and non-toxic bacteria.
Our results demonstrate that extracellular toxicity is a potent antipredator strategy, and dramatically reduces predator consumption. However, in mixed populations the passive prey switching of the predators resulted in an under consumption of the non-toxic strain. At high bacterial densities, the toxins of the wild type strain moreover inhibited the predators, reducing thus also the predation pressure on the whole population.
Consequently, toxic populations offer an optimal niche for low amounts of undefended bacteria and may thus select for rapidly evolving cheater phenotypes.

ECV10
Bacterial community structure and activity at the air-sea interface: Studies from the Baltic Sea

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The sea-surface microlayer (SML) is located at the air - water interface and plays a pivotal role in transport processes between the atmosphere and the hydrosphere. It is stated to be enriched in organic and inorganic components compared to the underlying water (ULW). However, this potential benefit for the microbial community (bacterioneuston) is opposed by harsh conditions within the habitat (e.g., UV-radiation, organic pollutants). In order to elucidate general patterns of the bacterioneuston, SML samples were taken throughout three years in the southern Baltic Sea and bacterial abundance, diversity (16S rRNA and 16S rRNA gene fingerprints) and productivity (^{3}H-thymidine-incorporation) were studied. Additionally, mesocosm experiments were conducted to assess responses of the bacterioneuston to an artificially calmed sea surface.
The SML was found to be generally enriched in organic carbon and nitrogen. Yet, this did not fuel bacterial activity as the abundance of highly active (CTC-positive) cells was comparable to the ULW and bacterial productivity was generally reduced in the SML. This reduction was not due to a change of the bacterial community composition, because only minor changes of abundant members among the communities were observed. Only when a visible surface film ("slick") with a high accumulation of organic material due to very calm weather conditions developed, significant enrichments in cell numbers and productivity occurred and were accompanied by strong changes in bacterial community structure. A similar pattern was observed in the mesocosm experiments under artificially reduced wind conditions. Our results imply that the SML is generally not a favorable habitat for bacteria, despite the accumulation of potential organic substrates. Thereby, the bacterioneuston community is strongly influenced by freshly imported, especially particulate, material.

ECV11
Dominant bacterioplankton groups respond differently to changes in $^1O_2$ and H_2O_2 exposure in a humic acid rich lake

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The photolysis of dissolved organic matter (DOM) is an important process in freshwater habitats enhancing the bioavailability of recalcitrant DOM. Although bacterioplankton species benefit from carbon substrates generated by this process they have to cope with reactive oxygen species (ROS) generated simultaneously. We performed *in situ* experiments to investigate the effects of increased singlet oxygen steady state ($[^1O_2]_{SS}$) and hydrogen peroxide (H_2O_2) concentration on the bacterioplankton composition in the SW basin of the humic acid rich lake Grosse Fuchskuhle. DGGE fingerprints and OTU distribution in 16S rRNA gene clone libraries showed that different bacterioplankton groups respond in a different manner to $^1O_2$ and H_2O_2. *Sphingomonadaceae* and *Burkholderiaceae* including *Polynucleobacter* spp. were positively affected by enhanced $[^1O_2]_{SS}$ in a concentration dependent manner but were sensitive to H_2O_2 exposure. In contrast freshwater *Actinobacteria* were sensitive to $^1O_2$ but positively effected by H_2O_2 exposure. Our results indicate that the generation of different ROS by DOM photolysis may have specific effects on dominating bacterioplankton species/groups and thereby affect the bacterial community composition.

ECV12
Ammonia availability affects the competition between ammonia-oxidizing bacteria and ammonia-oxidizing archaea in simulated creek ecosystems - a flow channel experiment

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Nitrogen transformation processes in creek ecosystems play an important role in the discharge of nitrogen compounds to streams and estuaries. The first and rate-limiting step of nitrification, the oxidation of ammonia to nitrite, is a key process of nitrogen cycling and is carried out by ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA). In order to assess the effect of different ammonium concentrations on AOB/AOA ratios and on AOB and AOA community composition in simulated creek ecosystems, we incubated experimental flow channels with planktonic and biofilm-associated nitrifying communities over a 12-week period. The flow channels were run at near *in situ* ammonium concentrations with water obtained from three different creeks. Biofilm-associated potential nitrification activity increased with ongoing incubation. Community composition of AOB and AOA was analyzed targeting the *amoA* gene, which encodes ammonia mono-oxygenase, the key enzyme of ammonia oxidation. Ammonia availability had a clear effect on the community composition of AOA and AOB while compartment – water column versus biofilm – did not. AOA were equally abundant or more numerous than AOB at the creek field sites. For both planktonic and biofilm-associated communities, AOB/AOA ratios increased during the experiment, indicating that AOB were more competitive than AOA under the incubation conditions of the flow channels. The highest AOB/AOA ratios were found at the highest concentration of ammonium (350 μM).

EKP01
Recognition and processing of *Aspergillus fumigatus* conidia by alveolar macrophages

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Immunsupressive treatment of patients represents a major risk factor for systemic infections. Among the overall number of systemic infections the prevalence of systemic fungal infections has drastically increased over the last decades. The mould *Aspergillus fumigatus* is the main causative agent of invasive pulmonary aspergillosis in immunocompromised patients. The infection route starts with the inhalation of *A. fumigatus* conidia that germinate in the lung. Alveolar macrophages in the lung alveoli are able to phagocytose and kill conidia. Their ability to engulf and degrade conidia is a prerequisite for efficient clearence of fungi from the lung. This clearance also depends on the release of chemokines and cytokines in order to trigger neutrophil migration at the site of infection. Until now, little is known about the molecular mechanisms employed by alveolar macrophages to detect and process *A. fumigatus* conidia. In the immunocompromized host, at least some conidia are able to evade macrophage degradation, resulting in germination and outgrowth of intracellularly residing spores. Therefore, conidia must be able to evade recognition and processing by phagocytes. The avirulent *pksP* mutant of *A. fumigatus* lacking the melanin layer present on wild-type conidia exhibited increased phagocytosis by macrophages apparently due to the loss of masking glucan-structures that are recognized e.g. by the dectin-1 receptor. Furthermore, here by analysing phagocytosis rate and phagolysosome fusion we show that intracellular processing of *pksP* mutant conidia is drastically increased in comparison to wild-type conidia, suggesting that *A. fumigatus* conidia actively decrease the phagolysosomal fusion, similar to obligate human pathogens like *Legionella sp*. or *Mycobacterium sp*.. The process by which wild-type conida mediate inhibition of phagolysosome fusion seems to be connected to the surface structure of conidia but is independent of the presence of a functional RodA-derived rodlet layer. Moreover, inhibition of phagolysosome fusion by macrophages is controlled by the fungal cAMP signalling pathway.

EKP02
First epidemiological survey of oral colonisation/infection with *Candida* in HIV infected Patients in Sarh, Chad

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Oral candidiasis is a common side effect and often the first hint of a HIV infection. In the general population of Chad, HIV seroprevalence amounts to approximately 3.5% (UNAIDS). This study presents the first systematic epidemiologic survey of oral candidiasis in Chad : swabs were taken from the oral cavity of 391 HIV positive patients (75% female, 25% male) and a control group of 69 non-HIV patients (56% female, 44% male) in the Medical Center of Maingara, Chad. The overall colonisation rate was 26% in the HIV positive patients and 33% in the control group. Species distribution in carriers and infected was 67% *Candida albicans*, 11% *C. tropicalis*, 6.3% *C. krusei*, 3.4% *C. glabrata*, 2.8% *S. cerevisiae* 1.1% *C. parapsilosis* and 8.6% others. Unexpectedly, no C. dublinensis</i> was found among our samples, although this species is frequently associated with HIV positive patients in other studies. Resistance testing for amphotericin B, nystatin, fluconazol, itraconazol and caspofungin was conducted by microdilution according to the CLSI M24-7A protocol. No significant resistance for neither amphotericin B, nystatin nor caspofungin was observed. In contrast, high intrinsic resistance to fluconazol in *C. krusei* and *C. glabrata* was observed. The influence of antiretroviral therapy (ART) and CD4 cell count on the colonisation/infection rate has been analysed and the outcome is presented here.

EKP03
Characterisation of the hypoxic adaptation of the human-pathogenic fungus *Aspergillus fumigatus* by proteomic approaches

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Aspergillus fumigatus is a ubiquitously distributed saprophytic mould that has become the most important airborne fungal pathogen. Upon inhalation, *A. fumigatus* spores germinate, undergo hyphal growth and spread in the lungs causing pulmonary invasive aspergillosis in immunocompromised patients. At the site of infection, the partial oxygen pressure presumably drops down due to granulocyte infiltrations and damage of the host tissue. Most recently it was shown that the loss of the transcription factor SrbA, which mediates the hypoxic response in *A. fumigatus* , leads to attenuation in virulence. To characterise the hypoxia sensing and adapting pathways more in detail we performed differential in gel electrophoresis (DIGE) analyses of an *A. fumigatus* strain in response to low oxygen partial pressure. For this purpose, we established an oxygen-controlled fermenter system. Mycelial proteins involved in glycolysis, amino acid biosynthesis, stress response and respiration showed an increased standard abundance under hypoxic conditions. In contrast, proteins involved in sulphate assimilation and acetate activation were down-regulated. To get a deeper insight into the changes in protein expression of the respiration machinery, we isolated mitochondria of *A. fumigatus* grown under normoxic and hypoxic conditions and performed DIGE and two dimensional Blue-Native PAGE. Many proteins of the respiratory complexes I-IV were strongly up-regulated under hypoxia.Surprisingly, enzymes involved in the biosynthesis of the secondary metabolite pseurotin A were up-regulated under low oxygen partial pressure as well. These finding was also confirmed on the transcriptional level and by HPLC analysis.

EKP04
Modulation of murine Plasmodium infections by activation of toll-like receptor pathways

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Recognition of pathogen-associated molecular patters through Toll-like receptors (TLRs) plays a pivotal role in first line defense against various pathogens. It has been shown that TLRs are also triggered during malaria infection by parasite-derived components. However the contribution of innate responses to the pre-erythrocytic stages of *Plasmodium* and to the subsequent clinical outcome of a malaria blood stage infection is not well understood.
In order to assess the role of enhanced TLR-signalling on *Plasmodium* infection we systematically examined the effect of agonist-primed immune responses to parasite inoculation in the *Plasmodium berghei* rodent malaria parasite model. In these studies we could identify distinct effects on the course of infection after stimulation with TLR-ligands. While single agonists are capable of inducing robust parasite killing in the liver, priming with other agonists leaves the liver stage unaffected and translates into strong protection against cerebral malaria.
Our findings suggest that activation of TLR signalling pathways might play a beneficial role in anti-malaria vaccine strategies. Adjuvant systems that elicit TLR-signalling may elicit robust antigen-independent responses against *Plasmodium* liver stages. The protective roles of TLR-signalling in severe forms of malaria may also offer an explanation for clinical effects observed with pre-erythrocytic vaccine approaches against disease outcome caused exclusively by erythrocytic stages.

EKP05
Identification and functional characterization of a plant-like UDP-Sugar pyrophosphorylase from *Leishmania major*

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The glycocalyx of Leishmania parasites is rich in galactose-containing glycoconjugates that are synthesized by specific glycosyltransferases which use UDP-galactose as a glycosyl donor. UDP-galactose biosynthesis is thought to be predominantly a *de novo* process involving epimerization of the abundant nucleotide sugar UDP-glucose by the UDP-glucose 4-epimerase, although galactose salvage from the environment has been demonstrated for *L. major*. Here we report the cloning, purification and characterization of a UDP-sugar pyrophosphorylase (USP) from *L. major* that constitutes a salvage pathway to UDP-galactose in this organism. Although *L. major* UDP-sugar pyrophosphorylase preferentially activates galactose-1-phosphate and glucose-1-phosphate, the enzyme is able to act on a variety of hexose-1-phosphates as well as pentose-1-phosphates but not hexosamine-1-phosphates and hence presents a broad *in vitro* specificity tested enzymatically. Interestingly Leishmania USP homologs are found in plants and display very similar substrate specificity, but are not found in other organisms suggesting a horizontal gene transfer from plants to Leishmania. The newly identified enzyme exhibits a low but significant homology with UDP-glucose pyrophosphorylases and conserved in particular is the pyrophosphorylase consensus sequence and residues involved in nucleotide and phosphate binding. Saturation Transfer Difference (STD) Nuclear Magnetic Resonance (NMR) spectroscopy experiments confirm the importance of these moieties for substrate binding.

EKP06
AfMkk2 is required for cell wall integrity signaling, adhesion, and full virulence of the human pathogen *Aspergillus fumigatus*

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The fungal cell wall is an important drug target for antifungal therapy. The cell wall integrity (CWI) pathway transduces stress signals from the cell surface to the nucleus. We analyzed the importance of AfMkk2, a CWI signaling kinase, for virulence and antifungal therapy in the human pathogen *A. fumigatus*. A mutant lacking AfMkk2 is less adherent to cell culture surfaces and suffers increased sensitivity to cell wall perturbing agents, alkaline pH stress and antifungals. Rather than AfMpkA, the target kinase of AfMkk2, AfMpkB is activated in the mutant under cell wall stress. In agreement with its sensitivity to moderate temperatures, a mutant lacking AfMkk2 is less virulent in a murine infection model.

EKP07
Microbial quorum sensing molecules induce acrosome loss and cell death in human spermatozoa

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Infertility in men and women is frequently associated with genital contaminations caused by different microorganisms. The molecular basis of this correlation remains still elusive, and little attention has been paid on potential direct influences of commensal or uropathogenic microbes on human gametes. Because many microorganisms are known to release distinct signaling molecules in substantial amounts, we raised the question whether such molecules can directly affect human spermatozoa. Here we show that the quorum sensing molecules farnesol and 3-oxododecanoyl-L-homoserine lactone employed by the opportunistic pathogenic yeast *Candida albicans* and the gram negative bacterium *Pseudomonas aeruginosa*, respectively, induce multiple damages in human spermatozoa. A reduction in the motility of spermatozoa coincided dose-dependently with apoptosis and necrosis at concentrations which were non-deleterious for dendritic-like immune cells. Moreover, sublethal doses of both signaling molecules induced premature loss of the acrosome, a cap-like structure of the sperm head which is essential for fertilization. This work uncovers a new facet in the interaction of microorganisms with human gametes, and at the same time sheds new light in the phenomenon of quorum sensing, a microbial communication system which may impact not only interkingdom signaling and pathogenicity but also host fertility.

EKP08
Microevolution of a nonfilamentous *Candida albicans* mutant during co-incubation with macrophages

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The commensal fungus *Candida albicans* is a member of the microbial flora of humans. Normally, *C. albicans* colonizes the host without causing damage. However, under certain circumstances, such as when the host defense is weakened, *C. albicans* can become a pathogen and cause disease. During the infection process, *C. albicans* has the potential to adapt to different host niches and to survive the attack of phagocytes, such as macrophages. In fact, *C. albicans* does not only survive ingestion by macrophages, but can also kill these immune cells and escape from them by producing hyphae.
In this study, we used an experimental microevolution approach to identify factors and activities necessary for survival within, and escape from, macrophages. First, we identified for hyphal-deficient mutants, which cannot escape from macrophages. Eight mutants lacking regulators of hyphal formation were tested for their behavior during co-incubation with macrophages as compared to wild type cells. Four hours after infection, more than 50 % of wild type cells escaped from the macrophages. In contrast, none of the tested mutants achieved such high escape rates. Two mutants (*ras1Δ* and *efg1Δ/cph1Δ*) were not able to escape. Since the *efg1Δ/cph1Δ* mutant is unable to produce hyphae under almost all tested conditions, we choose this mutant for our microevolution experiment and investigated the adaptation of this mutant within macrophages during a series of passages. After 23 passages, *efg1Δ/cph1Δ* exhibited a dramatic phenotypic alteration and formed filaments. These filaments enabled the modified mutant (strain P23) to escape from macrophages. Surprisingly, strain P23 also formed filaments under several other tested hyphal inducing conditions. This phenotype was stable, indicating that a microevolutionary event has bypassed the mutations of the two key hyphal regulators *CPH1* and *EFG1*. We have begun to elucidate the genetically stable modifications of P23 by using microarrays and karyotype analysis.

EKP09
Identification of novel antifungal compounds using a HTS activity-selectivity assay

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Fungal infections represent a serious health problem in industrialized countries. Especially immune suppressed patients are highly susceptible to life-threatening infections by opportunistic fungi. In addition transplant therapy and anticancer drugs have provided an opportunity for fungi to cause serious infections. Treatment of fungal infections largely relies on chemotherapy and is limited by the high cost of the most potent antifungals as well as by the reported emerging resistance to some antifungals. To find novel compounds with broad selective antifungal activity we have developed an assay to identify, evaluate and optimize tolerable and potent antimicrobial agents in compound libraries for drug therapy. This assay covers all potential *in vitro* targets of the pathogen and the host simultaneously. Moreover it constitutes the smallest unit of a natural infection by incubating host cells in the presence of antimicrobial compounds and the pathogen, e.g. *Candida* species. Host cell survival is determined to asses the efficiency and selectivity of the respective compound. In addition to the minimal inhibitory concentration for the pathogen, this test system provides the tolerability of the active compound by the host cells, expressed as selectivity index. Using this assay about 100 000 compound derived from pre-selected basic chemical structures have been screened. One of the hits identified in the compound library was investigated in more detail by chemical modification of the lead structure and target identification using transcriptional profiling of *C. albicans*. Tissue models derived from primary cells are used as second test system providing further information about tissue penetration and tolerability of the test compound. Furthermore the most effective compound is currently tested against other *Candida* and *Aspergillus* species. Transcriptome analyses of the fungi treated with the novel compounds are in progress to reveal the cellular targets of the new substances and their mode of action.

EKP10
1-hydroxy-2-acyl-4(1)quinolones are potent inhibitors of *Toxoplasma gondii* replication

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The apicomplexan parasite *Toxoplasma gondii* was recently shown to be effectively inhibited by the quinolone-like compound 1-hydroxy-2-dodecyl-4(1)quinolone (HDQ). Based on the structure of HDQ, novel 1-hydroxyquinolones were synthesized and tested against *T. gondii*. Compounds A and B displayed a 10- and 5-fold lower IC50 than HDQ, revealing that the dodecyl site chain can be localized at either C-2 or C-3 of the 1-hydroxyquinolone moiety without loosing the anti-parasitic activity and furthermore showing that a methyl-moiety at C-3 improves the anti-Toxoplasma activity of HDQ. These two compounds and HDQ were tested for *in vivo* activity in a mouse model of acute toxoplasmosis. All three drugs significantly reduced the percentage of infected peritoneal cells. Compound B possessed the highest potential to reduce the parasite load in lung and liver and also showed a tendency towards lower parasite loads in brains in a mouse model of toxoplasmic encephalitis. The *in vivo* anti-Toxoplasma activity of HDQ derivatives characterize these compounds as promising novel drugs which should be carefully evaluated for their therapeutic potential in future. Due to their structural similarity with ubiquinone, HDQ derivatives are believed to interfere with the ubiquinone binding site of respiratory chain enzymes. In fact, we could demonstrate that HDQ is an inhibitor of a type II NADH dehydrogenase (NDH2) in *T. gondii*. We are currently investigating whether NDH2 inhibition is the mode of action, which is responsible for the remarkable low IC50s of HDQ derivatives.

EKV01
Functional Analysis of *Candida albicans* Nik1p in *Saccharomyces cerevisiae*

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Fungal histidine kinases are considered to be attractive targets for antimycotics, as human cells do not contain homologue enzymes. In *Candida albicans*, the most important human fungal pathogen, as yet three histidine kinases were identified. There is a homologue to the enzyme Sln1, which is a sensor protein in the HOG-osmotic stress defense pathway in *S. cerevisiae*. Homologues of the second histidine kinase, CaNik1, were shown to be the target of a number of fungicides used in agriculture. However, its function in *C. albicans* is only partly understood, but it seems to work via the same pathway as the homologue to Sln1.
The fungicides fludioxonil, iprodione, ambruticin VS-3 and jerangolide target homologues of CaNik1 in fungal plant pathogens, whereas *S. cerevisiae* is resistant to these compounds, as there is no homologue to these enzymes in *S. cerevisiae*. Thus, we transformed *S. cerevisiae* with a pYES2-vector comprising a redesigned Ca*NIK1*-sequence. Expression of the protein was proven via MALDI-TOF, as well as by western blot analysis using a flag-tagged version.
Only *S. cerevisiae* transformed with CaNik1p stopped growth when treated with these fungicides in a concentration dependent manner.
To elucidate the essential protein domains in CaNik1p one and two of the N-terminal HAMP domains of CaNik1p were deleted. This shifted the effective fungicide concentrations to higher values in the transformed *S. cerevisiae* strains, but the deletion of a single HAMP-domain could not abolish the fungicidal activities. The effects of domain deletions differed between the chosen fungicides, which suggests differences in the submolecular site of interaction.
Fungicide treatment indeed activated the HOG-osmotic stress defense pathway in *C. albicans* as well as in the transformants. However, effects on growth were more severe in the transformants. Thus we performed comparative transcriptome analysis to obtain deeper insights into details of the CaNik1-dependent network.

EKV02
Investigation of the relationship of genotype and cell wall composition in the human pathogenic fungus *Candida glabrata*

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The human pathogenic fungus *Candida glabrata* is the second leading cause of systemic candidosis in immunocompromised patients after *C. albicans*. Its intrinsic resistance to clinically used azole antimycotics, the very dynamic cell wall structure and its highly flexible genome, conferring adaptive plasticity when invading the human host, renders *C. glabrata* a fungal pathogen of rising importance.
We investigated possible relationships between genotype, phenotype, biochemical structure of the cell wall in two *C. glabrata* collectives, one containing 10 MLST-isogenic laboratory reference strains and the other consisting of 124 heterogenous clinical isolates. Both groups were characterized phenotypically with cell wall perturbing and antimycotic agents, genotypically via electrophoretic karyotyping and MLST and FACS analysis to study cell wall composition.
Within the laboratory strains we were able to identify three karyotypic groups, each displaying characteristic phenotypes, as well as a specific cell wall composition.
However, phylogenetically closely related clinical isolates of the same karyotype showed already a wide variability in phenotypic characteristics and cell wall compositions. Nevertheless, one MLST clade consisted mostly of fluconazole hyposusceptible isolates, which shared a specific chromosomal aberration and an elevated glucan content of the cell wall. Additionally, most isolates which clustered in this clade showed hyposusceptibility towards cell wall perturbing agents. Interestingly, the characteristic chromosomal alteration of these isolates could be found more frequently among all hyposusceptible phenotypes than other ones.
This data suggests that there may be a linkage between geno- and phenotypic characteristics, as well as a spezific cell wall composition, which in turn that might contribute to fungal resitance, adaptability and virulence.

EKV03
Candida albicans BIS1 mediates stress adaptation and virulence

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Candida albicans belongs to a small group of opportunistic fungal pathogens which are able to cause life-threatening infections in immunocompromised humans. This ability to infect host tissue and evade the immune system is reliant on certain genes, such as those involved in hyphal formation, adhesion to host surfaces and invasion and damage of host cells.
However, it is likely that many, if not most, *C. albicans* infection-associated (IA-) genes have not yet been identified and characterised. Using a transcriptomics approach for different *C. albicans* infection models, we identified novel putative IA-genes of unknown function. One gene was chosen for further investigation as it featured a strong upregulation during liver infection, interaction with neutrophils and macrophages and under oxidative stress.
In silico analysis revealed that this uncharacterised gene encodes a putative small heat shock protein (sHSP). Based on its strong upregulation in the blood infection model and the function as a possible sHSP, the gene was named *BIS1* (blood induced stress protein 1).
A primary screen for growth of a *bis1Δ* knock out mutant revealed that this gene is required for tolerance against specific stresses including thermal, oxidative, DTT and ethanol stress. Interestingly, osmotic stress bypassed *BIS1* dependent thermal tolerance.
The *bis1Δ* mutant displayed reduced survival during interactions with macrophages. Moreover, *BIS1* was required for full virulence in an *in ovo* infection model.
Furthermore, *BIS1* was demonstrated to also be important for damage of both epithelial and endothelial cells.
Taken together these results provide novel links between stress adaptation and fungal pathogenicity.

EKV04

Candida albicans colonizes the intestinal tract of *Caenorhabditis elegans* and establishes a persistent lethal infection

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Candida albicans, a physiological commensal microorganism, is a causal agent of opportunistic human candidiasis. Infections with *C. albicans* account for 70 to 90% of all invasive mycoses. *C. albicans* hyphal formation as a response to the host environment is regulated by transcription factors like Tec1p and orchestrates the expression of important virulence factors. We used a nematode worm *Caenorhabditis elegans* as an infection model to assess virulence of *C. albicans* and examined hyphal formation and survival of the nematode host. Previously, *C. elegans* has been successfully used as a model host for human infections to understand the genetic mechanisms of virulence of various pathogenic microorganisms. In this study, a wild type *C. elegans* strain N2 and wild type *C. albicans* strain SC5314 were used for *C. elegans*-*C. albicans* interaction. *C. albicans*, provided as a food source in parallel to standard diet *E. coli* OP50, was ingested by *C. elegans* and generated a persistent lethal infection in the *C. elegans* intestine accompanied by deep tissue invasion. Interestingly, wild type *C. albicans* cells formed hyphae at 25°C only after getting ingested by the worm and being exposed to the worm's intestinal environment, whereas *C. albicans* cells remaining outside the worm showed no hyphal development and replicated as yeast cells. From our results we hypothesize that *C. elegans* detects the presence of *C. albicans* and activates its defence mechanisms. As an evasion strategy, the yeast cells undergo morphogenetic development and form hyphae, which in turn results in aggressive tissue destruction and death of the nematode host. A series of strains with modifications of the Tec1p transcription factor will now be evaluated for their phenotype in this new *in vivo* infection model.

EKV05

Production of Neutrophilic Extracellular Traps against *Aspergillus fumigatus in vitro* is influenced by the conidial surface protein hydrophobin RodA

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Aspergillus fumigatus is the most important airborne fungal pathogen. Conidia as the infectious agent infiltrate the lungs and get in contact with alveolar macrophages and neutrophil granulocytes, which repesent the first line of defense. From *Candida albicans* it is known that neutrophils are able to attack the pathogen by the formation of neutrophilic extracellular traps induced by an ROI dependent beneficial suicide (Brinkmann and Zychlinsky, 2007) called NETosis. These sticky filaments consist of nuclear DNA decorated with histones and fungicidal proteins. Here, we show that human, unstimulated neutrophils produce NETs *in vitro* when encountering both *A. fumigatus* conidia and hyphae. In time-lapse movies NET production was a highly dynamic process which, however, was only exhibited by a sub-population of cells. NETosis was maximal against hyphae, but reduced against resting and swollen conidia. By using fungal mutants and purified proteins we demonstrate that hydrophobin RodA, a surface protein rendering conidia immunologically inert, led to reduced NET formation by neutrophils encountering *Aspergillus*.
Taken together, NET formation is morphotype- and strain-dependent, but the killing of *A. fumigatus* condia is not influenced by the amount of released extracellular DNA. Our data suggest that NETs prevent further spreading, but apparently do not represent the major factor for killing.

EKV06

Candida albicans pH-regulated antigen 1 is a potent fungal protein for complement evasion

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During infection the pathogen *Candida albicans* has to overcome the complement system, which forms the first defense line of innate immunity. To evade toxic complement activity *C. albicans* expresses several virulence factors such as the cell wall bound and secreted protein pH-regulated antigen 1 (Pra1). Aiming to characterize the role of Pra1 in immune evasion mechanisms at a molecular level the single protein was recombinantly expressed and purified from *Pichia pastoris* culture supernatant. Recombinant Pra1 binds complement protein C3 and several C3 activation products as well as the complement regulator factor H (FH). Moreover, investigating functional activity in hemolytic assays with both activated normal human serum (NHS) and FH-depleted serum Pra1 potently inhibits alternative pathway of complement activation at 30 and 90 %, respectively. In conclusion these results suggest that Pra1 inhibitory action in hemolytic assays is independent of FH binding. As the C3 convertase represents the central enzyme of the complement cascade, we analyzed whether Pra1 specifically modulates complement at this level. Interestingly, upon activation of complement by zymosan and yeast cells Pra1 inhibits both C3a generation and C3b deposition in a dose dependent manner. Thus, decreased C3b-mediated opsonization results in reduced adherence and phagocytosis of yeast cells by human macrophages as shown by flow cytometry. In summary, Pra1 is a multifunctional protein displaying potent complement regulatory function thereby modulating host immune effector functions. Pra1 represents an important virulence factor and hence could be considered as a promising candidate for future vaccine design aiming to control and prevent life threatening disseminated Candidiasis particularly in immunosuppressed and other susceptible clinical patients.

EKV07

Design of transgenic Plasmodium parasites that express an agonist of the innate host defense

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Malaria is the most important vector-borne infectious disease and causes more than one million deaths annually, mainly in children from sub-saharan Africa. Cerebral malaria is one of the most serious and often fatal complications of severe malaria. The exact pathogenesis of cerebral malaria remains unclear, but parasite sequestration to endothelial cells in the brain, the secretion of pro-inflammatory cytokines, and expansion of pathogenic T-cells are thought to play major roles. Recent findings also indicate the contribution of Toll-like receptors (TLRs) to disease pathogenesis and/or parasite clearance, but the exact role of TLR-mediated signaling in disease outcome remains inconclusive.
Towards the generation of improved vaccine strategies we tested the effect of a non-malaria TLR agonist in disease progression. We could show that triggering of the innate immune system with a purified, recombinant protein prolonged the time to blood stage infection and partly protected C57/Bl6 mice from cerebral malaria in sporozoite-induced infection. These results prompted us to generate transgenic Plasmodium berghei parasites that express the TLR agonist under the control of two strong, endogenous promoters. These parasite lines were tested for sporozoite-induced infections. We could show that these transgenic lines show a robust delay in liver stage development, and, perhaps as a consequence, are protected against cerebral malaria.
Our study may lead to a better understanding of the role of TLR-agonists in the interaction with and modulation of the host's immune system and may offer new perspectives for anti-malaria intervention strategies.

EKV08
Gel free analysis of the Proteome of intracellular *Leishmania mexicana*

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The proteome of intracellular Leishmania amastigotes has recently been investigated based on exploitation of fluorescence activated particle sorting. We employed this technology in combination with gel free analysis to to describe 1764 proteins of which 741 had not been identified before. Protein abundance was calculated to rank individual proteins according to their amount *in vivo*. Using the LeishCyc resource a metabolic overview was produced that integrated protein abundance. Bioinformatic analysis identified 143 proteins possibly secreted by *L. mexicana* amastigotes, half of which have no known function. The data were used as a resource to select a particular metabolic pathway for further analysis with the aim to identify new targets for drug development.

EKV09
Evaluation of lysine biosynthesis as antifungal drug target

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Lysine biosynthesis of fungi has been assumed as an optimal target for new antifungal drugs, because (i) the pathway is absent in humans and (ii) several fungal mutants with deletions of lysine biosynthetic genes display strongly attenuated virulence in murine infection models. Here, we investigated an *Aspergillus fumigatus* strain with deletion of the first key enzyme in fungal lysine biosynthesis, the homocitrate synthase. This mutant can only grow in the presence of lysine. However, we assumed that proteins might provide a major carbon source during infection. Since proteins contain lysine, this might become released and support growth to complement virulence of the deletion mutant. To solve the question of a strongly attenuated virulence of the deletion mutant, despite lysine availability from protein degradation, we performed *in vitro* and *in vivo* studies. Growth tests revealed that conidia of the deletion mutant were unable to grow on intact proteins, whereas mycelium formed additional biomass. *In vivo* studies confirmed an importance of lysine biosynthesis especially during the onset of infection. Complementation of virulence of the deletion mutant by additional feeding of lysine strengthens the assumption that *de novo* biosynthesis of lysine is essential for the onset, but not for manifestation of infection. A consolidated view of these results indicates that drug targets directed against lysine biosynthetic enzymes would only be useful to prevent conidia germination but are not useful for therapy of an already manifested infection.

EKV10
A single UDP-Galactofuranose transporter is crucial for galactofuranosylation in *Aspergillus fumigatus*

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The human pathogenic fungus *Aspergillus fumigatus* is responsible for the severe and often fatal disease invasive aspergillosis. Antifungal agents launched so far primarily target membrane or cell wall biogenesis, but are still very limited. One major component of the *A. fumigatus* cell wall is galactomannan, a polysaccharide decorated with chains of galactofuranose (Gal*f*). This sugar is present in glycoconjugates of many pathogenic organisms including bacteria, fungi and parasites, but absent from higher eukaryotes. We have recently demonstrated that the absence of Gal*f* attenuates the virulence of *A. fumigatus* and *Leishmania major*.
The donor for galactofuranosylation reactions, UDP-Gal*f*, is provided by UDP-galactopyranose mutase, an enzyme that has recently been identified and localized to the cytosol. Galactofuranosylation of N-glycans and glycolipids, however, takes place in the lumen of the Golgi apparatus. Our aim was to characterize translocation of UDP-Gal*f* into the organelles of the secretory pathway.
In the genome of *A. fumigatus* we identified 16 nucleotide sugar transporter genes. One of them, referred to as *glfB*, encodes a Golgi localized integral membrane protein exclusively transporting UDP-Gal*f*, as shown by *in vitro* nucleotide sugar transport assays. Targeted deletion of the *glfB* gene yielded a mutant, Δ*glfB*, completely devoid of galactomannan, indicating that biosynthesis of galactomannan, in contrast to chitin and glucan, seems to take place along the secretory pathway. Moreover glycolipids and N-glycans were purified and analyzed by high performance thin layer chromatography and capillary electrophoresis, respectively. These analyses revealed a complete Gal*f* deficiency of the Δ*glfB* mutant, demonstrating that the UDP-Gal*f* specific nucleotide sugar transporter GlfB is crucial for all galactofuranosylation reactions in *A. fumigatus*.

EKV11
Farnesol misplaces tip-localized Rho proteins and inhibits cell wall integrity signaling in *Aspergillus fumigatus*

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The isoprenoid farnesol is a quorum sensing molecule of *C. albicans* and is known for inducing apoptosis in some fungi and mammalian cells. To evaluate its potential role as an antifungal agent, we studied its impact on the human pathogen *Aspergillus fumigatus*. We found that growth of *A. fumigatus* wild type is inhibited, but two cell wall mutants, Δmnt1 and ΔglfA, are much more susceptible to farnesol. This susceptibility is partially rescued by osmotic stabilization, suggesting that farnesol is a cell wall perturbing agent. However, farnesol does not activate but inhibit the cell wall integrity (CWI) pathway. Remarkably, a mutant lacking AfMkk2, a kinase essential for CWI signaling, is also highly susceptible to farnesol, suggesting that its mode of action goes beyond inhibition of CWI signaling. Farnesyl derivatives are known for interfering with the function of prenylated proteins. We analyzed the subcellular localization of two prenylated Rho family GTPases, AfRho1 and AfRho3, which are implicated in controlling CWI and the cytoskeleton. We found that under normal growth conditions AfRho1 and AfRho3 predominantly localize to the hyphal tip. After farnesol treatment this tip localization is rapidly lost, which is accompanied by swelling of the hyphal tips. We propose farnesol impairs the biological function of AfRho1 and AfRho3.

EKV12
Candida albicans GPI-anchored proteases modulate fungal cell wall functions and interaction with phagocytes

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Secreted aspartic proteases (Saps) are important virulence factors of the human pathogenic yeast *Candida albicans*. Two Sap family members, Sap9 and Sap10, are, in contrast to other Saps, not secreted to the extracellular space but glycosylphosphatidylinositol (GPI)-anchored on the cell surface. We have previously shown that both proteases play a role in cell wall integrity and during interaction with epithelial cells.
To analyse which *C. albicans* cell surface proteins are proteolytically targeted by Sap9 and Sap10, we digested isolated cell walls with recombinant Saps. Using LC/MS/MS we detected the release of seven GPI-anchored proteins following protease digestion. These potential target proteins have distinct functions from cell wall remodelling to host cell adhesion. For two candidates, Rbt5 (role in haem-iron utilization) and Cht2 (chitinase), functional assays revealed that protein activities are reduced in the absence of Sap9 or Sap10.
Since the cell surface is the major contact point between *C. albicans* and the immune system, we expected that Sap9 and Sap10 activities influence *C. albicans*-phagocyte interactions. Indeed, we showed that the lack of *SAP9* causes an increased ingestion of *C. albicans* by human macrophages. This effect can be explained by an enhanced recognition of β-1,3-glucan. This fungal cell wall component is normally masked by a mannoprotein layer and gets exposed in a *Δsap9* deletion mutant.
Another key attribute of *C. albicans* virulence is hyphal formation. While Sap10 seems to be dispensable for this process, we discovered that Sap9 is involved in the regulation of filamentation under hypoxic conditions. Sap9 could therefore function in hypoxic adaptation and morphological regulation during infection. Our data provide evidence for a regulatory action of Sap9 and Sap10 on specific proteins of the fungal cell surface, modulating distinct cell wall functions and host cell interactions, thus contributing to the pathogenicity of *C. albicans*.

EKV13
Sexual stage specific adhesion proteins of *Plasmodium falciparum* as potential targets for transmission blocking vaccines

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Future strategies in combating the tropical disease malaria have to target multiple life cycle stages of plasmodial parasites in order to reduce not only the infection, but also spread of the mosquito-born disease. The sexual reproduction of the malaria pathogen *Plasmodium falciparum* mediates parasite transition from the human to the mosquito and thus represents a crucial step in the propagation of the disease. Upon uptake of infected human blood by the female Anopheline mosquito the differentiation of gametocytes to gametes and the following fertilization events are triggered in the insect midgut. During gametocyte formation in the human blood, the six members of the *Pf*CCp protein family, characterized by the possession of multiple adhesion domains, are expressed. The proteins are associated with the parasites plasma membrane leading to their exposure on the surface of macrogametes after activation in the midgut. Recent co-immunoprecipitation and co-elution binding assays indicated that the six *Pf*CCp proteins form multi-protein complexes on the parasite surface, where they further interact with additional sexual stage-specific adhesion proteins, like *Pf*s230, *Pf*s48/45 and *Pf*s25. We propose that these adhesive protein complexes either mediate binding of macrogametes to factors of the blood meal or promote contact between microgametes and macrogametes prior to fertilization. Recently we could show that antibodies against select *Pf*CCp proteins are able to block the emergence of microgametes after *in vitro* activation of gametocytes in a complement-dependent manner. A newly established insectary at the Research Center for Infectious Diseases in Würzburg gives us the opportunity to transfer our findings to the *in vivo* model and evaluate sexual stage adhesion proteins as possible candidates for transmission blocking vaccines.

FGP01
The Genomic Encyclopedia for Bacteria and Archaea (GEBA) Project: An Update

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Though the wide variety of microbial sequencing projects undertaken throughout the world has created a rich, diverse collection of microbial genomes, strong biases in what has been sequenced thus far are evident. Following the recent recommendation to coordinate an effort to construct draft genome sequences of each of the roughly 8000 known type strains, the Genomic Encyclopedia for Bacteria and Archaea (GEBA) project by JGI and DSMZ is aimed at systematically filling in the gaps in sequencing along the archaeal and bacterial branches of the tree of life. It also represents the first attempt to use the tree of life itself as a guide to sequencing target selection. To demonstrate the feasibility of the GEBA approach, we here provide the results from a phylogenomic analysis of the first 56 complete genomes (released to the community through a web site: http://www.jgi.doe.gov/programs/GEBA/index.html). Despite recent claims that Archaea and Bacteria do not form a part of the tree of life, inferring trees from whole genome sequences appears to mainly present technical challenges but not inherent difficulties regarding their biological interpretation. Significant differences between the whole-genome and the 16S rRNA phylogeny are likely. We thus anticipate that the bulk of type strain genomes to be released in the next years will be of great value for a better understanding of the evolutionary history of microbial species.

FGP02
γ-EC and Other Low Molecular Weight Thilos Compensate Loss of Glutathione in *Escherichia Coli*

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Reduced oxygen forms very toxic Reactive Oxygen Species (ROS). The presence of too much ROS is called oxidative stress. An important self-defence system against ROS in bacteria like *E. coli* is the glutathione (GSH) system. GSH is the main thiol-redox buffer in *E. coli*. It is thought to protect cells against the negative impacts of ROS, like damage of DNA, lipids, or proteins, while maintaining the thiol-redox state of cells. Δ*gshA*- and Δ*gshB*-mutants, with a disrupted biosynthesis of glutathione, however, show no apparent growth phenotype under standard conditions, when compared to wildtype. This suggested to us that other Low Molecular Weight Thiols (LMWT) in *E. coli* could be compensating for the loss of GSH in these mutants. Measurements of LMWT in Δ*gshA* and Δ*gshB* knock out mutants confirmed this hypothesis. The total concentration of LMWT is higher in the mutants while the ratio between reduced and oxidized LMWT change to a higher amount of oxidized LMWT. HPLC analyses confirmed the absence of glutathione in both mutants. However, our analyses showed an increased level of γ-Glu-Cys, a GSH-precursor, in the Δ*gshB*-mutant. Enzymatic tests with glutathione reductase revealed that γ-Glu-Cys unlike other LMWT commonly found in *E. coli*, including cysteine and homocysteine, acts as a substrate to this enzyme with a K_m of 604 μM. These experiments suggest that other LMWT, such as γ-Glu-Cys, can partially assume the function of glutathione in *E. coli*.

FGP03
Principles of vertical and lateral gene transfer in *Streptococcus mutans*

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Streptococcus mutans (SM), a major contributor to dental caries and infective endocarditis, is a genetically highly diverse species consisting of numerous (>90) multi-locus-sequence-types and four serotypes (c, e, f, k). Recently, the second complete genome of an SM-strain belonging to serotype c (NN2025, isolated 2002 in Japan) has been sequenced and analysis has shown a large genomic inversion across the replication axis in comparison to the reference genome sequence UA159 (isolated 1982 in the US). More genome information will soon be generated in our BioInSys-project (MedSys, BMBF 0315411) leading to a better understanding or even definition of what the SM core-genome versus the SM pan-genome might be. For selecting strains to undergo genome sequencing we have sequenced the 16S rRNA gene from 45 SM-strains along with closely related streptococci (i.e. *S. ratti, S. criceti, S. macacae, S. sobrinus*) that had been collected in our laboratory from clinical samples or were obtained from academic or public strain collections. Nine strains with distinct genetic alterations based on sequence analysis of the nearly complete 16S rRNA gene and with variation in phenotypical characteristics (e.g. bacteriocine-production, susceptibility to biofilm-inhibitors, epidemiological and clinical background) were finally selected. Results about the genome structures and gene variation, especially those related to adhesion and cariogenic properties, will be presented. Furthermore we have designed species-specific PCR-primers to test our strain collection for the presence of mobile elements, such as plasmids. Since, SM-strains are known to possess a natural defense mechanism (CRISPR) against phages, plasmids -together with IS and transposon-like elements- were found to be the important elements responsible for lateral gene transfer.

FGP04
Phenotypic *Pseudomonas aeruginosa* landscape uncovers new functional relatedness of genotypes

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Recent technical advances in acquiring genome-wide data has lead to substantial progress in identifying gene functions. However, we still do not know the function of a large number of genes and, even when a gene product has been assigned to a functional class, we cannot normally predict its contribution to the phenotypic behaviour of the cell or organism – the phenome. Here we provide evidence that most genotypes in the pathogenic bacterium *Pseudomonas aeruginosa* are not phenotypically discrete but rather form a part of a continuum of related phenotypes, and that phenotypic overlap reflects the activities of homologous genes that specify related functions. In this study, a genome-wide simultaneous analysis of 116 distinct growth-related phenotypes provided a comprehensive *P. aeruginosa* phenome and identified overlapping phenotypic traits that link complex mutant phenotypes through key shared features. Description of the phenomic landscape of an organism functionally links its genomics, systems biology and phenotype, by defining complex trait phenotypes that cover genetically homogenous categories and revealing unpredicted genetic links.

FGP05
The Role of *Escherichia coli* Alkyl Hydroperoxide Reductase Subunit F in Detoxification of Nitric Oxide

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Escherichia coli alkyl hydroperoxide reductase subunit F (AhpF) is able to reduce toxic peroxides together with AhpC *in vivo*. For intramolecular electron transfer it harbours two highly conserved redox active CXXC motifs and two further cysteins which likely do not contribute to enzyme activity. In a previous study AhpF was shown to respond to nitric oxide stress by an increase in its thiol oxidation state. The N-terminal domain of AhpF shows homology to the N-terminal domain of eukaryotic protein disulfide isomerase (PDI), a protein that is able to denitrosylate other proteins. We, therefore, wanted to test if AhpF is directly involved in detoxification of nitric oxide through denitrosylation of cysteins. Phenotypic studies revealed that spermine NONOate treatment inhibited *E. coli ahpF* knockout as well as *E. coli* wildtype but no difference between the strains could be shown. A spectrophotometrical measurement of hypothetical S-nitrosoglutathione reduction by AhpF showed no denitrosylation activity. These findings suggest a more indirect role of AhpF in the defence against nitric oxide stress.

FGP06
Serratia odorifera 4Rx13 – volatile emission and genome analysis

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The rhizobacteria *Serratia odorifera* 4Rx13 emits a complex bouquet of organic as well as anorganic volatiles (1, 3), which are known to inhibit the growth of various organisms during co-cultivation (2, 5). Approximately 100 volatiles are emitted, one major compound comprises approximately 45 %, 10 compounds contribute each between 2 to 10 %, and the majority of ca. 70 compounds were emitted at very low levels (< 1 %). The main compound of *S. odorifera* was recently structurally elucidated ('sodorifen', 6), but many volatile structures remain unknown. To unravel and elucidate the underlying biosynthetic pathway of 'sodorifen' and other volatiles, we initiated the sequencing of the genome of *S. odorifera*. Pyrosequencing employing the Titanium technology (Roche 454) revealed the complete genome of *S. odorifera* 4Rx13. It is comprised of 5.36 Mbp and has a GC content of 56.16 %. One plasmid was found. The sequences were submitted into the NCBI data base. Automated and manual annotations revealed ca. 4500 ORFs of which ca. 1200 ORFs were without known or defined sequence/gene information. The latter ORFs may help to identify pathways involved in the biosynthesis and regulation of new volatiles.

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FGP07
The diversity and abundance of PAH-ring hydroxylating dioxygenase genes studied by a novel PCR detection system revealed soil type-dependent responses to phenanthrene

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Cultivation-independent studies on the occurrence and diversity of polycyclic aromatic hydrocarbon ring hydroxylating dioxygenase (*pah-rhdα*) genes in environmental samples are impeded by limitations of existing primer systems. Here we report a novel PCR primer system that targets a wide range of *pah-rhdα* genes of both gram-positive and gram-negative bacteria. The specificity and target range of the primers predicted *in silico* was confirmed experimentally with isolates carrying different *pah-rhdα* genes and by cloning and sequencing of amplicons from soil DNA. The novel primer system was used to study changes in *pah-rhdα* genes in a Cambisol from Ultuna, Sweden, and a Luvisol from Scheyern, Germany, in response to phenanthrene. At Day 21, *pah-rhdα* genes were only detected in the Luvisol, and at Day 63, in both soils. In controls not spiked with phenanthrene the genes remained below the detection limit. Cloning and sequencing showed the dominance of *phnAc* genes in the contaminated Luvisol. In contrast, in the Cambisol a high diversity of *pah-rhdα* genes responded to phenanthrene contamination. Genes known from both gram-positive and gram-negative bacteria as well as previously undescribed genes could be detected due to the broad specificity of the novel PCR system. Quantitative PCR based on the same primers revealed that 63 days after spiking *pah-rhdα* genes were one order of magnitude more abundant in the Luvisol than in the Cambisol. In conclusion, the soil type determined the response to phenanthrene with respect to diversity and abundance of *pah-rhdα* genes.

FGP08
Development and verification of viability tests for granulated microorganisms

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This project is part of the joint research project WIGRATEC which deals with fluidized bed granulation. Aim of the present study is to develop new methods for testing the viability of granulated microbial starter cultures.
To test the viability and activity of microorganisms currently methods like colony forming units (CFU) and the Live/Dead BacLight Assay (Invitrogen) are used. During production and as a quality control tool for granulated starter cultures it would be beneficial to have a fast method for detecting bacterial viability. Therefore within the framework of this project new markers for setting up alternative assays are developed by means of protein analysis (1D- and 2D-electrophoresis) and MALDI-TOF-MS (Matrix-assisted laser desorption/ionization time of flight mass spectrometry). These assays will then be used to test different charges of granulated microorganisms to determine their viability. Aim of the project is to develop an assay for viability detection that is able to fulfill the requirements of an assay used in routine process control.
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FGP09
Characterization of RepMp1-containing genes in *Mycoplasma pneumoniae*

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Mycoplasma pneumoniae (M.p.) is a frequent cause of infections of the human respiratory tract. Despite a remarkable reduction of the genome size 5% of the genome consist of repetitive sequences (RepMp). Four types of RepMp's (1, 2/3, 4, 5) differing in number of copies, size and sequence can be found all over the genome of the completely sequenced strain M129. One copy each of RepMp2/3, RepMp4 and RepMp5 are localized in the P1 operon coding for the adherence-associated and immunodominant proteins P1, P90 and P40. The subtypes and variants of M.p. vary in the RepMp copies of the P1 operon suggesting a modification of surface proteins by recombination. Regarding the further genes containing RepMp sequences limited information is available with respect to function, localization and genotype-specific differences of the proteins. In the present study the known 16 RepMp1-carrying genes were characterized. In strain M129 (subtype 1) all genes were transcribed and can also be detected in the other subtypes and variants of M.p.. Sequence analysis resulted in differences of the RepMp1-containing genes between the genotypes. After exchange of the TGA codons the recombinant proteins reacted with an antiserum against the cytosolic fraction of M.p. proteins. Consequently, only few sera of test animals obtained after infection with M.p. and sera of patients with confirmed infection with M.p. showed a reaction with the recombinant proteins. Polyclonal sera against the RepMp1-containing recombinant proteins reacted only partly with M.p. whole cell preparations indicating a low concentration of the proteins in the M.p. cell. Nevertheless, the antiserum against the product of the RepMp1-carrying gene mpn130 reacted strongly with an up to now unknown 45 kDa protein. The results provide for the first time a systematic insight into occurrence, sequence differences and localization of RepMp1-containing proteins in M.p..

FGP10
Prediction of genes involved in *Campylobacter* colonisation of chickens using a bioinformatic approach

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A novel approach was followed to predict which bacterial genes potentially contribute to a complex phenotype, using multiple genome sequences. In the presented test case, *Campylobacter* genes required for commensal colonisation of poultry were predicted. First, the literature was screened for *Campylobacter* knockout genes that had shown to have a negative effect on chicken colonisation. Next, a database was generated containing all protein-coding genes from 11 genomic *Campylobacter jejuni* and *C. coli* sequences. The relevant KEGG and GO terms for each gene were added to this database in such a way that these could be mined for association. Thus, by use of the KEGG and GO terms of proven colonisation genes, alternative genes were identified that had identical or similar terms. Three tested GO systems and the KEGG approach each identified large numbers of genes, but by combining all four approaches into one selection, a shortlist of 189 *Campylobacter* genes was produced that were possible colonisation gene candidates. This list was further shortened for genes that were conserved as single copies in every sequenced *Campylobacter* genome analysed. This resulted in a shortlist of 24 genes, present and conserved in the 11 analysed genomes, each of which shared 3 GO terms and KEGG descriptions with at least one known colonisation gene. The approach illustrates how genomic datamining can result in predictions of gene function in complex phenotypes, that can initiate hypothesis-driven laboratory work.

FGP11
Functional analysis of the carotenoid gene cluster of *Thermus thermophilus* HB27

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Many members of the hyperthermophilic genus *Thermus* are able to produce carotenoid pigments. The main end products of the carotenoid biosynthesis in *T. thermophilus* HB27, thermozeaxanthins and thermobiszeaxanthins, have been characterized and identified as being zeaxanthin mono- or diglucoside fatty acid esters (Yokoyama et al., 1996). The unique structural features of these thermoxanthins have been connected with the establishment of appropriate membrane fluidity for growth at high temperatures and/or protection of the membranes against a photosensitized reaction.
Analysis of the genome of *T. thermophilus* HB27 has revealed that a cluster of about 13 carotenogenic genes (car cluster) is present on the megaplasmid pTT27 (Henne et al., 2004). Apart from the key gene *crtB*, coding for phytoene synthase, the precise function of these genes has not been studied. It is also unknown if the carotenogenesis in T. thermophilus is constitutive or subjected to regulation, nor what are the signals and factors that are involved.
We have constructed gene deletion and gene overexpression strains for 6 of the ORFs of the vicinity of the carotenogenic cluster. Analysis of the phenotypes of these strains suggests that carotenoid biosynthesis in *T. thermophilus* is regulated. For example, the deletion of the gene for a transcriptional regulator of the Crp family (TT_P0055) as well as the overexpression of a neighboring transcriptional regulator of the MerR family (TT_P0056) led to a colorless cell phenotype. We could also identify a small protein (65 amino acids) of unknown function (TT_P0053) that is required for the complete formation of carotenoid pigments. Among the other car cluster genes whose functions we are investigating are TT_P0061 and TT_P0062, coding for putative acetyltransferase and glycosyltransferase, respectively. Deletion of TT_P0062 led to no visible color phenotype or growth defects, however, the carotenoids formed showed a significantly altered behavior when analysed by thin-layer chromatography. Structural studies of these presumable thermoxanthin intermediates as well as further investigation of the detected regulatory circuits are underway.

FGP12
Biomineralization of gold in *Cupriavidus metallidurans* CH34

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The heavy metal resistant bacterium *Cupriavidus metallidurans* CH34 forms biofilms on Au grains. The formation of such secondary Au grains is commonly attributed to abiotic processes. But it was shown that the bacterium rapidly accumulates toxic Au(III)-chloride complexes from solution. This promotes Au toxicity, and *C. metallidurans* reacts by inducing a number of general oxidative stress- and metal resistances- (*ohr, cup, cop, mer, ars*) gene clusters and a Au-specific (Rmet_4682-4687) cluster to promote cellular defense. Microarray analysis leads to the identification of these general and specific Au gene clusters. All genes of the cluster (Rmet_4682-4687) were highly expressed by Au(III)-complexes which was verified by RT-PCR. It could be determined that four genes (Rmet_4682-4685) form an operon, which is under control of the ECF sigma factor RpoQ.

C. metallidurans seems to catalyze formation of gold particles by active biochemical processes. The biomineralization of Au nano-particles in </i>C. metallidurans</i> CH34 is the result of Au-regulated gene expression leading to this energy-dependent reductive precipitation of toxic Au(III)-complexes. As a result, Au detoxification is mediated by a combination of efflux, reduction and possibly methylation of the Au-complexes, leading to the formation of Au(I)-C-compounds and the deposition of metallic Au particles within the cells. Transmission electron microscopy (TEM) analyses showed nanoparticles of Au inside of the bacterial cell after incubation in Au(III) hydrochloride. Similar Au particles are common in bacterial biofilms on natural Au grains, indicating that Au biomineralization actively contributes to the formation of secondary Au grains in surface environments.
Growth at different Au-complexes were tested due to their toxic effects to *C. metallidurans* CH34 cells on solid Tris buffered mineral medium.

FGP13
Genomic islands of *Legionella pneumophila* Corby

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Exchange of genetic information by horizontal gene transfer is an important mechanism for the evolution of bacterial genomes. Horizontal gene transfer was also observed in *Legionella pneumophila* (*Lp*) and other *Legionella* species. Recently we identified and characterized a new *trb/tra* conjugation type IVA secretion system in the genome of *Lp* Corby. Two similar versions of this conjugation system are localized on two seperate genomic islands (Trb-1 and Trb-2) integrated within the tRNAPro gene and the tmRNA gene, respectively. Both islands exhibit an *oriT* region and encode all essential *trb/tra* genes require for conjugation. We also could determine that Trb-1 as well as Trb-2 can exist in an integrated chromosomal or an episomal circular form. For Trb-1 we could demonstrate that the whole island can be transferred to other *Legionella* strains and integrated site-specifically into the genome of the transconjugants. By analysing the genome of *Lp* Corby we identified two additional genomic islands (Lpc-GI3 and Lpc-GI4). GI3 and GI4 are flanked by a tRNA gene and exhibit several putative integrases. Both islands exhibit a *Legionella vir* region (*lvr*) encoding a CsrA homologue, the genes *traG, traD, virB4, pilT* and a *hel* gene locus. In addition, GI4 contains genes encoding an ATP synthase and various efflux systems for heavy metals and other toxic substances. Similar genomic islands are also present in the genomes of *Lp* Philadelphia, Paris and Lens. We could demonstrate that both genomic islands can exist in an integrated chromosomal and an exercised episomal form, similar to Trb-1 and Trb-2. Additionally, we analysed the distribution of genes, characteristic for the described genomic islands, within the genus *Legionella* by Southern hybridization. On the basis of these results we could conclude that similar or identical regions also present in various *Legionella* strains.

FGP14
Comparative Genomics of the *Myxococcales elucidates* the evolutionary history of a complex developmental program

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Objectives: To investigate the evolution of the molecular mechanisms involved in Myxococcales development, we compared the genomes from four species: *M. xanthus, S. aurantiaca, A. dehalogenans*, and *S. cellulosum. A. dehalogenans* genome is approximately half the size of most Myxococcales, and it does not develop, allowing us to use it to identify developmentally important proteins conserved only in the other genomes.
Orthologous proteins pairs between each set of species were identified using a best-match reciprocal BLAST strategy. This information was then used to analyze the conservation of: 1) 94 genes from *M. xanthus* known to be directly involved in development; and 2) 837 *M. xanthus* genes that are transcriptionally regulated during development, identified using microarray analyses.
Results: We defined a core Myxobacterial proteome consisting of 1328 proteins, of which 1069 were also found in non-Myxococcales species. Conservation levels of the *M. xanthus* proteins paralleled the phylogenetic relationships with the other proteins, with the highest conservation seen with the most closely related species and much lower conservation seen with the most distantly related species. Analysis of proteins conserved specifically amongst the three developing species revealed no significant bias for either the known development proteins or those transcriptionally regulated during development. However, we did observe a significant bias of conservation of the *M. xanthus* development proteins in *S. aurantiaca*.
Conclusions: These comparisons suggest the *M. xanthus* and *S. aurantiaca* developmental programs share a significant core and discredits the hypothesis that *M. xanthus, S. aurantiaca* and *S. cellulosum* share a significant common genetic program leading to fruiting body formation.

FGP15
Parallel genomic evolution of Candidatus „Endomicrobium trichonymphae“ genomes from Trichonympha protists in termites

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Candidatus „Endomicrobium trichonymphae“ (CET) are vertically transmitted endosymbionts of *Trichonympha* protists in the hindgut of lower termites. Genome sequencing has revealed the presence of many pseudogenes and numerous gene duplications in the genome of CET strain Rs-D17, the endosymbiont of *Trichonympha agilis* in *Reticulitermes speratus*. In this study, we compare the genome of strain Rs-D17 with the genome fragments of other CET lineages from a large metagenome library obtained from the assemblage of *Trichonympha protists* in *Zootermopsis nevadensis*.
The genome fragments of the metagenome showed the same patterns of functional preservation as strain Rs-D17 including complete pathways for amino acid and cofactor biosynthesis. Likewise, similar genes are depleted in the different endomicrobia strains, such as those involved in outer membrane biosynthesis. Nevertheless, strain Rs-D17 and the endomicrobia represented in the metagenome differ in their levels of pseudogenization and in the positions of individual mutations in homologous genes. This indicates that similarities in the genomes are not inherited characteristics but similar selective pressure led to parallel genomic evolution of CET in different *Trichonympha* hosts. Together with the small genome of CET, the large number of pseudogenes and the evidence for genome rearrangements suggests that the „Endomicrobium“-*Trichonympha* symbiosis is still developing towards a stable integration of endosymbiont and host.

FGP16
Functional genome analysis of the purine-utilising bacterium *Clostridium acidiurici*

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Clostridia are Gram-positive, anaerobic, and endospore-forming bacteria. These organisms are found in a wide range of different environments such as soils and the intestinal tract of humans and animals.
Sequencing of the *C. acidiurici* genome was done by the Goettingen Genomics Laboratory employing the 454 GS FLX XLR Titanium pyrosequencing technology. Sequences were assembled into contigs using the Newbler assembly tool from Roche. The estimated genome size and the GC content are 3 Mb and 29.74%, respectively. These values are in the range that is described for other clostridia (2.5 to 4.5 Mb and 28 to 32%, respectively). To close remaining gaps and to identify misassembled regions caused by repetitive sequences, different PCR-based techniques are currently employed. To elucidate the genome content and the unique metabolism of *C. acidiurici* annotation and genome comparisons are performed.
Clostridium acidiurici is a purine-utilizing Clostridium. It is able to use purines like uric acid and xanthine as sole carbon, nitrogen, and energy source. The major fermentation products from these substrates are ammonia, carbon dioxide, and acetic acid. It is unable to degrade complex nitrogen-containing substrates such as tryptone or yeast extract.
Automatic annotation indicates the existence of common metabolic pathways like glycolysis/gluconeogenesis and pentose phosphate pathway.
However, *C. acidiurici* revealed no growth on different hexoses and pentoses, such as glucose, fructose or ribose, thereby indicating an inability to use these metabolic pathways.

FGP17
New cellulolytic and hemicellulolytic enzymes derived from a thermophilic microbial community

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In order to find new cellulolytic and hemicellulolytic enzymes, but also to get insight into the microbial diversity in certain thermophilic habitats, we constructed metagenome DNA libraries and subjected them to functional and sequence analysis. In particular, a fosmid library constructed with DNA from a naturally heated (67°C) and slightly alkaline (pH9.3) sample from the crater of Avachinsky in the Kamchatka region was screened for cellulase, xylanase and β-glucosidase activities. Analysis of the fosmids conferring xylanase and cellulose activity by low coverage shotgun sequencing and primer walking led to the identification of an ORF coding for a potential xylanase and an ORF coding for a potential cellulase, Xyn1015 and Cel29E. BLAST analysis showed that the closest homolog of Xyn1015 was a GHF 10 protein from *C. saccharolyticus* (40 % identity) and of Cel29E a GHF 5 protein from *Thermus caldophilus* (60 % identity). Both genes were cloned in the expression vector pET101 and the encoded enzymes were overexpressed in *E. coli* BL21.
Recombinant Xyn1015 (50 kDa) was active against diverse xylans, with beech wood xylan being the best substrate and Cel29E (38 kDa) showed activity against CMC, lichenan and barley beta-glucan. The maximum hydrolytic activity of Cel29E and Xyn1015 was found to be at 96°C. Xyn1015 was active over a pH range from 6-8. The pH optimum of Cel29E was at pH7. Hydrolysis products indicated that Xyn1025 acts as an endoxylanase, Cel29E as an endocellulase. The therm stability against thermal inactivation was investigated. Cel29E had a half life of 4 min at 96°C and at 106°C a half life of 1.3 min. At 86°C it had a half life of 16 min. Xylanase Xyn1015 showed a long term stability against thermal inactivation with a half life of 22 hours at 95°C. Both enzymes displayed, partially, a high hydrolytic activity and stability in different ionic liquids (ILs).

FGP18
Comparative Genomics of Green Sulfur Bacteria

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Eleven completely sequenced Chlorobi genomes were compared in oligonucleotide usage, gene contents and synteny. The green sulfur bacteria are equipped with a core genome that sustains their anoxygenic phototrophic lifestyle by photosynthesis, sulfur oxidation and carbon dioxide fixation. Whole-genome gene family and single gene sequence comparisons yielded similar phylogenetic trees of the sequenced chromosomes indicating a concerted vertical evolution of large gene sets. Chromosomal synteny of genes is not preserved in the phylum Chlorobi. The accessory genome is characterized by anomalous oligonucleotide usage and endows the strains with individual features for transport, secretion, cell wall, extracellular constituents and a few elements of the biosynthetic apparatus. Giant genes are a peculiar feature of the genera Chlorobium and Prosthecochloris. The predicted proteins have a huge molecular weight of 10 million or more and are probably instrumental for the bacteria to generate their own intimate (micro)environment.

FGP19
Genome Diversity of *Pseudomonas aeruginosa* PAO1 laboratory strains

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Pseudomonas aeruginosa PAO1 is the most commonly used strain for research on this ubiquitous and metabolically versatile opportunistic pathogen. Strain PAO1, a derivative of the original Australian PAO isolate, has been distributed worldwide to laboratories and strain collections. Over decades discordant phenotypes of PAO1 sublines have emerged. Taking the existing PAO1-UW genome sequence (named after the University of Washington which led the sequencing project) as a blueprint, the genome sequences of reference strains MPAO1 and PAO1-DSM (stored at the German Collection for Microorganisms and Cell Cultures (DSMZ)) were resolved by physical mapping and deep short read sequencing-by-synthesis. MPAO1 has been the source of near-saturation libraries of transposon insertion mutants and PAO1-DSM is identical in its SpeI-DpnI restriction map with the original isolate. The major genomic differences between MPAO1 and PAO1-DSM in comparison to PAO1-UW are the lack of a large inversion, a duplication of a mobile 12 kb prophage region carrying a distinct integrase and protein phosphatases or kinases, deletions of 3 to 1006 bp in size and at least 39 single nucleotide substitutions, 17 of which affect protein sequences. The PAO1 sublines differed in their ability to cope with nutrient limitation and their virulence in an acute murine airway infection model. Subline PAO1-DSM outnumbered the two other sublines in late stationary growth phase. In conclusion, *P. aeruginosa* PAO1 shows an on-going microevolution of genotype and phenotype that jeopardizes the reproducibility of research. High-throughput genome re-sequencing will resolve more cases and could become a proper quality control for strain collections.

FGP20
A C-terminal hyphal activation motif (CHAM) of the transcriptionfactor Tec1p involved in protein – promoter interactions is essential for morphogenetic development of *Candida albicans*

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Candida albicans is the most frequent human fungal pathogen and infections range from superficial mucosal lesions to life-threatening invasive mycoses with a high mortality rate. Hyphal growth of *C. albicans* and the transcriptional regulation of putative virulence traits for adaptation to the host environment are key issues during pathogenesis. *C. albicans* Tec1p is a member of the TEA transcription factor family, which shares a conserved DNA-binding TEA domain in the N-terminal part of the proteins. Tec1p has previously been shown to be involved in the signalling events that lead to hyphal formation, and a *tec1/tec1* deletion mutant does no longer form hyphae *in vitro* and is avirulent *in vivo*. In order to define a structure function relationship of the *C. albicans* Tec1p protein, we integrated modified open reading frames (ORFs) of the wildtype *TEC1* under the control of the *C. albicans* -adapted reverse Tet-dependent transactivator (rtTA) into the *tec1/tec1* mutant CaAS12 and used Tet-induced hyphal formation as a read-out for Tec1p-activity. Transformation of CaAS12 with ORF constructs either containing a deletion of the C-terminus from 637 to 744 aa or a deletion of the TEA domain from 216 to 244 aa could no longer confer TET-inducible hyphal growth, while transformation with ORF constructs without any deletion or a deletion of the N-terminal 150 aa restored hyphal growth during incubation with TET. After expression of modified ORFs of *TEC1* in *E. coli*, we tested for binding of recombinant rTec1p to the promoter consensus sequence TCS. A C-terminal Deletion of 480 to 744 aa abolished gel retardation of a TCS probe. In conclusion, we show that a C-terminal hyphal activation motif (CHAM) is essential for Tec1p function, because it is necessary for the protein-promoter interaction.

FGP21
Studies on *Gluconobacter oxydans* 621H by DNA microarray analyses

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Gluconobacter oxydans is a strictly aerobic α-proteobacterium that plays an important role in industrial biotechnology due to its capacity to incompletely oxidise a wide variety of sugars, alcohols and polyols. It is used e. g. for production of vitamin C, ketogluconic acids, dihydroxyacetone or vinegar and numerous further applications can be envisaged. Despite its industrial importance, knowledge of the metabolism of *G. oxydans* and its regulation, but also of its stress responses, is still very scarce. Using oligonucleotide-based DNA microarrays, we studied two aspects of *G. oxydans* physiology, the response to iron-limiting conditions and the influence of carbon substrates with a different oxidation state on global gene expression.
It was shown that during iron limitation several genes that encode proteins for iron uptake are highly upregulated, such as ABC transport systems. Interestingly, genes for flagella synthesis and chemotaxis were also significantly upregulated, indicating that iron limitation has an influence on the motility of *G. oxydans*.

When mannitol is used as carbon source by *G. oxydans*, it is rapidly and almost exclusively oxidised to fructose in the periplasm, accompanied by a high oxygen consumption rate. When fructose is used as carbon source, growth behaviour was shown to be very different. The growth rate was halved, oxygen consumption was much lower, but carbon dioxide production was higher. The latter result indicated a higher flux through the pentose phosphate pathway (PPP) and thus higher sugar uptake rates. Transcriptome analyses comparing cells grown on fructose with cells grown on mannitol supported this assumption. In particular, genes of the PPP were upregulated on fructose as well as genes for transporter systems that could participate in fructose uptake. In contrast, genes of the Entner-Doudoroff pathway were downregulated.

FGP22
The transcriptome of meningococci under *in vivo* mimicking conditions

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Neisseria meningitidis (the meningococcus) is a commensal of the upper airways in about 10% of the healthy human population. For reasons that are still mostly unknown, meningococci can sometimes cause life threatening infections especially in infants and young adults such as septicemia or acute bacterial meningitis. Based on their capsular polysaccharide they can be divided into 12 serogroups. Strains of serogroup B are of particular concern because they are a major cause of invasive disease in Europe and the United States, and there is currently no licensed vaccine available. We first wanted to analyze the changes in the transcriptional pattern in the invasive meningococcal serogroup B strain MC58 as well as the non-pathogenic serogroup B carriage strain α522 under *in vitro* conditions mimicking those encountered by the bacteria within the human host during invasive infection using oligonucleotide based whole genome microarrays. In addition, the samples were also subjected to ultra-deep sequencing to further characterize the transcriptomes with respect to the small non-coding RNAs not covered by conventional microarrays. As animal models covering all aspects of meningococcal carriage and disease are still lacking, we first established an *in vitro* system mimicking the conditions encountered by the bacteria on their route from the human nasopharynx to the human meninges and allowing for the preparation of sufficient amounts of bacterial RNA for further analyses. Accordingly, the two strains MC58 and α522 were exposed to human saliva representing the nasopharyngeal niche, human whole blood representing the blood stream niche, and human cerebrospinal fluid representative of the meningeal niche. RNA from the two strains after exposure to the various human materials were isolated and sequenced and microarray analyses were preformed. The results of the ongoing analyses will be discussed in the present study.

FGP23
Proteomic studies of conditional cell division mutants

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Because of the advanced spread of antibiotic-resistant bacteria new antibiotic agents are urgently needed. Existing antibiotics mostly target a small number of essential cellular functions such as cell wall, DNA, protein, or RNA biosynthesis. Some of the other essential cellular processes have not yet been clinically exploited to any meaningful extent. One of these – cell division - represents an attractive new target for inhibitors [1]. To evaluate the utility of several cell division gene products as potential targets we created *Bacillus subtilis* conditional mutants. In the cell, down-regulation of the target gene simulates protein inhibition by inhibitors [2]. To this end, the gene of interest was placed under the control of a glucose-repressible promoter while the gene at the original locus was knocked-out. The resulting mutants were analyzed under permissive and repressing conditions. First, *ftsL* and *divIC* mutants were characterized microscopically. We then examined the proteome of the cell division mutants and wild type using 2D-gelelectrophoresis. This approach facilitates visualization of the global changes in the cytosolic protein complement. The resulting proteomic response patterns can serve as references patterns and aid the identification of the mechanism of action of novel cell division inhibitors [3].

[1] D. J. Haydon *et al.*, Science, 321: 1673-5 (2008)
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FGP24
Functional genome analysis of *Paenibacillus larvae*, the causative agent of the American Foulbrood of honey bees (AFB)

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Paenibacillus larvae is a rod-shaped and spore-forming Gram-positive bacterium causing American Foulbrood of honey bees. First *P. larvae* has been described as *Bacillus larvae* in 1906. Recently, it was shown that the species *P. larvae* comprises different genotypes differing in virulence at the individual insect and at the colony level [1]. *P. larvae* is able to infect honeybees and honeybee larvas via the spores, but only kills the latter. The way of infection and killing is still poorly understood. It has been shown, that approximately 10 infectious spores from virulent strains are sufficient to cause mortality [2].
Raw-sequencing of the *P. larvae* genotype ERIC II genome was done by using 454-pyrosequencing. The obtained sequences were assembled and analyzed. The genome size and the GC content are approximately 3.6 Mb and 45 %, respectively. To close remaining gaps and sort the contigs, whole genome shotgun libraries were created in plasmid vectors and sequenced. Additionally, a fosmid-library was constructed. The annotation of the genome sequence provided new important insights into genes involved in pathogenesis.
In addition, the prokaryotic diversity analysis of the honeybee larval midgut and the brood comb honey (i.e. stored honey used as larval food) were performed by employing 16S -rRNA gene-analysis. The gut contained members of the γ-Proteobacteria, Firmicutes, and Cyanobacteria and Chloroplasts, while the brood comb honey contained only members of the Firmicutes.

[1] Genersch *et al.*, Int. J. Syst. Evol. Microbiol. 56, 501-511 (2006)
[2] Brodsgaard *et al*., Apidologie 29, 569-578 (1998)

FGP25
Functional anaylses of conserved hypothetical chloroplast open reading frames: the *Synechocystis ycf34* gene

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Plant and algal chloroplast genomes mainly contain genes involved in photosynthesis and housekeeping of the organelle. The remaining genes include open reading frames of unknown function and have been designated *ycf* for hypothetical chloroplast open reading frame. No final conclusion can be illustrated about these *ycfs* without functional analysis of the resulting gene products. Some of these *ycfs* are highly conserved in all plastid genomes of higher plants and algae. The gene *ycf34* is a hypothetical chloroplast open reading frames that occurs only in a limited number of algal plastid genomes and is highly conserved in all lineages of cyanobacteria. We report here on the functional analysis of the cyanobacterial *ycf34* gene product using an insertion mutant generated in *Synechocystis* sp. PCC 6803. We show that Ycf34 is a new small protein possibly involved in the assembly of the cyanobacterial light harvesting antenna, the phycobilisomes. The mutant has a significantly lower level of phycocyanin as revealed by 77K fluorescence spectroscopy under light conditions, which require changes in the composition of the phycobilisomes. The expression of an epitope-tagged Ycf34 fusion protein restored the wild-type phenotype. Genetically engineered strains of *Synechocystis* expressing the FLAG-tagged Ycf34 fusion protein will be further used for the identification of interacting protein by co-immunoprecipitation and analysis of the localisation of Ycf34.

FGP26
Genome reconstitution of the ammonia-oxidizing archaeon "*Candidatus* Nitrososphaera gargensis"

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Novel ammonia-oxidizing *Archaea* (AOA) have been identified as the key players for the first step of nitrification in thermal environments [1]. The moderately thermophilic ammonia oxidizing crenarchaeote "*Candidatus* Nitrososphaera gargensis" was originated from a biofilm of the Garga hot spring in the Baikal rift zone [2] and shows high ammonia-oxidizing activities at elevated temperatures. It grows at an incubation temperature of 46 °C in a community with different bacteria and was enriched to a final ratio of more than 90 %. Nine bacterial species were detected in the enrichment. The complex metagenome of this microbial consortium was sequenced by 454 pyrosequencing and ~ 200 Mb data were generated in order to assemble and annotate the whole genome of "*Cand.* Nitrososphaera gargensis". 32 of the 6.700 contigs with a size of ~ 2.6 Mb were binned as contigs of "*Cand.* Nitrososphaera gargensis" and annotated. Only 10 % of the coding sequences were definitely related to known functions and nearly 60 % were of unknown functions. Furthermore, we constructed a metagenomic fosmid library containing 2.500 clones. End sequencing of 500 fosmid clones allowed to physically map 27 contigs into one large scaffold with a size of 2.57 Mb. Current work focuses on closing the gaps and binning of the obtained contigs to assemble the whole genome of "*Cand.* Nitrososphaera gargensis". This will be one of the first assembled and annotated genomes of a moderately thermophilic ammonia-oxidizing crenarchaeote.

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[2] Hatzenpichler, R.; Lebedeva, E. V.; Spieck, E.; Stoecker, K.; Richter, A.; Daims, H. and Wagner, M. (2008). A moderately thermophilic ammonia-oxidizing crenarchaeote from a hot spring. PNAS, Vol. 105, No. 6: 2134-2139

FGV01
Swarming of *Pseudomonas aeruginosa* is controlled by a complex regulatory network

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Pseudomonas aeruginosa is capable of swarming on semi-solid (viscous) surfaces. Our recent studies have indicated that swarming is more than just a form of locomotion and represents a complex adaptation resulting in changes in virulence gene expression and antibiotic resistance. In this study, we used a comprehensive *Pseudomonas aeruginosa* PA14 transposon mutant library to investigate how the complex adaptation process of swarming is regulated. A total of 247 *P. aeruginosa* PA14 transposon mutants were verified as having alterations in swarming motility. These swarming-associated genes functioned not only in flagella or type IV pili biosynthesis, but also in processes as diverse as transport, secretion, and metabolism. Thirty-three swarming deficient and 2 hyper-swarming mutants had transposon insertions in transcriptional regulator genes including those encoding two-component sensors and response regulators; 27 of these were newly identified. Of the 25 regulatory mutants highly impaired in swarming motility (79-97%), only 1 (PA1458) had a major defect in swimming suggesting that this regulator might influence flagella synthesis or function. Twitching motility, which requires type IV pili, was strongly affected in only 2 regulatory mutants, pilH and PA2571 and moderately affected in 3 others algR, ntrB, and nosR. Microarray analyses and follow up experiments were performed for selected regulatory mutants to identify the corresponding regulon. We also identified more than a dozen swarming mutants with insertions in genes encoding for diguanylate cyclases and phosphodiesterases which are involved in the synthesis and degradation of the second messenger molecule cyclic diguanylate (c-di-GMP), respectively. These mutants were further characterized with respect to virulence, motility and biofilm formation. Overall, our data provide evidence that swarming is controlled by a complex regulatory network and may be part of an alternative growth state for *P. aeruginosa*.

FGV02
Identification and functional analysis of putative Hfq dependent ncRNAs in Synechocystis sp. PCC 6803

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Hfq is a versatilely acting, ubiquitous bacterial RNA chaperone that manages multiple regulatory processes. One of its best studied molecular functions is the support of post-transcriptional regulation events mediated by small non coding RNAs (ncRNAs). Orthologues of Hfq were detected in a variety of cyanobacteria [1]. Our attempts to identify novel cyanobacterial ncRNAs that might be targeted by Hfq in *Synechocystis sp.* PCC 6803 included comparative transcriptional analysis on a high-density oligonucleotide microarray, covering one third of the genome. This approach revealed four putative ncRNAs from intergenic regions (IGRs) with drastically reduced transcript levels in the hfq knock-out mutant over the wild-type cells. Northern Blot analyses could demonstrate that cellular levels of these RNAs increase upon heat stress and in the stationary growth phase when Hfq is present. This data suggest a physiological relevance of these ncRNA candidates in *Synechocystis sp.* PCC 6803 under environmental stress conditions. The low amount of discovered ncRNA candidates is in line with the relatively restricted phenotypic alteration of the Δ*hfq* mutant [2], still it might be expanded by using a more comprehensive microarray design. Furthermore, an RNA co-immunoprecipitation approach with a FLAG-tagged Hfq-protein followed by deep sequencing analysis [3] is in the pipeline.

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FGV03
Helicobacter pylori genome evolution during human infection

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H. pylori is one of the most diverse and variable bacterial species. Its remarkable allelic diversity is generated by a high mutation rate, combined with frequent recombination during mixed infection. In order to elucidate genomic changes occurring during human infection, we used Roche/454 sequencing technology to determine genome sequences of five pairs of sequential *H. pylori* isolates. Four pairs of strains were isolated sequentially (time interval, 3 years) from Colombian individuals. The fifth pair consisted of challenge strain BCS 100, used to infect a volunteer in a vaccine trial performed in Germany, and one reisolate (8A3), cultured after three months of infection.
454 sequencing was performed with 20-30 fold coverage, and contigs were arranged as virtual genomes for each strain using the published genome of *H. pylori* J99 as a scaffold. These genomes were aligned in pairs in order to identify and quantify single nucleotide polymorphisms (SNPs), and clusters of nucleotide polymorphisms (CNPs), which are a hallmark of recombination.

The pairs from Colombian individuals differed by 27 to 241 SNPs, and by 17 to 442 CNPs. The average length of CNPs was ~400 bp, consistent with earlier model-based estimates of import length in *H. pylori* (Falush et al., PNAS 2001). By contrast, the pair obtained from the vaccine trial showed no CNPs and only three SNPs.
The data reveals genome-wide recombination in *H. pylori* strains from a high-prevalence country, leading to replacement of a major fraction of the chromosome during chronic infection. These imports were not distributed randomly over the chromosome but were partially grouped, suggesting that in some cases large DNA fragments may enter the bacterial cell and subsequently be integrated into the chromosome in multiple small pieces. Annotation of the import events provided evidence of selection operating on the family of outer membrane protein genes.

FGV04
Methods for the phylogenetic inference from whole genome sequences and their use in prokaryote systematics

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To establish a novel species, the currently dominating, pragmatic species concept for prokaryotes requires to demonstrate that the similarity between its genomic DNA and the DNA of closely related reference species (represented by their type strains), as inferred using DNA-DNA hybridization methods, is lower than 70%. However, this technique is cumbersome and cannot easily be made reproducible and thus is currently carried out in only a few molecular labs in the world. Due to the recent staggering advances in DNA sequencing technology, prokaryotic genomes can be obtained in steadily decreasing time and at steadily decreasing costs. Hence, it is likely that routine sequencing of nearly complete genomes will become an integrated part of biodiversity research on prokaryotes within the next few years. However, beyond sequencing techniques, algorithms to calculate distances and similarities between partial or full genome sequences need to be devised that are both reasonably fast and able to reflect biologically sensible differences. We here describe several approaches to infer genome-genome distances and discuss them regarding their correlation with DNA-DNA-hybridization values, their computational speed, their robustness regarding the use of only partially sequences genomes, and their ability to infer whole-genome phylogenetic trees in addition to pairwise distances. The techniques have been implemented as a user-friendly web service to calculate the similarity between reference and query genomes available at http://www.gbdp.org/species/ .

FGV05
Uncovering the interaction in mixed species biofilms: The use of a new 5 species expression array

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Biofilm communities represent an ubiquitous lifestyle of many bacteria, also of commensals and pathogens of the oral cavity. Bacterial commensal species can cause serious illnesses like heart disease in susceptible and immunocompromised human hosts. Pathogenic oral bacteria are the cause of infectious diseases like periodontitis. For our studies we selected *S. mutans, S. mitis, S. intermedius, S. sanguinis, S. salivarius, M. micros, P. gingivalis, F. nucleatum* and *A. actinomycetemcomitans* representing an assortment of oral streptococci and anaerobic oral pathogens. Not much is known on the transcriptional level during the setup and establishment of such mixed species biofilms. In order to monitor transcriptional changes during the lifespan of mixed species biofilms we developed a five species expression array based on the NimleGen® platform. The full genomes of *S. mutans, S. sanguinis, P. gingivalis, F. nucleatum* and *A. actinomycetemcomitans* were represented on these arrays. Investigation of two species biofilms of *S. mutans* combined with *S. mitis*, and *P. gingivalis* combined with *S. salivarius*, using these novel arrays uncovered many transcriptional changes of genes for metabolism as well as environmental information processing and genetic information processing categories.
The co-cultivation of *S. mutans* with *S. mitis* results in up regulation of 41 genes and down regulation of 4 genes in *S. mutans*. In parallel phenotypic assays an increased biofilm mass in the combination compared to single species biofilms was observed. The majority of regulated genes were involved in carbohydrate metabolism and membrane transport. The combination of *P. gingivalis* and *S. salivarius* showed the same phenotype accompanied by the regulation of more than 600 *P. gingivalis* genes.
These new arrays are a powerful tool for understanding the complexity of bacterial mixed species biofilms. The introduction of further species could clarify the molecular background of microbial cohabitation in such biofilms.

FGV06
A Blueprint of Ectoine Metabolism from the Genome of the Industrial Producer *Halomonas elongata* DSM 2581^T

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The halophilic γ-proteobacterium *Halomonas elongata* DSM 2581^T thrives at high salinity by synthesizing and accumulating the compatible solute ectoine. Ectoine can be used as a stabilizer for enzymes and as a cell protectant in skin and health care applications and is thus produced annually on a scale of tons in industrial processes using *H. elongata* as producer strain. Here we present the complete genome sequence of *H. elongata* (4,061,296 bp) and experiments and analysis identifying and characterizing the entire ectoine metabolism, including a newly discovered pathway for ectoine degradation and its cyclic connection to ectoine synthesis. The degradation of ectoine (doe) proceeds via hydrolysis of ectoine (DoeA) to Nα-acetyl-L-2,4-diaminobutyric acid, followed by deacetylation to diaminobutyric acid (DoeB). In *H. elongata* diaminobutyric acid can either flow off to aspartate or re-enter the ectoine synthesis pathway, forming a cycle of ectoine synthesis and degradation. Interestingly, genome comparison revealed that the ectoine degradation pathway exists predominantly in non-halophilic bacteria unable to synthesize ectoine. Based on the resulting genetic and biochemical data, a metabolic flux model will be introduced, which provides a basis for a model-driven improvement of industrial ectoine production. As indicated by the model, mutagenesis of the degradation pathway resulted in a strain of *H. elongata* with higher volumetric productivity for ectoine.

FTP01
From Saulus to Paulus: "The Bacterial Effector Protein YopM Inhibits the Production of Molecules Relevant in Rheumatoid Arthritis (RA)"

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Previously, we identified and characterized the ability of the bacterial effector protein YopM of pathogenic *Yersinia* to enter host cells independent of translocation by a type III secretion system. Furthermore, once inside the cells, YopM is able to down-regulate major pro-inflammatory cytokines such as TNFα. These novel findings suggest a potential immunotherapeutic application. Hence, we were interested to investigate whether YopM might have potential as a "self-delivering" immune therapeutic agent by exhibiting a beneficial effect on inflammation and cartilage destruction linked to rheumatoid arthritis (RA). RA is a chronic, systemic autoimmune disorder that causes inflammation and tissue damage in joints. RA-synovial fibroblasts (RASFs) are active drivers of joint destruction and maintain the inflammatory conditions in RA. Therefore, we used activated RASFs as model cells and analyzed that recombinant YopM can penetrate these cells by confocal laser scanning microscopy. Furthermore, we investigated the inhibitory effect of YopM on the TNFα-induced production of the cartilage-degrading matrix-metalloproteinases (MMP-1 and MMP-3) by ELISA. Interestingly, YopM is also capable of preventing structural damage by inhibition of RANKL-induced osteoclastogenesis of murine bone marrow cells.

These combinatory effects of YopM might be applicable in antirheumatic therapy to reduce inflammation and structural damage. With respect to a potential *in vivo* application of YopM for the treatment of RA, we investigated the distribution of Cy5-conjugated YopM by Fluorescence Reflection Imaging (FRI) in mice after intra-articular (i.a.) injection into the knee joints of the hind legs. Apparently, YopM-Cy5 remained at the site of injection and did not distribute systemically during a time period of 48 h. These results combined with the possibility for focussed application in skeletal joints enhance the potential of YopM as a novel therapeutic agent for the treatment of inflammatory RA.

FTP02
Zinc uptake in *Cupriavidus metallidurans* CH34

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Zinc is an essential cofactor in many enzymes. *Cupriavidus metallidurans* strain CH34 is a well-characterized metal-resistant bacterium known for its content of multiple efflux systems for transition metal cations. But informations concerning the transition metal uptake are scarce. *C. metallidurans* lacks the highly efficient high-affinity Zn(II)-uptake system ZnuABC, probably due to its adaption to environments containing already high zinc concentrations. So, how does *C. metallidurans* take up zinc?
C. metallidurans contains a member of the ZIP (Zrt/Irt-like, TC 2.A.5) protein family, an ortholog of ZupT from *Escherichia coli*. $ZupT_{Cm}$ seems to transport Zn(II), Mn(II) and Co(II) ions. In *C. metallidurans* and in contrast to *E. coli*, *zupT* gene expression was induced by metal chelators like EDTA, TPEN or DTPA. Additionally, *C. metallidurans* contains the unusual high number of four members of the MIT (CorA metal ion transporter, TC 1.A.35) protein family, which usually transport many different transition metal cations in addition to the main substrate Mg(II). One of these, $CorA_1$, was induced by magnesium starvation. Finally, transition metal cations like Zn(II) can enter the cell in the form of phosphate complexes, which are imported by the low affinity inorganic phosphate transporter PitA (TC 2.A.20.1.1). To determine the contribution of the four CorA-like proteins, ZupT and PitA to Zn(II) uptake in *C. metallidurans* single and multiple gene deletions were constructed using the Cre-Lox system. The resulting mutants were characterized in growth and metal uptake experiments as well as by quantification of gene expression under high and low metal conditions.

FTP03
Starvation-influenced carbonylation patterns of the initial enzyme from the herbicide-degrading strain *Delftia acidovorans* MC1

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A huge spectrum of compounds is released to the environment by the chemical industry and finally the consumers. For example phenoxyalkanoates such as 2,4-dichlorophenoxyacetate (2,4-D) and (*RS*)-2-(2,4-dichlorophenoxy) propionate ((*RS*)-2,4-DP) have been applied as herbicides for about 70 years. Anyhow herbicide degrading microorganisms were found, what raises the question how they adapt to structures that exhibit a xenobiotic character but also how microbes behave under fluctuating pollutant conditions. Growth of *Delftia acidovorans* MC1 on the herbicide (*RS*)-2,4-DP followed by starvation resulted in the appearance of different variants of the α-ketoglutarate-dependent dioxygenase RdpA. As key enzyme RdpA initiates the degradation of (*RS*)-2,4-DP. The RdpA variants, made visible by 2D gel electrophoresis, differ in their isoelectric points (pI). They obviously originated from posttranslational modification, since there is only one *rdpA* gene in the genome. To understand this phenomenon, the hypothesis was tested that RdpA modification resulted from carbonylation by reactive oxygen species (ROS), which are known side products of dioxygenase reactions. Starvation leads to an increase of oxidative stress and thus an enhanced release of ROS. The consequences were followed up during starvation considering changing enzyme activities and enzyme patterns. Carbonylated amino acids in proteins of starved cells were specifically derivatized with 2,4-dinitrophenylhydrazine. Mass spectrometry was used to verify the presence of carbonylated peptides in RdpA peptide mass fingerprints.

FTP04
Lipid binding mechanism of DivIVA revealed by genetic and crystallographic analyses

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DivIVA is a highly conserved lipid binding protein and functions in various morphogenetic processes in Gram-positive bacteria. In *Bacillus subtilis*, the protein localizes to the cell division site and the cell poles. It is essential for the correct placement of the septum at midcell, and also for sporulation. Although lipid binding activity could be attributed to the 60 aa N-terminal lipid binding domain (LBD) [1], the molecular mechanisms by which DivIVA binds to or is inserted into the phospholipid bilayer are far from being understood. Here we present the crystal structure of *B. subtilis* DivIVA. We demonstrate that DivIVA forms a tetrameric parallel coiled coil with unusual knotted loop structures at the N-terminal ends of the tetramer. Using genetic and biochemical approaches we could show that the knot structure is essential for membrane binding and thus for *in vivo* activity. From one *divIVA* mutant that specifically interferes with lipid binding but leaves the structure of the lipid binding domain intact we have isolated an intragenic suppressor mutation that restores lipid binding and localisation. Based on our findings we present a new model describing how proteins carrying DivIVA-like LBDs interact with phospholipid bilayers.

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FTP05
Control of lipopolysaccharide biosynthesis by regulated proteolysis of LpxC

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The outer leaflet of the outer membrane of Gram-negative bacteria consists of phospholipids and lipopolysaccharides (LPS) and its composition has to be tightly regulated [1]. In *E. coli* the deacetylase LpxC is the key enzyme in biosynthesis of lipid A, the hydrophobic membrane anchor of LPS. Degradation of LpxC by the ATP-dependent and essential AAA^+-protease FtsH adjusts the amount of LPS [2]. Using heterologous LpxC proteins we learned that degradation of LpxC is a common but not entirely conserved mechanism in Gram-negative bacteria. This makes LpxC a potential drug target for new antibiotics.
Proteolysis of LpxC requires a C-terminal degradation signal (LAxxxxxAVLA). Pointmutations in or the absence of this degradation signal resulted in increased stability of LpxC [3, 4]. The stability of LpxC is related to the growth rate. At slow growth rates LpxC shows a half-life of about 10 minutes. At higher growth rates the half-life increases up to 10-12 fold. We assume that this stabilization ensures the correct synthesis of the LPS, even if the cells grow rapidly. The influence of factors that might contribute to growth phase regulation of LpxC like polyphosphates or ppGpp is under investigation.

[1] Raetz (1990) *Annu. Rev. Biochem.*, 59: 129-170
[2] Ogura *et al* (1999), *Mol. Microbiol.*, 31: 833-44
[3] Führer *et al* (2006), *Mol. Microbiol.*, 59: 1025-3
[4] Führer *et al* (2007), *J. Mol. Biol.*, 372: 485-96

FTP06
Analysis of oxidative Stress in *Corynebakterium glutamicum* and the impact of metal ion homeostasis

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Since the appearance of cyanobacteria on the planet earth oxiative stress is a daily problem for most of the bacteria and means the occurrence of ROS (*reactive oxygen species*) including hydrogen peroxide (H_2O_2), Superoxide (O_2) or the hydroxyl radical ($HO^{\cdot}$). Interestingly, the response towards oxidative stress was addressed in several bacteria including *E. coli* or *B. subtilis* but is poorly characterized in *C. glutamicum*. We aim to identify sources and targets of ROS in *C. glutamicum* as well as to understand the bacterial stress response.

We constructed a mutant lacking catalase, the enzyme that is responsible for the removal of H_2O_2. We characterized the sensitivity of this mutant towards H_2O_2 as well as a-biotic stress factors like heat or alterations of the pH. Additionally, we observed a strong impact of metal ion homeostasis on growth of the catalase mutant and therefore on the appearance of H_2O_2. We addressed the impact of iron, manganese, copper, zinc and nickel on growth of *C. glutamicum*. Whereas for most of them no specific toxic effect on the catalase mutant was observed in comparison to WT cells, iron and manganese availability were critical for survival of the *C. glutamicum* catalase mutant. We set up experiments in order to identify transport systems involved in the uptake and excretion of iron and manganese. We will present data on an ABC-type transport system and a secondary active transporter of the NRAMP family including the impact of these transporters on uptake of ions and the oxidative stress response of *C. glutamicum*.

FTP07
Genome analyses of *Bdellovibrio bacteriovorus*HD100 - mutant strains possessing a host-independent lifecycle

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Bdellovibrio bacteriovorus HD100 is a small and motile bacterium, which is characterized by predatory behaviour. *Bdellovibrios* attack other Gram-negative bacteria, penetrate into their periplasm, multiply within the periplasmatic space and finally burst the cell envelope of their host bacteria to start the cycle anew.
Although *Bdellovibrios* possess an obligatory parasitic life cycle, it is possible to isolate variants that no longer require living host cells for growth. These host-independent variants complete the transition from attack phase to growth phase and back again on heat-killed prey bacteria or even on standard complex bacteriological media.
In 1992 T.W. Cotter & M.F. Thomashow detected, that host-independent mutants of the strain *B. bacteriovorus* 109J, which grow axenically on rich media, possess frame shift mutations in a small open reading frame within the *hit* locus (*host interaction locus*). By contrast, another study in 2001 reported that sequence alterations within this region occurred only in three out-of seven mutants.
In our previous studies we isolated 30 host-independent mutants of the strain *B. bacteriovorus* HD100 in two different ways and analysed the *hit* gene (*Bd0108*) sequence. The obtained results indicated frame shift mutations of *Bd108* in every mutant.
To get a better insight into the ongoing processes, which causes the *Bdellovibrio* lifecycle switch, more comprehensive genetical data are necessary. Therefore we analysed the complete genome sequence of the mutant strain *B. bacteriovorus* HD100-M1. The sequencing results indicated mutations in four different genes.
Referring to these data we are currently analysing the genome sequence of five more HD100-mutant strains. The comparison of the resulting data should help us to understand the genetical process that enables these bacteria to variegate their unique lifecycle.

FTP08
Statistical validation of protein phosphorylations in cell signalling from high throughput MS/MS data

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Current MS technology has become highly accurate and can sport tens of thousands of spectra in automated peptide sequencing experiments (LC-MS/MS). In addition, it has become possible to detect post-translational modifications (PTMs) of a sequence like phosphorylations. These phosphorylations are known to mediate intra cellular signal transduction. Therefore, the regulation of an individual modified peptide often reveals the biological activity of the corresponding protein. Fortunately, isotopic labelling strategies such as iTRAQ basically allow to quantify proteins at the peptide level. However, beside established statistical concepts to validate protein and phosphosite identifications the robustness of regulatory peptide information is still incompletely characterized. Thus data mining usually relies on artificial thresholds to claim significant regulations instead of utilizing rational statistical approaches.
We have developed a means to model these uncertainties statistically and to calculate the likelihood of regulation for any given peptide detected by mass spectrometry. This method already opened new avenues for signal transduction studies revealing participating signalling components by significantly regulated post-translational modifications. Noteworthy, this concept gives insight both into altered modification and expression levels which are the prerequisite for its application also in cancer research. In conclusion, our method provides a novel, statistically sound validation for high-throughput MS data targeting the dynamics of PTMs in parallel to the relative protein expression levels.

FTP09
Survival of different animal viruses in the gut of leeches (*Hirudo medicinalis*)

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The introduction of leeches as a therapeutic in human medicine is currently considered to be of high value in the treatment of vascular diseases. Since animal blood is used to feed the parasites, they possibly harbour different pathogens including viruses. Consequently, the possibilily to get infected by subsequent medical application was discussed. This risk may be decreased by keeping the leeches in quarantine prior to use. Therefore, the aim of this study was to determine the time in quarantine necessary to effectively reduce the number of selected viruses in the gut of inoculated leeches. The parasites were divided into different groups and fed with porcine blood spiked with defined loads of bovine parvovirus (BPV), feline calici virus (FCV), equine arteritisvirus (EAV) and equine herpesvirus type 1 (EHV1), respectively. The groups were sampled prior to feeding and at regular time points post feeding. The leeches were held in aquariums at 10°C throughout the whole sampling period. However, a part of them was held at 30°C after an initial 6 week phase at 10°C. Analysis of virus titres of parasites continuously held at 10°C demonstrated a decrease below the detection limit within 22 weeks (FCV), 23 weeks (EAV), 27 weeks (EHV1) and 29 weeks (BPV), respectively. Increasing the temperature up to 30°C resulted in a shorter survival time of the viruses. Virus titres dropped below the detection limit within 15 weeks (EAV, EHV1), 21 weeks (FCV) and 27 weeks (BPV), respectively. Thus, keeping leeches at 30°C was of low time saving effect, as it shortened the quarantine for only two weeks regarding the results obtained with BPV. Based on these findings and adding another 2 week period for safety reasons, we suggest keeping leeches potentially contaminated with viruses in quarantine for at least 31 weeks at 10°C. Regarding the high initial virus load used in the experiments this procedure is believed to represent a very stringent safety standard.

FTP10
Purification and characterization of a novel sulfite dehydrogenase in *Ruegeria pomeroyi* DSS-3

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Sulfite tends to cause oxidative stress, and sulfite-oxidizing enzymes (SOEs) detoxify it to sulfate. The relevant SOE, whose electron transport is understood, is the periplasmic, heteromultimeric sulfite dehydrogenase (SorAB) in *Starkeya novella* [1]. SorA interacts with the molybdenum cofactor, and the electron acceptor is cytochrome *c* (SorB). Periplasmic SorT in *Sinorhizobium meliloti* also interacts with the molybdenum cofactor, but presumably interacts with a membrane-bound electron acceptor [2], as does the SorT in *Delftia acidovorans* and the SorA from *Cupriavidus necator* [3]. SorA (cd02114) and SorT (cd02110) cluster in related clades of "sulfite oxidases" generated by NCBI, and the nascent protein has a molecular mass of about 44 kDa. Aerobic, marine *Ruegeria pomeroyi* DSS-3 utilizes L-cysteate and taurine as the sole source of carbon and energy for growth, and an SOE is involved in detoxifying sulfite [4]. However, no orthologue of *sorA* or *sorT* was found on the genome. The SOE was ferricyanide-coupled and was purified 57-fold to homogeneity. The monomer, molecular mass 11 kDa, could be identified in the genome sequence. The 11 kDa corresponded to the mature protein after cleavage of a predicted leader peptide, so the soluble enzyme is presumably periplasmic. The enzyme was trimeric and removed sulfite from reaction mixtures to form sulfate. The specific activity was 113 mkat sulfite oxidised/kg protein. In reverse transcriptase PCR experiments, transcription of the 3-gene cluster was inducible. We presume that we have isolated a novel sulfite dehydrogenase.

[1] Kappler *et al.* (2000) J Biol Chem, 275: p. 13202-13212
[2] Kappler *et al.* (2009) BBA-Bioenergetics, 1787: p. 1516-1525
[3] Denger *et al.* (2008) Microbiology, 154: p. 256-263
[4] Denger *et al.* (2006) Biochem J, 394: p. 657-664

FTP11
Myrosinase activity in lactic acid bacteria

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Glucosinolates are secondary metabolites found in cruciferous vegetables of the genus Brassica such as Brussels sprouts, kohlrabi, broccoli and cabbage. They are broken down after a tissue damage–triggered activation by specialized β-thioglucosidases (myrosinases) in plants or after consumption in the small intestine or in the colon. Myrosinase cleaves off the glucose moiety and the remaining molecule then quickly converts to an isothiocyanate, a nitrile, or a thiocyanate. Due to their anti-carcinogenic and antimicrobial properties of metabolic products glucosinolates have generated considerable interest for the food and pharmaceutical industry.
In bacteria myrosinase activity has been only scarcely reported for e.g. the microbiota of the human intestine or a single strain of Lactobacillus. Neither enzymes nor genes are published. Moreover, plant myrosinase genes do not give significant hits blasting bacterial genomes.
In this study we screened 26 strains mainly lactobacilli for degradation of the two glycosinolates Sinigrin and Sinalbin isolated from the sprouts of black mustard (Brassica nigra) and Sinalbin isolated from white mustard seeds (Sinapis alba), respectively.
Glycosinlate degradation was monitored by enzymatic measurement of glucose or detection of volatile metabolites (isothiocyanate, nitriles) by headspace-SPME-GC-mass spectrometry.
Sinigrin cleavage activity was detected in strains of *L. brevis, L. agilis, L. buchneri, L. crustorum, L. paralimentarius* and *L.pentosus*.
Sinalbin cleavage activity was detected in *L. agilis*, *L.brevis, L. buchneri, L. casei, L. crustorum, L. kimchi, L. paracasei, L. pentosus, L. plantarum, L. reuteri, L. rhamnosus, L. rossiae*.
Preincubation of strains with glycosinolate containing plant extracts lead to induction of myrosinase activity in certain strains but was also negatively correlated (repression) in other strains.

FTP12
Role of flagella and motility for *Shewanella oneidensis* MR-1 community formation

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The γ-proteobacterium *Shewanella oneidensis* MR-1 is motile by means of a single polar flagellum. Depending on the environmental sodium-ion concentration, the flagellar motor is driven by Na^+- or proton gradients. It has been shown previously that the initiation of cell-surface interactions and subsequent community formation of *S. oneidensis* MR-1 are strongly affected by flagella-mediated motility. However, the underlying mechanisms are still not understood. That prompted us to conduct a detailed study on the role of the flagellar filament, the motor, and chemotaxis in different stages of *S. oneidensis* community formation in static and hydrodynamic systems.
Analysis of corresponding deletion mutants revealed that all components are required for mediating initial adhesion. Mutants lacking the flagellar filament by deletion of the flagellin encoding genes (Δ*flag*) were more severely affected in attachment to the substratum, suggesting an adhesive role for the filament. Interestingly, highly motile chemotaxis mutants (Δ*cheA*) were also affected in the transition from planktonic to sessile life style. For initial attachment it was also not critical whether protons or sodium-ions were used as coupling ions for motor functions, as demonstrated by single mutants in the two alternative stator systems, PomAB and MotAB.
In hydrodynamic and static systems, mutants in motor functions and chemotaxis were unable to cover the surface and to form the distinct three-dimensional structures typical to the wild type. In contrast, non-filamented Δ*flag* mutants formed an unstructured three-dimensional community and accumulated more biomass than wild type cells. Staining with fluorophor-coupled lectins ConA and WGA revealed that Δ*flag* mutants produced large amounts of sugar-containing EPS, most likely exopolysaccharides. Notably, this only occurred during surface attachment and not during planktonic growth of the mutants.
From the results we conclude that the filament, flagellar rotation and chemotaxis are involved in initial adhesion and further structural development, moreover, the presence of the filament is required to modulate EPS production.

FTP13
Characterisation of the self-processing Bradyrhizobium japonicum protein NopE1

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Bradyrhizobium japonicum is the symbiont of soybean and several other legumes. We have shown that *B. japonicum* secretes proteins (Nops, nodulation outer proteins) upon induction by the plant signal genistein. We are interested in the type III-secreted effector protein NopE1 (484 amino acids), which exhibits autocleavage activity in the presence of calcium ions [1].
For this purpose NopE1 and its derivatives were expressed in *E. coli* as GST fusion proteins and purified using glutathione-sepharose affinity chromatography. The two cleavage sites are located in two similar domains of unknown function (DUF1521). Site-specific mutagenesis was used to exchange eight amino acids next to the C-terminal cleavage site ($IKGD_{359}$'PHVD) with alanine. Only the exchange of aspartate (D_{359}) or proline (P_{360}) resulted in non-cleavable protein variant. To determine the minimal domain with autocleavage activity, N- and C-terminal truncated NopE1 derivatives were created, indicating that the minimal domain comprises less than 200 amino acid residues. For further characterisation, different reaction parameters (temperature, pH and divalent metal ions) are tested in ongoing experiments. First results indicate that cleavage takes place within a wide temperature and a limited pH range.
This autocleavage attributes hold the potential to develop NopE1 as a tool for different applications in biotechnology.

[1] Wenzel *et al.* (2010). The type III-secreted protein NopE1 affects symbiosis and exhibits a calcium-dependent autocleavage activity. Mol. Plant-Microbe Interact., in press

FTP14
Bacterial sulfoglycolysis: the degradation of dihydroxypropanesulfonate

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2,3-Dihydroxypropanesulfonate (DHPS) and 3-sulfolactate (S-Lac) are natural products which were detected in plants and algae [1]. They originate from the metabolism of sulfoquinovose (SQ, the polar head group of plant sulfolipid), which was named "sulfoglycolysis" [1]. The ubiquity of the sulfolipid in plants and algae highlights the importance of degradative pathways for SQ and sulfoglycolytic products derived from it. In the bacterial degradation of SQ, DHPS can be excreted quantitatively but transiently, and S-Lac is an end product [2].
A bifurcated degradative pathway for S-Lac is now known [3], and we have evidence that DHPS is degraded *via* S-Lac. Our hypothesis involves two dehydrogenases converting racemic DHPS into the correct enantiomer, and a third dehydrogenase oxidising the latter to S-Lac. The work on S-Lac degradation revealed a widespread gene cluster, which we supposed to encode enzymes for the uptake of DHPS and its conversion to S-Lac. Bioinformatic analyses yielded 28 genome-sequenced bacteria sharing this cluster. All seven tested organisms grew with DHPS as carbon source, excreting sulfate. Anion exchange chromatography of cell extracts of *Cupriavidus pinatubonensis* JMP134 grown with DHPS showed three separable DHPS dehydrogenase activities. Peptide mass fingerprinting confirmed their correlation to the genes mentioned above. The S-Lac forming dehydrogenase was purified and characterized, and its product identified as S-Lac by MALDI-TOF-MS. The transcription of candidate genes involved will be studied by reverse transcription experiments, which might also shed light on transporters involved. Thus, together with the evidence for the presence of the known degradative pathway for S-Lac, we might be able to close another gap in the biological sulfur cycle.

[1] Benson and Lee (1972) BJ 128:29P
[2] Roy *et al.* (2003) AEM 69:6434
[3] Denger *et al.* (2009) JB 191:5648

FTP15
SuperScent - A comprehensive bacterial volatile collection

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Volatiles are efficient mediators of chemical communication acting universally as attractant, repellent, or warning signal in all kingdoms of life. Recently it was demonstrated that also bacteria are a rich source of volatile metabolite emission comprising compounds of known but also unknown structure. Chemical elucidation revealed substances with structures that are new to science. The vast number and diversity of volatile compounds lead to the establishment of the public database „SuperScent". This database supplies users with detailed information on the variety of odor components. The version of the database presented here comprises the 2D/3D structures of approximately 2,100 volatiles and around 9,200 synonyms as well as physicochemical properties, commercial availability, and references. The volatiles are classified according to their origin, functionality, and odorant groups. The information was primarily extracted from the literature and web resources. „SuperScent" offers several search options, e.g. name, Pubchem ID number, species, functional groups, or molecular weight.
„SuperScent" is available online at: http://bioinformatics.charite.de/superscent.

FTP16
Characterization of steroid degrading bacteria from the Baltic Sea at Kiel Germany

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Steroid contamination of sea water is an ever growing problem and impacts population dynamics of all kinds of sea animals. We have long experience with the soil bacterium *Comamonas testosteroni* which is able to catabolize a variety of steroids and polycyclic aromatic hydrocarbons, and which might be used in the bioremediation of contaminated soil. For our studies we use 3α-hydroxysteroid dehydrogenase/carbonyl reductase (3α-HSD/CR) as a reporter enzyme, since it is the key enzyme in steroid degradation. Moreover, the expression of the corresponding gene, *hsdA*, is induced by environmental steroids. In previous investigations we have identified and described several genes being involved in *hsdA* regulation. In this work we isolated several bacterial strains from the Baltic Sea at Kiel, Germany, which degrade steroids and which are able to use steroids as carbon source. Two of them, strain S19-1 and H5, were characterized as being gram negative. 16S-rRNA analysis showed that S19-1 belongs to *Buttiauxella noackiae* and H5 is similar to *Vibrio porteresiae*. They could be best grown in SIN medium supplemented with 0.6 – 5.1 % NaCl and at 20°C. Both S19-1 and H5 can use testosterone, estradiol or cholesterol as a carbon source in minimum medium. In *Comamonas testosteroni* about 20 enzymes could be induced by 0.5 mM testosterone. A new plasmid pKEGFP-2, suitable for metagenomic studies, and pGEM-EGFP were prepared for isolation of steroid inducible genes in S19-1 and H5. A 4.610 kb DNA fragment which contains the 3α-HSD/CR gene and its regulation elements from *Comamonas testosteroni* was cloned into plasmid pGEM-EGFP and pKEGFP-2. The result showed that testosterone induction could be detected by a microplate fluorescence reader after the plasmids were transformed into *E. coli* HB101 cells. Therefore, the system could be used to isolate steroid degradation and steroid regulatory genes from S19-1 and H5. In addition, the exact characterization and systematic classification of these marine steroid degrading bacterial strains is envisaged. The strains might be used for the bioremediation of steroid contaminations in sea water.

FTP17
Structures of the arm-type DNA binding domains of P4-like integrases encoded by genomic islands

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Genomic islands play a significant role in bacterial evolution. The horizontally acquired non-replicating islands have to rescue themselves by *attP* x *attB* recombination with the host chromosome. The integrase and associated *attP* recombination site are responsible for this interaction with conserved *attB* on the chromosome. The vast majority of the integrases of genomic islands possess high similarity to the P4 phage-like integrase and are grouped with P4-like recombinases. We have shown before that short (80AA) N-terminal integrase domain determines specific interaction with left and right parts (arms) of *attP* recombination site.
The structures of the N-terminal domains of two integrases of closely related but not identical asn tDNA-associated genomic islands, *Yersinia* High-Pathogenicity Island (HPI), and *Erwinia carotovora* genomic island HAI7, have been resolved. Both integrases utilize a novel four strand β-sheet DNA-binding motif in contrast to the known proteins that bind their DNA targets by means of the three-stranded β-sheets. Moreover the β-sheets in Int_{HPI} and Int_{HAI7} are longer than in other integrases and the structured helical N-terminus is positioned perpendicular to the large C-terminal helix. All these differences strongly support a proposal that the integrases of the genomic islands make up a distinct evolutionary branch of the site specific recombinases that utilize a unique DNA binding mechanism.

FTP18
Light by Spin Centers. A Magnetic Resonance Study of Bioluminescence in Bacteria

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Bioluminescence is one of nature's most remarkable and most visible reactions catalyzed by enzymes. It was discovered that the production of light is not accompanied by heat (Boyle, 1668), which was the first indication of the high efficiency with which light is produced. The emission of colored light by marine bacteria is generated by the oxidation of $FMNH_2$ and a long-chain aliphatic aldehyde, catalyzed by the luciferase [1]. Once $FMNH_2$ binds to the enzyme, it is protected from auto-oxidation. In the presence of oxygen, a flavin-hydroperoxide is formed that is still bound to luciferase, after which the reaction is proposed to continue *via* several radical intermediate states, in which the long-chain aldehyde participates [2]. The crystal structures of bacterial luciferases have become available only recently. The protein consists of a heterodimer of which the alpha and beta subunits have a slightly different molecular weight [3].
The cells from *Vibrio fischeri* are grown under aerobic conditions and the luciferase is purified *via* FPLC, whereby DEAE Sepharose turned out to be the most suited purification material. A light emitting reaction caused with decanal and reduced flavin gave evidence for the activity of the examined luciferase. UV-VIS spectroscopy of purified protein furthermore showed an absorption maximum at 411 nm and is a strong indication of the luciferase-bound flavin. The electronic structure of the reaction intermediates are investigated by electron paramagnetic resonance (EPR) spectroscopy.

[1] Baldwin T. O. & Ziegler M. M. (1992), in *Chemistry and Biochemistry of Flavoenzymes* (Müller F., ed) Vol. 3, pp. 467-530, CRC Press, Inc., Boca Raton, FL
[2] Macheroux P., Ghisla S., Hastings J. W. (1993) *Biochemistry*, 32, 14183-14186
[3] Fisher A. J. *et al.* (1995) *Biochemistry*, 34, 6581-6586

FTP19
SubtiPathways: Understanding Bacillus metabolism

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The Gram-positive soil bacterium *Bacillus subtilis* is a well-studied model organism. The great amount of accumulated research data needs to be presented in way easy to understand. Diagrams are one way to visualize these data. Already existing diagrams are a useful tool for system biologists but are not very helpful for lab work. The aim of this work was to generate a graphical system that can be used by system biologists and „bench biologists" as well. To recreate the metabolic pathways we used an easy to understand symbol language called SBGN [1]. We used the program CellDesigner that is based on SBGN to create maps of metabolic pathways of *B. subtilis*. The basis for this work, were already existing diagrams (KEGG, Biocyc), Databases (DBTBS, SubtiWiki) and hundreds of publications from PubMed. To grant access to scientists we built the website *Subti*Pathways (http://subtipathways.uni-goettingen.de) using the Google Maps™ technology and the programming language Python. Thereby an intuitively usable interface was created. To make the diagrams a useful tool, special features were added. Intermediates and enzymes were linked to external databases giving background information.

Besides these links - to the PubChem database and the *B. subtilis* specific wiki *Subti*Wiki [2] - known 3D protein structures and a short description were integrated for the enzymes. So far 25 diagrams covering different aspects of the metabolism like the carbon flow, amino acid metabolism or stress responses were created and are accessible. The interactive visualizations of this work provide a good overview for *Bacillus'* metabolism for "bench biologists" and can also serve as a fundament for further systems biology *in silico* reconstructions. In addition *SubtiPathways* provide an easy direct access to further information.

[1] Kitano *et al.* 2005 Nat Biotechnol 23(8), 961-966
[2] Flórez *et al.* 2009 Database Vol. 2009: doi: 10.1093/database/bap012.

FTP20
KC 5/2, an unusual endocytobiont of acanthamoebae and monocytic cells

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Introduction: In an acanthamoeba strain C 5/2 from a potable water reservoir an endocytobiont of unknown species was found. It was described as endocytobiont KC 5/2 (1-1.5 µm), maintained on NN-Agar according to Page, then transferred to axenic medium which allowed further characterisation. Extracellular growth could not be established. Infected amoebae died by cell rupture within 2-5 days after phagocytosis. Multiplication by fission with the formation of septa as known from prokaryotes or budding as known from viruses was not observed in our earlier studies.
In the present study the influence of KC 5/2 on the immune function of human monocytes and lymphocytes was investigated. In addition, physiological and structural data are provided.
Methods and results: Lymphocyte transformation tests (LTT) and cytokine production assay (ELISpot) in cocultures of human peripheral blood mononuclear cells (PBMC): Phythohaemagglutinin stimulation in the presence of KC 5/2 increased the interferon-gamma production and enhanced the cell proliferation of peripheral blood mononuclear cells. Antigen tetanus toxoid stimulated proliferation was significantly decreased.
Mass spectra obtained by MALDI-TOF-MS analysis of KC 5/2 did not match with spectra of any other microorganism.
Bacterial fatty acids were not found by gas chromatographic analysis (GLC).
KC 5/2 was not susceptible to antibiotics in therapeutical concentrations.
Electron microscopy revealed that KC 5/2 multiplicates in the cell lines THP-1 and U-937. Like amoebae, the infected cells died within 3-5 days.
In light microscopy the organisms could not be stained by Gram or nucleic acid stains, but by protein stains.
So far, nucleic acid analysis gave no evidence for microbial DNA or RNA.
Discussion: KC 5/2 is an obligate endocytobiont that kills acanthamoebae and monocytic cells. The unusual morphology and proliferation does not resemble any other microorganism.

FTP21
DipM, a cell division-related murein hydrolase in *Caulobacter crescentus*

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Cell wall peptidoglycan is a structural element that protects the cell from the effects of internal osmotic pressure. It is essential for the maintenance of cell shape and serves as a scaffold for the attachment of extracellular proteins. In the late stages of cell division, prior to the division of the daughter cells, new peptidoglycan has to be formed at the division site. This process requires a delicate balance between peptidoglycan synthesis and hydrolysis. Peptidoglycan hydrolases are essential for cleavage of the septum, but our knowledge about the regulation of their activity has remained limited. To elucidate the processes involved in the late stages of cell division in the model organism *Caulobacter crescentus*, we investigated proteins involved in peptidoglycan remodeling during cell division. To this end, we focused on potential hydrolases that localize to the site of constriction. This screen identified a novel peptidoglycan hydrolase, DipM. To investigate the function of DipM in cell division, mutant derivatives of the protein were analyzed using fluorescence microscopy, electron microscopy, and biochemical approaches. A mutant strain lacking DipM displays strong filamentation and aberrant polar morphology, suggesting that DipM plays a critical role in cell division and cell pole development. DipM was confirmed to be a peptidoglycan hydrolase active in the late stages of cell division, which requires essential cell division proteins for localization to the division site. Our data suggest that DipM is directly involved in septum cleavage during cell division and the generation of fully differentiated poles, and thus in the establishment of cellular polarity.

FTP22
Verification of data – is it really that important and are there consequences?

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Like all experimental sciences biology requires the collection and evaluation of data. Technological advances mean that there is currently a data flood with the associated problems of evaluation, storage and administration of the data. However, in most instances the data are taken at face value and the question is never asked whether the data or information associated with them are correct. The simple principle of verifying the data or even being able to detect from the outset that a dataset is faulty is something that requires two elements. Firstly the original biological material on which that study was based must be available and secondly the data must be brought into the context of the organism(s) from which it was obtained. It is not a difficult task for experts to find errors in databases and in the literature, and some will be presented. However, datasets are also used by non-experts and errors may not detected. The consequences are by no means trivial. A key issue that should be addressed by all those involved in the production and evaluation of data is how reliable is that data and how can one deal with unreliable data? The independent verification of data must also be addressed, especially where doubts are raised about the reliability of the data.

FTP23
N54 – The key amino acid in 5-Aminolevulinate synthase determines the transient α-amino-β-ketoadipate-intermediate in *Rhodobacter capsulatus* heme biosynthesis

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There are three different intermediates in the 5-Aminolevulinate synthase (ALAS) reaction mechanism, the first quinonoid, the α-amino-β-ketoadipate intermediate and the second quinonoid. The first two intermediates are not detectable because of a fast reaction cycle. Here we provide evidence trapping the second substrate, α-amino-β-ketoadipate by generating a variant where this intermediate is not converted into the product 5-aminolevulinate (ALA). Two variants of ALAS were generated, N54Q and N54D, respectively. N54 coordinates glycine via a hydrogen bond. The non-conservative amino acid exchange, asparagine to aspartic acid, shows no activity while the conservative one leads to a higher V_{max} than the wild-type. Nevertheless, they have a related catalytic turnover. Furthermore, we present results in detection of the second quinonoid that is visible at a wavelength of 510 nm. By generating a conservative and a non-conservative exchange for N54 we obtained inactive variants which indicates that the N54-residue controls the catalytic reaction leading to the product 5-ALA. We also present the results for single-turnover experiments where the reation is slower in comparison to the wild-type enzyme through a tighter binding resulting in a slower 5-ALA release. Finally, the mutagenesis studies of the key amino acid N54 mutant variants allows us to elucidate the ALAS catalysis in detail.

FTP24
Comparison of oxacillin MICs and zone diameters in the assessment of *mecA*-mediated methicillin resistance among coagulase-negative staphylococci from bovine mastitis

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Background: Based on CLSI-approved clinical breakpoints, coagulase-negative staphylococci (CoNS) are classified as methicillin-resistant by oxacillin MICs of ≥ 0.5 mg/L and zone diameters (1 µg disk) of ≤ 17 mm and as methicillin-susceptible by oxacillin MICs of ≤ 0.25 mg/L and zone diameters of ≥ 18 mm. Little is known about the correlation between pheno- and genotypic tests in the assessment of methicillin resistance among CoNS from animals.
Methods: In the present study, 123 bovine CoNS isolates from cases of mastitis were investigated for oxacillin susceptibility/resistance by disk diffusion and broth microdilution. Scattergrams were constructed by plotting MICs against zone diameters. Isolates classified by either method as methicillin-resistant were tested by PCR for *mecA* and – if positive – for the SCC*mec* type.
Results: Sixteen isolates were classified as methicillin-resistant and 96 isolates as methicillin-susceptible by both methods. Eleven isolates showed oxacillin MICs of 0.5 or 1 mg/L but had zone sizes of 18–28 mm. The *mecA* gene was identified in 15 of the 16 resistant isolates. The single *mecA*-negative resistant isolate had an oxacillin MIC of 1 mg/L and a zone diameter of 17 mm. A SCC*mec* type V cassette was seen in 5 *Staphylococcus haemolyticus*, a type III cassette in 1 *S. saprophyticus*, a type IVa cassette in 5 *S. epidermidis* and 1 *S. capitis*, a type IVa cassette with an additional *ccrA/B4* gene complex in 2 *S. epidermidis*, while 1 *S. epidermidis* carried a SCC*mec* element non-typeable by the PCR approach used. All strains with SCC*mec* type IVa or non-typeable cassettes exhibited low oxacillin MICs of 1–4 mg/L whereas strains with type III or V cassettes had MICs of ≥ 16 mg/L.
Conclusion: With 0 of 8 and 3 of 7 CoNS with MICs of 0.5 and 1 mg/L, respectively, being *mecA*-positive and only 1/16 CoNS with zone diameters of ≤ 17 mm being *mecA*-negative, zone diameters seem to be the more accurate indicators of *mecA*-mediated resistance in CoNS than oxacillin MICs.

FTP25
Determinants of receptor-transporter interactions of the histidine ABC transporter $HisQMP_2$ from *Salmonella enterica* serovar Typhimurium

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The histidine ABC transporter $HisQMP_2$ of *Salmonella enterica* serovar Typhimurium is composed of the membrane-integral subunits HisM and HisQ, forming a heterodimer as translocation pore, two copies of the ATP-binding subunit HisP, and the solute binding proteins HisJ and LAO [1]. Although well-characterized biochemically, no crystal structure of the complete transporter is available and little is known about interaction between the binding proteins HisJ/LAO with the transmembrane domains HisQ/M.
Interaction of HisJ/LAO with HisQM was investigated by assaying binding-protein-dependent stimulation of ATPase activity of transporter variants reconstituted in proteoliposomes. Sequence alignment with close relatives suggested a possible role of conserved positively charged residues in the second periplasmic loop of HisQ and HisM which were consequently changed to glutamate and glutamine.
Replacing of K178 and K185 in HisQ led to four- and twofold lowered stimulation of the ATPase activity, respectively, compared to the wildtype. In HisM, exchange of K187 and R190 resulted in a complete loss of activity. These results are consistent with a role of the selected residues in receptor-transporter interaction.

[1] Ames, G. F.-L. 1986. Annu. Rev. Biochem. 55:397-452.

FTP26
Purification and characterization of the A_1A_O ATP synthase/ ATPase of the hyperthermophilic Crenarchaeon *Ignicoccus hospitalis<k/>*

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Ignicoccus hospitalis is a hyperthermophilic crenarcheal chemolithoautotroph that serves as host organism for *Nanoarchaeum equitans* thereby forming the first and only known intimate association of two Archaea. The cells exhibit a highly unusual ultrastructure with two membranes and a huge intermembrane compartment (periplasmic space) [1].
Highly unexpected and based on immuno-EM analyses of ultrathin sections and immunofluorescence experiments with whole *I. hospitalis* cells it was demonstrated that the ATP synthase and the H_2:sulfur oxidoreductase complex of this organism are located in the outer membrane [2]. These two enzyme complexes are mandatory for the generation of an electrochemical gradient and for ATP synthesis. To get insights into the structure and function of the A_1A_O ATP synthase we started to purify this complex. Cells were disrupted by French Press and subsequently solubilisation of the membrane protein with DDM was performed. In a second approach DDM was directly added to the cells. Purification of the ATP synthase was performed by ion exchange chromatography followed by gel filtration. Purified complexes were analysed by high-resolution clear native gel electrophoresis (hrCNE) and checked on their ATP hydrolyzing activity by in-gel assays. The obtained active complexes were separated by SDS-PAGE and identified by Western Blot analyses and confirmed by MALDI-TOF-MS. Thus, all annotated subunits of the ATP synthase could be identified.

[1] Junglas B. *et al.* Arch Microbiol 190:395-408 (2008)
[2] Küper U. *et al.* PNAS, submitted

FTP27
Applying resampling frameworks on housekeeping gene comparisons

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Hierarchical network methods or clustering schemes are subject to deficiencies, due to which, unambiguous tree representations are not always obvious.
We introduce a new method providing reliability values for branches based upon uncertainty of data and degeneracy of tree solutions. Rather than being implemented within an algorithm, the method builds a statistical framework around the algorithm.
The proposed workflow generates a number of resampled data sets from the input data. Each resampled data set is clustered using the chosen clustering method. The resulting trees are summarized into a consensus network by a summary factory. The resampling framework is the entire framework that includes data factory, the encapsulated clustering algorithm and the summary factory.
The current study focuses on the comparison of housekeeping gene sequences from prokaryotes which were analyzed by maximum parsimony combined with bootstrap resampling on the one hand and by means of a minimum spanning tree combined with permutation resampling on the other hand.
The resulting plots illustrate the influence of resampling methods and/or summary methods on the outcome of data comparisons, and may advise on general requirements for qualitative, meaningful datasets.

FTP28
Regulation at the two divergent promoters P_{pA} and P_{pD} involved in gas vesicle synthesis in *Halobacterium salinarum*

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The formation of gas vesicles is used as model system to study gene regulation in halophilic Archaea. The formation of gas vesicles in *Halobacterium salinarum* requires the expression of the 14 genes *gvpACNO* and *gvpDEFGHIJKLM*, which are located in two opposite clusters in the vac region. The two divergent promoters P_{pA} and P_{pD} driving the expression of p-vac are separated by 35 nt only. Both promoters are activated by the transcription activator GvpE. Two 20-nt sequences (UAS) consisting of two conserved 8-nt sequence portions located upstream of P_{pA} and P_{pD} BRE are required for GvpE-mediated activation, with the distal 8-nt portions almost completely overlapping in the centre of the P_{pD}-P_{pA} region.
The activation of P_{pD} and P_{pA} are different – with P_{pA} driving the expression of the gas vesicle structural protein GvpA and GvpC 2.5-fold stronger as P_{pD}. To analyse the contribution of TATA-Box, BRE and GvpE-UAS to the observed promoter activities, promoter mutants were constructed by inverting the intergenic region or exchanging the respective promoter sequence elements. The expression was analysed using a dual reporter construct which contains P_{pA} fused to the *bgaH* reporter gene (encoding an enzyme with β-galactosidase activity), and P_{pD} fused to an altered version of p-*gvpD* (3 UGA-stop codons introduced in p-*gvpD*) to determine the amounts of p-*gvpD* mRNA.
The exchange of the TATA-Box did not affect the promoter strength, whereas the strength was strongly affected when BRE-sequence was replaced. Furthermore an influence on the promoter activities was observable when the orientation of the intergenic region was altered.

FTP29
Soil microcosms for determination of growth by *Streptomyces mirabilis* P16B1 in heavy metal contaminated soil

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Streptomycetes are a dominant group of soil bacteria which belong to the group of Actinobacteria. They are known for their complex life cycle, including mycelial growth and spore production, as well as their production of secondary metabolites, among them a large number of antibiotics. In comparison to pristine soils, heavy metal contaminated soils show much higher numbers of Gram positive bacteria, with bacilli and streptomycetes dominating over Gram negative proteobateria or firmicutes. The former uranium mining site WISMUT in Eastern Thuringia, Germany, shows extreme environmental conditions, such as scant nutrients, intense salt load and low pH, followed by high metal content. The banks of the creek Gessenbach are characterized by exceedingly high concentrations of heavy metals as a consequence of the permanent inflow of acid mine drainage water over several decades of mining activity. Actinobacteria isolated from this hostile environment show high resistances against a range of heavy metals, including nickel, cobalt, cadmium or zinc. The extremely heavy metal resistant strain *S. mirabilis* P16B1 shows the ability to grow in highly contaminated soil from sample site K7 without the addition of any media ingredients even though there is a significantly higher content of a range of heavy metals, including mobile and adsorbed fraction metals as determined by sequential extraction methods. Scanning electron microscopy was used to detect the mycelium of the strain and spore production on the surface and in the interior of the soil as well as highly abundant patchy organic cover material. It could be shown that inoculation with the strain has an effect of heavy metal availability in mobile and adsorbed fraction. The superoxide-dismutase-production of *S. mirabilis* P16B1 under natural conditions in microcosms was investigated by SOD-activity-assay and protein expression under artificial conditions with and without high nickel content.

FTP30
Analysis of the cytoskeleton protein MreB from *Chlamydia pneumoniae*

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In rod-shaped bacteria the actin-like protein MreB is thought to direct incorporation of cell wall material into the side-wall and the tubulin homologue FtsZ is acknowledged to tether incorporation of cell wall building blocks at the developing septum. Surprisingly, chlamydiae lack the essential division protein FtsZ but harbor, despite their spherical shape, MreB.
We started to investigate the interaction of MreB with key components in cell division and cell wall biosynthesis.
MreB, MurG and MraY from *Chlamydia pneumoniae* were overproduced in *Escherichia coli* and functionally analyzed. We previously demonstrated that MraY-His_6 and MurG-His_6 exhibit *in vitro* activity, synthesizing lipid I and lipid II respectively (Henrichfreise, Schiefer *et al.*, Mol Microbiol. 2009. 73:23). Here we prove that Strep-tagged chlamydial MreB is functional and polymerizes *in vitro* using light scattering assays.
A deeper insight into the function of cytoskeleton proteins and their role in cell division and cell wall biosynthesis on molecular level will provide valuable information for the design of new anti-chlamydial antibiotics.

FTP31
Dynamics and Regulation of *Bacillus subtilis* SMC *in vivo*

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Chromosomes must be compacted to fit into the bacterial cell, and the topology of the DNA must be regulated to allow transcription and replication to occur in an efficient manner. The compaction of the DNA is regulated by a number of proteins including histone-like proteins, topoisomerases and the SMC complex.
The *Bacillus subtilis* SMC complex consists of a SMC (bsSMC) dimer, an ABC-transporter related ATPase, and a subcomplex of the two accessory proteins ScpA and ScpB. This complex is essential for chromosome segregation and condensation. Deletion causes severe defects in chromosome organization, whereas overexpression of bsSMC in *Bacillus subtilis* leads to an excessive overcondensation of the nucleoid. Heterologous overexpression of the bsSMC in *Escherichia coli* leads to a hyper-compaction of the *E. coli* chromosome as well. Expression of an ATPase deficient mutant (D1117A) amplifies this effect. These results imply that the accessory proteins ScpA and ScpB are not necessary for the activity of SMC *in vivo* and that the ATPase activity may play an important role in regulation.
The SMC complex localizes cell cycle dependent in a bipolar manner to discrete centers on the nucleoid. Fluorescence Recovery after Photobleaching (FRAP) experiments of SMC-GFP, ScpB-GFP and ScpA-YFP, performed in exponentially growing cells, showed a full recovery of the foci after a few minutes. To recover the full intensity, there must be a complete exchange of the components in the complexes within minutes, indicating the transient formation and dissipation of the complex on the chromosome. These experiments have a profound impact on the view of the mode of action of the SMC complex.

FTP32
Subcellular localization of the tetrachloroethene reductive dehalogenase (PceA) and the proteins PceB and PceT in *Desulfitobacterium hafniense* Y51

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Desulfitobacterium hafniense Y51 is a strictly anaerobic, gram-positive bacterium, which can grow with aliphatic chlorinated compounds, such as tetrachloroethene (PCE), as electron acceptors. PCE is reductively dechlorinated to *cis*-1,2-dichloroethene. The enzyme mediating the dechlorination of tetrachloroethene is the PCE reductive dehalogenase, a corrinoid cofactor and Fe/S-cluster containing protein. The cytoplasmic precursor of this enzyme (prePceA) includes an N-terminal Tat (*twin arginine translocation*) signal peptide. The gene encoding the PCE reductive dehalogenase, *pceA*, is organized in the *pceABCT* operon. The gene *pceB* encodes a putative membrane integral protein, *pceC* shows homology to transcriptional regulators, and the gene product of *pceT* is a chaperone interacting with prePceA (1).
In this study we investigated the subcellular localization of the PCE dehalogenase in *D. hafniense* Y51 cells grown in the presence or absence (15 subcultivations) of PCE. When PCE was present, the enzyme was attached to the exoplasmic face of the cytoplasmic membrane. In PCE depleted cells we found prePceA aggregates in the cytoplasm using freeze-fracture immunogold-labeling. The protein aggregates were enriched and characterized in more detail via electron microscopy. Furthermore, the localization studies on PceA were extended by the examination of the subcellular localization of the *pceB* and *pceT* gene products. With respect to the localization patterns of both proteins, the proposed function of PceB and PceT will be discussed.
(1) Morita, Y., Futagami, T., Goto, M., Furukawa, K. (2009). Functional characterization of the trigger factor protein PceT of tetrachloroethene-dechlorinating *Desulfitobacterium hafniense* Y51. *Appl Microbiol Biotechnol* 83:775-81

FTP33
Analysis of volatile emission of *Serratia odorifera* and the biological impact

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Bacteria have the ability to release complex spectra of volatile organic compounds (VOCs). The volatile mixtures of the rhizobacterium *Serratia odorifera 4Rx13* inhibit the growth of various fungi, plants and protozoa during co-cultivation (1, 2). GC-MS analysis of the VOC mixture of *S. odorifera* depicted approximately 80 compounds. Twenty additional volatiles were presented by PTR-MS (proton transfer reaction mass spectrometry). The latter method preferentially identified small molecular mass compounds such as methanol, ethanol, methanethiol and dimethyl disulfide. Furthermore, temporal volatile emission profiles were obtained by PTR-MS. The dynamic emission profiles of eight volatiles indicated an acute burst during logarithmic growth.
Co-cultivations with individual (commercially available) volatiles and determination of IC50 concentrations were performed to identify biologically active compounds.

[1] Vespermann *et al.* (2007) Applied and Environmental Microbiology 73:5639-5641
[2] Kai *et al.* (2009) Applied Microbiology and Biotechnology 81:1001-1012

FTP34
From unicellular to multi-cellular stress response strategies: a case study on *B. subtilis* sporulation

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Bacterial stress responses primarily serve to increase the fitness of the individual bacterium. However, we begin to recognize that their induction may be modulated in a multi-cellular context to increase fitness of the community. Here we analyze the temporal differentiation dynamics within starving micro-colonies of *B. subtilis* as a model system for analyzing more complex temporal strategies of stress response induction. We follow the population dynamics with single cell resolution using fluorescence time-lapse microscopy in conjunction with quantitative image analysis. We find that upon starvation a fraction of the population initiates the formation of spores while a subpopulation of sister cells maintains growth. Within the later population the transcriptional activation of a "delay" element stabilizes growth against the pressure to sporulate. Upon completion of the sporulation program matured spores are released. The lysis event correlates with an increase of the cellular growth rate in sister cells. This suggests that nutrients are released from one subpopulation that benefit the other. By means of a mathematical model we theoretically analyze to what extent the observed differentiation dynamics – in particular, population diversification by means of a stochastic activation of a "delay" element- could reflect a social survival strategy.

FTP35
Assembly and disassembly of the *Bacillus subtilis* divisome

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Cell division in *Bacillus subtilis* is initiated by assembly of a cytokinetic ring followed by recruitment of different transmembrane proteins. While a whole set of membrane spanning proteins have been identified to be recruited to the divisome, a clear function is only known for the penicillin-binding protein 2B (PBP-2B). It contributes to the synthesis of the new cell wall material. The three bitopic proteins FtsL, DivIC and DivIB are supposed to play a more structural and/or regulatory role during cell division. So far it was controversially discussed, in which manner these proteins interact and how exactly they influence each others stability.
FtsL is degraded by the intra membrane protease RasP. Co-expression of FtsL and RasP in *E. coli* led to rapid degradation of FtsL. Co-expression of FtsL and an inactive mutant of RasP, however, had no effect on FtsL stability. This system was now used to analyse the impact of DivIC and DivIB on FtsL stability. Surprisingly, an effect could be observed not only on stability, but also on the polymerisation state of FtsL. Together with additional bacterial two-hybrid studies to further investigate the protein-protein interactions, these results provided new insights into the assembly and disassembly of the divisome membrane complex.

FTP36
CzcI has a negativ effect on *czc*-mediated metal resistance in *Cupriavidus metallidurans* CH34

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The *czc* resistance determinant is located on plasmid pMOL30 of the β-proteobacterium *Cupriavidus metallidurans* CH34. Gene products of *czc* are the RND-driven transenvelope protein complex CzcCBA that transports the divalent cations Co^{2+}, Zn^{2+}, Cd^{2+} from the periplasm to the outside, the efflux ATPases and the CDF-proteins get these cations from the cytoplasm to the periplasm , the two-component regulatory system CzcRS, and a variety of gene products with uncertain functions. Among these are the periplasmic metal-binding proteins CzcE, CzcJ and CzcI. Related bacterial strains such as *C. eutropha* H16 and JMP134 contain an operon orthologous to *czcICBA* and interrupted loci are still present on chromosome 2 of *C. metallidurans*. This defines *czcICBA* is kind of a "core" determinant. Possibly, *C. metallidurans* inherited *czcICBA* from its ancestor, duplicated this operon onto plasmid pMOL30, and added *czcN* upstream of *czcICBA* as well as *czcD*, *czcRS*, *czcE*, *czcJ* and *czcP* downstream during its evolution. Although these findings place CzcI at the core of this most important metal resistance determinant of *C. metallidurans*.

The *czcI2* gene was deleted on chromosome 2 of the plasmid-free *C. metallidurans* strain AE104, strain AE128(pMOL30), and CH34(pMOL28, pMOL30) wild type. Additionally, *czcI* was also deleted in the two pMOL30-containing strains. Deletion of *czcI* but not of *czcI2* increased zinc and cadmium resistance of strains AE128 and CH34 on solid medium. Zinc and cobalt resistance increased in both strains carrying *czcI* or *czcI2* deletions, and the effect was additive here. Deletion of *czcI2* in the plasmid-free strain AE104 increased cobalt and cadmium but not zinc resistance. Thus, CzcI has a negativ effect on *czc*-mediated metal resistance in *Cupriavidus metallidurans* CH34.

FTP37
Analysis of unique cytoskeletal structures in magnetotactic bacteria

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Magnetotactic bacteria are able to orient and move along magnetic field lines. This capability depends on formation of specific organelles called magnetosomes. Magnetosomes consist of nanometer-sized magnetite or greigite crystals enclosed by a lipid membrane. To provide a magnetic moment strong enough to align a cell within a magnetic field, the magnetosomes need to be aligned into chains along the cell axis, and they need to be prevented from uncontrolled aggregation. Magnetosome assembly is controlled by several specific proteins that include MamK, an actin-like protein that polymerizes into cytosekeletal magnetosome filament structures. Presumably, the magnetosomes are tethered to the MamK-filaments by MamJ, a small acidic protein.
However, recent studies suggest that in addition to bacterial actins also tubulin-like proteins might be involved in magnetosome formation. In *Magnetospirillum gryphiswaldense*, two genes with similarity to *ftsZ*, encoding a structural protein known to be essential for cell division, are present. One of them (*ftsZm*) is part of the *mamXY* operon within the genomic magnetosome island (MAI) which harbours most of the genes required for biomineralization and chain assembly. Its co-localization with magnetosome genes and dispensable role for cell division suggest a function in magnetosome formation or chain assembly. Currently, we are analyzing the intracellular localization pattern of FtsZm together with MamK and other magnetosome-specific proteins by fluorescence microscopy in mutant and wildtype cells. Results of heterologous expression of *ftsZm* in *E. coli* suggest that the protein interferes with the endogenous FtsZ function and is able to co-localize with MamK.

FTP38
Bactofilins, a ubiquitous class of cytoskeletal proteins mediating polar localization of a cell wall synthase in *Caulobacter*

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The cytoskeleton plays a key role in the temporal and spatial organization of both prokaryotic and eukaryotic cells. Here, we report the identification of a new class of polymer-forming proteins, termed bactofilins, that are widely conserved among bacteria. In *Caulobacter crescentus*, two bactofilin paralogues cooperate to form a sheet-like structure lining the cytoplasmic membrane in proximity of the stalked cell pole. These assemblies mediate polar localization of a peptidoglycan synthase involved in stalk morphogenesis, thus complementing the function of the actin-like cytoskeleton and the cell division machinery in the regulation of cell wall biogenesis. In other bacteria, bactofilins can establish rod-shaped filaments or associate with the cell division apparatus, indicating considerable structural and functional flexibility. Bactofilins polymerize spontaneously in the absence of additional cofactors *in vitro*, forming stable ribbon- or rod-like filament bundles. Our results suggest that these structures have evolved as an alternative to intermediate filaments, serving as versatile molecular scaffolds in a variety of cellular pathways.

FTP39
A viral tool box of multiplex qPCR assays for tracking the sources of fecal contamination

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Microbial source tracking is increasingly being used to assist in tracing sources of faecal pollution in the environment. Human and animal faecal pollution of water and food presents a risk to human health because of the presence of pathogenic bacteria and viruses. It is therefore important that sources of faecal pollution are rapidly and accurately identified.
We have developed a suite of viral detection methods to identify specifically human and animal enteric and respiratory viruses commonly occurring in sewage and faeces. This 'viral tool box' comprises four multiplex real-time PCR assays for the detection of human and animal noroviruses, human and animal adenoviruses and for genotyping of F-RNA bacteriophage, a commonly used indicator of water quality.
The norovirus assay detects and distinguishes genogroups I (specific to humans), II (humans and pigs) and III (cattle and sheep), the two adenovirus assays detect and distinguish between human adenovirus C and F (both specific to humans), porcine adenovirus 3 and 5 (pigs), ovine adenovirus (sheep and cattle) and atadenoviruses (sheep, cattle, deer, goats). The F-RNA bacteriophage assay distinguishes between genogroups GI through GIV. Although reportedly less specific to either animal or human faecal sources, genogroups I and IV are generally associated with animal contamination and genogroups II and III indicate predominantly human fecal contamination or domestic sewage associated inputs. The sensitivity of this Viral Tool Box (VTB) was tested against 10-fold dilution series of DNA plasmids that carry the target sequences of the respective viruses and was shown to detect at least 10 plasmid copies for each assay. A panel of human and animal enteric and respiratory viruses show these assays to be highly sensitive and specific to their respective targets. The VTB was used to detect viruses in fecal and environmental samples, including raw sewage and biosolids from municipal sewage treatment plants, abattoir sewage and fecally contaminated shellfish and river water which were likely to contain animal or human viruses. The 'viral toolbox' will aid in microbial source tracking and so assist resource managers and public health officers to determine sources of pollution for effective management of shellfish growing waters.

FTP40
Biomethylation and volatilization of metal(loid)s

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Formation of volatile derivatives of metals and metalloids by microorganisms is widespread in anaerobic habitats like sewage-sludge, geothermal vents as well as intestinal tracts of mammals including humans. These methylated and hydrated metal(loid) compounds exhibit a higher toxicity than their inorganic educts. As recent studies suggest, methylation of metal(loid)s is an inherent feature of methanoarchaea [1].
Here, we focus on the biochemical mechanisms of the synthesis of these methylated derivatives by methanoarchaea. Two corrinoid-dependent methyltransferases MtaA and MtbA of the central energy metabolism of *Methanosarcina mazei* (MtaA and MtbA) were investigated regarding their capability to transform inorganic metal(loid)s. The results indicate that a broad range of metal(loid)s can be hydrogenated and methylated by these methyltransferases. By using the powerful tool of oxidation-state specific hydrid generation coupled to "purge&trap"-GC/ICP mass spectrometry we are able to follow the change of the oxidation state of various arsenic species in the course of their transformation processes [2]. The results derived from *in vitro* experiments with MtaA will be compared to recently discussed methylation mechanisms for arsenic.

[1] Meyer, J. *et al.* (2008), Systematic and Applied Microbiology, 31: 81-87
[2] Diaz-Bone, R.A. *et al.* (2009) Journal of Analytical Atomic Spectrometry, 24: 808–814

FTP41
Horiozontal DNA-transfer in *Bacillus subtilis* mediated by a plamid encoded conjugation system

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DNA transfer in Gram-negative and Gram-positive bacteria can occur by a mechanism called conjugation. This way, bacterial cells can gain novel genetic information, e.g. resistance genes, which is a clinically relevant problem.
In contrast to the well studied conjugation systems of Gram-negative bacteria there is a general lack of knowledge about these systems in Gram-positive bacteria. To investigate the conjugational DNA transfer in the model bacterium *Bacillus subtilis* we analysed the sequence of the natural fertility plasmid pLS20. This showed sequence homologies to five virulence genes (VirB1, VirB4, VirB11, VirD2, VirD4) of the well known conjugation system in the Gram negative bacterium *Agrobacterium tumefaciens*. These proteins are thought to be the basic components of the VirB/D4 translocation machinery.
We adressed the question, which other episomally encoded proteins are involved in this process and how the mechanism of DNA transfer occurs in Gram-positive bacteria. For this reason we performed several biochemical experiments inluding Tandem Affinity Purification and pull down assays as well as purification and biochemical characterization of already known complex components.
Additionally, by using fluorescence microscopy on live cells we studied the localization and dynamics of the involved proteins of the pLS20. Indeed we found that the DNA transfer machinery seems to assemble at the cell pole and that the assembly of the known complex-components seems to be highly dynamic. Even the localization pattern of the conjugative plasmid itself supports the idea of a highly dynamic process.

FTP42
Extending DNA sequence information of two *styA1/styA2B* gene clusters by adapting SiteFinding-PCR to strains of *Rhodococcus opacus*

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Members of the gram-positive genus *Rhodococcus* are of considerable biotechnological relevance due to their extraordinary high metabolic versatility. Available genome data indicate a distinctive gene redundancy which in combination with huge genome sizes favours these actinobacteria as a source for novel enzymes, e.g. monooxygenases [1]. PCR-based approaches are frequently used in order to identify corresponding genes. However, further methods are necessary in that case in order to identify whole genes or complete operons. Several genome walking methods have been described for that purpose, e.g. the SiteFinding-PCR [2].
In an attempt to identify the complete ORFs of two novel styrene monooxygenase gene clusters *styA1/styA2B* from *R. opacus* strain 1CP and MR11 available sequence information was extended by the method of SiteFinding-PCR. The original approach [2] had to be adapted in respect to the sitefinding oligo and the temperature program in order to meet the high GC content of rhodococci and to obtain new fragments of sufficient size. In contrast to the original protocol which resulted in 0.1 - 0.2 kb-large amplicons the presented adaptation now allows amplification of up to 1.35 kb of new sequence information. To our knowledge this is the first report that this simple and reliable method has been used on DNA templates originating from the actinobacteria group.
The complete ORFs of *styA1* and *styA2B* from strain MR11 could be identified by this protocol and comparison of the deduced proteins with StyA1 and StyA2B from *R. opacus* 1CP showed high homology.

[1] Tischler *et al*. 2009. Identification of a novel self-sufficient styrene monooygenase from *R. opacus* 1CP. *J. Bacteriol.* 191:4996-5009
[2] Tan *et al*. 2005. SiteFinding-PCR: a simple and efficient PCR method for chromosome walking. *Nucleic Acids Res.* 33:e122.

FTP43
Cell Division and Chromosome Segregation in *Corynebacterium glutamicum*

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A major player in cell division is the tubulin homologue, FtsZ, which forms a ring-like structure (Z-ring) at midcell, forming the divisome. The appearance of the FtsZ ring coincides with the termination of DNA replication. In *E. coli* and *B. subtils* nucleoid occlusion and the Min system control the placement of the Z-ring. *Corynebacteria glutamicum*, possess no positive or negative FtsZ regulators that are found in other rod-shaped bacteria. Therefore, cell division and growth in C. glutamicum is different to the conventional model organisms.*C. glutamicum* does possesses a *parAB* operon and an orphan *parA*-like gene. The Par proteins interact with each other, playing a role in plasmid or chromosome partitioning and replication initiation, although there is a lack of uniformity in their mode of action in different organisms. The *parABS* genes are almost invariably located close to the single origin of chromosome replication, *oriC*, from which the bidirectional replication of the whole chromosome is achieved. *parS* is a cis-acting DNA binding site that interacts directly with the trans-acting ParA and ParB proteins where they form various nucleoprotein complexes. Localization studies have shown that ParB localises in the vicinity of the oriC regioin, whereas the ParA proteins localise to the extreme tips of the chromosomes, forming bands at midcell and foci at the poles. Both single and double deletion mutants of the *par* genes demonstrate a dramatic increase in anucleate cells, altered cell length distributions and growth rates, and growth from abnormal sites. Initiation of chromosome replication is also altered in the par mutants, as demonstrated by flow cytometry. *In-vivo* and *in-vitro* analysis show an interaction of the Par proteins with FtsZ, suggesting a close link between cell division and chromosome segregation, ensuring stable genome transmission during cytokinesis.

FTP44
Localization of small regulatory RNAs and Hfq in prokaryotes

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The mechanisms of target regulation by regulatory RNAs are well studied. But an intriguing questions remains: is subcellular localization of sRNAs and the major RNA chaperone Hfq relevant for mRNA targeting?
A recent study revealed membrane localization of the major endoribonuclease RNase E, which is necessary for the degradation of the sRNA-mRNA complex [1]. Additionally it was shown that membrane localization of the *ptsG* mRNA that encodes for the major glucose transporter $IIBC^{Glc}$ is mandatory for the downregulation by the sRNA SgrS, which combats phosphosugar-stress by inhibiting translation of *ptsG* mRNA [2]. These findings strongly suggest that a specific subcellular localization of sRNAs is important for regulation.
To study the localization of regulatory RNAs we utilize three different aptamer tags: MS2-, boxB-, and eIFA4-tag. Whereas the MS2/boxB system is based on a fullsize GFP molecule that is fused to the tag-binding-protein/peptide [3, 4], the eIFA4 system uses a split GFP molecule. Both halves of the GFP are fused to one domain of eIFA4 [5]. Binding of these domains to the eIFA4-tag initiates a refolding of the GFP halves and thereby reconstitutes GFP fluorescence.
In order to get a complete view of the sRNA regulon we were interested in the localization of the major RNA chaperone Hfq. To this end we did an Hfq-GFP fusion. We can show that Hfq forms a helical structure in *E. coli*, which we think is a result of its interaction with RNase E.

[1] Khemici *et al.*, (2008) Molecular Microbiology 70(4):799-813
[2] Kawamoto *et al.*, (2005) Genes and Development 19(3):328-38
[3] Golding and Cox, (2004) PNAS 101(3):11310-5
[4] Daigle and Ellenberg, (2007) Nature Methods 4(8):633-6
[5] Valencia-Burton *et al.*, (2007) Nature Methods 4(5):421-7

FTP45
CzcI has a negativ effect on czc-mediated metal resistance in *Cupriavidus metallidurans* CH34

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The czc resistance determinant is located on plasmid pMOL30 of the β-proteobacterium *Cupriavidus metallidurans* CH34. Gene products of *czc* are the RND-driven transenvelope protein complex CzcCBA that transports the divalent cations Co^{2+}, Zn^{2+}, Cd^{2+} from the periplasm to the outside, the efflux ATPases and the CDF-proteins get these cations from the cytoplasm to the periplasm, the two-component regulatory system CzcRS, and a variety of gene products with uncertain functions. Among these are the periplasmic metal-binding proteins CzcE, CzcJ and CzcI. Related bacterial strains such as *C. eutropha* H16 and JMP134 contain an operon orthologous to *czcICBA* and interrupted loci are still present on chromosome 2 of *C. metallidurans*. This defines czcICBA is kind of a "core" determinant. Possibly, *C. metallidurans* inherited *czcICBA* from its ancestor, duplicated this operon onto plasmid pMOL30, and added *czcN* upstream of *czcICBA* as well as *czcD, czcRS, czcE, czcJ* and *czcP* downstream during its evolution. Although these findings place CzcI at the core of this most important metal resistance determinant of *C. metallidurans*. The *czcI2* gene was deleted on chromosome 2 of the plasmid-free *C. metallidurans* strain AE104, strain AE128(pMOL30), and CH34(pMOL28, pMOL30) wild type. Additionally, *czcI* was also deleted in the two pMOL30-containing strains. Deletion of *czcI* but not of *czcI2* increased zinc and cadmium resistance of strains AE128 and CH34 on solid medium. Zinc and cobalt resistance increased in both strains carrying *czcI* or *czcI2* deletions, and the effect was additive here. Deletion of *czcI2* in the plasmid-free strain AE104 increased cobalt and cadmium but not zinc resistance. Thus, *CzcI* has a negativ effect on *czc*-mediated metal resistance in *Cupriavidus metallidurans* CH34.

FTP46
Dynamic localization of DNA repair proteins in live bacteria

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All organisms, prokaryotes and eukaryotes, somehow need to maintain the integrity of their genome to ensure survival. A huge threat for the cells are double-strand breaks (DSBs) caused by irradiation or chemicals that modify DNA. These DSBs can be either repaired through non homologous end joining (NHEJ), where the broken ends are directly connected, or via homologous recombination (HR) using the intact sister chromosome as template for recombination.
HR requires a large number of proteins that are recruited to the repair centre in a discrete temporal order. Within the first 30 minutes upon DNA damage, the nucleoids are decondensed and RecN recognizes the break sites to initiate the response to repair DNA. The AddAB helicase/nuclease complex or alternatively RecJ endonuclease in concert with RecQ and RecS helicase act in endprocessing of the DNA at the DSBs.
Our intention was to investigate the localization of RecJ, RecQ and RecS in exponentially growing Bacillus subtilis cells after induction of DSBs with Mitomycin C (MMC). Therefore, we labelled the Rec proteins with several fluorescent proteins and determined the localization via fluorescence microscopy.
We found out that RecJ, RecQ and RecS form one focus at the replication machinery in uninduced cells indicating an additional role of these proteins in DNA replication. After induction of DSBs, all proteins started to form multiple foci on the nucleoid. In ΔrecN, ΔrecQ, ΔrecS and ΔrecQrecS cells RecJ localization was not severely effected. An identical localization pattern could be observed for RecQ and RecS in a recJ deletion background. Colocalization experiments of RecJ and RecN indicated a partial colocalization of RecJ with RecN. In time lapse experiments most of the RecJ foci revealed a dynamic behaviour while some foci remained static. This argues for a scanning mechanism of RecJ that moves along the DNA until it has found a DSB.

FTP47
Periplasmic electron transfer network in the dissimilatory iron reducer *Shewanella oneidensis* known proteins – new functions

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Dissimilatory iron reducers are challenged to perform electron transfer reactions from the cytoplasmic membrane through the periplasm to the outer membrane to bring the electrons into contact with the insoluble terminal electron acceptor. The γ-proteobacterium *Shewanella oneidenis* became a model organism to study this process. So far it was known, that the cytoplasmic membrane bound tetraheme cytochrome CymA, the periplasmic decaheme cytochrome MtrA, the outer membrane β-barrel Protein MtrB and the outer membrane decaheme cytochromes OmcA/B are essential for *S. oneidensis* under ferric iron reducing conditions. Furthermore it was shown that MtrA, MtrB and OmcB build a complex across the outer membrane. Possible electron transfer reactions under iron reducing conditions in the periplasm were so far not elucidated.
We identified MtrA and FccA as abundant soluble periplasmic cytochromes. FccA is annotated as the periplasmic fumarate reductase of *S. oneidensis* and was so far not discussed to be involved in the electron transfer to iron oxides. Furthermore, MtrA was so far believed to be solely located associated with the outer membrane. Our experiments showed a direct electron transfer between MtrA and FccA. Furthermore we have evidence for a FccA mediated reduction of the MtrA, MtrB/OmcB complex in the outer membrane [1]. These results imply that FccA is not only a periplasmic fumarate reductase but might rather be also part of the electron transport chain to ferric iron species. This is supported by growth experiments with a *fccA* deletion mutant, that suggest a role of FccA as a transient electron storage. In summary, our experiments provide evidence for an electron transfer network in the periplasmic space of *S. oneidensis* and demonstrate a possible electron transfer chain from the cytoplasmic membrane to insoluble iron.

[1] Schuetz B *et al.* (2009) Appl environ microbiol accepted

FTP48
Exploration of microbial chromate resistance strategies

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In the last decades chromium has become a wide spread pollutant in the environment. This is mainly due to anthropogenic factors, namely an often inadequate toxic waste management in leather tannery, dye-, car- and steel-industry. Consequently chromium has become the most important heavy metal pollutant in the European Union. Depending on its oxidation state chromium has varying levels of toxicity. Cr(VI) is the most toxic and biologically-mobile form, whereas Cr(III) is a highly insoluble metal species and therefore less toxic.
In this study the chromate resistance strategies of a new *Leucobacter* species were elucidated. This species is capable of tolerating more than 100 mM chromate and shows a distinct correlation between the chromate concentration in the medium and the production of aggregates. The detailed investigation of the extracellular polymeric substances (EPS) showed that sugars and extracellular DNA (eDNA) are the main components and that eDNA has a fundamental relevance concerning structural integrity of the aggregates. We could show that inhibition of aggregate formation via DNase I treatment severely influences chromate resistance. Furthermore we identified an intracellular chromate reductase activity that might be relevant for chromate resistance due to a depletion of chromate dependent radical formation. In addition, we observed that incubation with increasing chromate concentrations goes along with the enhanced production of a membrane-located red pigment which is considered to be α-carotene. These carotenes may have the ability to quench reactive oxygen species resulting from the intracellular chromate reduction.
We hypothesize that Cr(VI) reduction, radical quenching by carotenes and biofilm formation all together enable the studied *Leucobacter* strain to withstand these extremely toxic chromate concentrations.

FTP49
Identification of a TatA-interacting protein

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The twin-arginine-translocation (Tat) pathway transports folded proteins across the cytoplasmic membrane of most prokaryotes and thylakoid membranes of plant plastids. In *Escherichia coli* as well as in plant plastids, transport is achieved by multiple copies of the proteins TatA, TatB, and TatC. TatB and TatC form stable complexes that bind the transported proteins (Tat substrates) and TatA is believed to transiently interact with TatBC complexes when substrates are bound. TatA complexes can be very large and apparently have the ability to form tubes that might serve to enclose Tat substrates during the translocation process that is triggered by TatC. During purification of TatA complexes from *E. coli*, we detected a co-eluting protein that could be identified by mass spectrometry. Truncation analyses demonstrated that the N-terminus of TatA was sufficient and essential for the interaction. The interacting protein formed homo-multimeric complexes and most likely interacted with TatA only via a small subpopulation of its subunits. Interestingly, the production of the interacting protein was strongly induced by TatA. We will propose a likely functional role of this surprising new factor.

FTP50
Disproof of the postulated Tat-dependent quality control system

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The Tat system can transport folded proteins across biological membranes. It has been observed that several substrates of the Tat system were not transported when correct folding was abolished. On the other hand, it has also been shown that the Tat system can transport unfolded proteins if they lack hydrophobic patches on their surface and if they are not larger than 25 kDa in size. Recently, it has been claimed that the Tat system triggers the degradation of malfolded proteins [1, 2]. This claim was concluded from experiments in which the accumulation or degradation of recombinantly produced Tat substrates were compared in the *Escherichia coli* strain MC4100 and several tat deletion mutants thereof. Our attempts to reproduce these published quality control experiments failed. Using the original strains and plasmids of these studies, we found that the reason for the published observations were simple expression artifacts, caused by reproducibly distinct recombinant expression levels in the tested strains. We could clarify the reasons for these differences in expression levels and we could show that there exists no evidence for Tat-dependent degradation of malfolded Tat substrates so far.

[1] Matos CF, Robinson C, Di Cola A. (2008) EMBO J. 27, 2055-2063
[2] Matos CF, Di Cola A, Robinson C. (2009) EMBO Rep. 10, 474-479

FTP51
Distribution of Tat complexes in MinCDE and cardiolipin deficient strains

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The Tat system has the ability to translocate folded proteins across the bacterial cytoplasmic membrane. Three functionally different translocon components have been identified in *Escherichia coli*, namely TatA, TatB, and TatC. The subcellular distribution of the Tat components was analyzed in a *minCDE* deletion mutant at native non-recombinant expression level. Due to cell divisions at poles, the *minCDE* mutant strain forms minicells and rodcells. The minicells are exclusively generated from polar cell regions whereas rodcells contain also non-polar material. Tat components were detected by Western blot analyses with α-TatA, α-TatB, or α-TatC antibodies. TatB and TatC were more abundant in minicells than in rodcells, whereas TatA was equally abundant in both cell types during exponential growth. The distribution of TatB and TatC components was less polar in the stationary growth phase. Cardiolipin deficiency affected TatA distribution during exponential growth, resulting in higher polarity. TatB and TatC levels were not detectably influenced by cardiolipin levels. Using BN-PAGE analyses, the 440 kDa TatBC complex was detected in mini- and rodcells. A TatA signal was detected at 440 kDa in the cardiolipin deficient strain, suggesting the formation of a TatABC complex in this strain.

FTP52
The essential *E. coli* apolipoprotein N-acyltransferase (Lnt) exists as an extracytoplasmic acyl-enzyme intermediate

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Bacterial lipoproteins are unique combinations of polypeptide and fatty acids. They are universal to all bacteria and involved in a variety of biological processes including the biogenesis of the outer membrane, secretion, signal transduction and virulence. Lipoprotein modification requires at least the two enzymes Lgt and LspA, functioning as a prolipoprotein diacylglyceryltransferase and a signal peptidase, respectively. As shown for Gram-negative bacteria and actinomycetes a third enzyme in the bacterial lipoprotein modification pathway, the apolipoprotein N-acyltransferase (Lnt), transfers an acyl chain from *sn*-1-glycerophospholipid to the free α-amino group of the N-terminal cysteine of apolipoproteins, resulting in mature triacylated lipoprotein. In *E. coli* all three membrane-bound lipoprotein modification enzymes are essential, but their catalytic mechanisms as well as substrate recognition are largely unknown.
Here we report that the transfer of acyl chains by Lnt proceeds in two steps via an acyl-enzyme intermediate in which a palmitoyl group forms a thioester bond with the thiol of the active site residue Cys387. The stable acyl-enzyme intermediate allowed us to determine which essential residues in the catalytic domain of Lnt affect the formation of the acyl-enzyme intermediate, or transfer of the acyl chain to the apolipoprotein substrate. Like all residues of the catalytic triad (Cys387, Glu267 and Lys335), Glu343 is involved in the first step although it is not located in close proximity to the active site. Trp237, Tyr388 and Glu389 play a role in the second step of the reaction since acyl-Lnt is formed but transfer of the acyl group to the protein substrate does not occur. We are currently developing an *in vitro* assay to study the kinetics of N-acylation and the first results with the non-functional Lnt mutants are discussed.

FTP53
A closer investigation of flagella and membrane proteins of *Pyrococcus furiosus*

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Flagella of the archaeon *Pyrococcus furiosus* have been described as multifunctional organelles, not only made for swimming but also adhesion to various surfaces and the formation of cell-cell-contacts. Previous studies of this organism and other representatives of the domain archaea have revealed flagellar genes to be arranged in an operon. Although the function of most of these genes has not been definitely understood so far, it has been shown for *Methanococcus maripaludis* that they are necessary for proper assembly and function of flagella.
Due to an absent detectable homology to bacterial counterparts we were mainly interested in the anchoring and motility mechanisms of archaeal flagella. Therefore a protocol for lysis of stationary *Pyrococcus* cells under hypotonic conditions was established allowing the detection of flagella-associated proteins in membrane fractions. Electron microscopy was used throughout this approach to monitor the integrity of flagellar and membrane ultrastructure. Extracted complexes were subjected to SDS-PAGE to finally identify the obtained protein bands by MALDI-TOF. For further analysis and characterization those proteins were successfully cloned and expressed in *E. coli*. Generated antibodies against the recombinant proteins will enable the localization of the corresponding structures with immunolabeling techniques and improve the understanding of archaeal flagella in general.

FTP54
Analyses of the Mth60 fimbriae of *Methanothermobacter thermoautotrophicus*

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Up to now, nearly nothing is known about the ultrastructure or the mode of assembly of archaeal fimbriae. In contrast, bacterial fimbriae and pili are well analyzed and characterized. The P-pili of uropathogenic *Escherichia coli* strains e.g. are composed of six different proteins which are assembled in a certain pattern. Two further proteins are responsible for correct folding and assembly of these subunits. The genes encoding these eight proteins are clustered.
The euryarchaeon *Methanothermobacter thermoautotrophicus* possesses fimbriae with a diameter of 5 nm. These cell surface appendages were among the first detailed characterized archaeal fimbriae. They were shown to (mainly) consist of 16 kDa glycoprotein Mth60 and to function as adhesins.
Reverse transcription PCR and Northern Blotting analyses revealed that the fimbrin gene *mth60* is part of two operons: on the one hand it is cotranscribed with *mth58* and *mth59*, on the other hand it is also cotranscribed with *mth61*. Using bioinformatical methods it was possible to prove the homology of Mth59 to PapD, a periplasmatic chaperon involved in P-pili formation. The homology of Mth59 to PapD might indicate that archaeal and bacterial fimbriae have a related mode of assembly. In order to confirm the chaperon-character of Mth59 (via co-immunoprecipitation experiments), antibodies against this protein are generated at the moment. With the help of immulolabeling technique the localization of Mth59 could be clarified.
As former experiments showed, cloning of *mth60* alone is lethal for *E. coli*. Very interestingly, expression of the complete region between *mth61* and *mth58* was successful. The resulting *E. coli* strain is a very valuable tool to start a definition of the assembly machinery of the Mth60 fimbriae.

FTP55
Oxidative and nitrosative stress defence in *Wolinella* succinogenes, an exceptional Epsilonproteobacterium devoid of catalase and bacterial haemoglobins

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Microorganisms employ diverse mechanisms to withstand physiological stress conditions exerted by reactive oxygen and nitrogen species like hydrogen peroxide, organic peroxides, superoxide anions, nitric oxide, nitrite, hydroxylamine, nitric oxide and NO-generating compounds. This study identified components of the oxidative and nitrosative stress defence network of *Wolinella succinogenes*, an Epsilonproteobacterium that lacks catalase and haemoglobins.
Various gene deletion mutants were constructed and subjected to disc diffusion, growth and viability assays under stress conditions. It was demonstrated that two periplasmic *c*-type cytochromes, namely dihaem cytochrome *c* peroxidase (Ccp) and pentahaem cytochrome *c* nitrite reductase (NrfA), mediate resistance to hydrogen peroxide. A minor contribution to hydrogen peroxide tolerance was observed by a putative cytoplasmic non-haem iron protein (Rrc) that appears to be specific for Epsilonproteobacteria.
Two AhpC-type peroxiredoxin isoenzymes and thioredoxin reductase (TrxB) were shown to be involved in protection against organic peroxides. In contrast, individual deletion of the peroxiredoxin-encoding *bcp* or *tpx* genes had only minor effects toward peroxide stress sensitivity.
The phenotypes of two superoxide dismutase mutants lacking either SodB or SodB2 implied that these two enzymes functionally replace each other in superoxide and oxygen stress defence although they are proposed to be located in different cell compartments.
NrfA and a cytoplasmic flavodiiron protein (Fdp) were identified as key enzymes for nitric oxide detoxification. In the absence of NrfA, cells were also more susceptible to nitrite and hydroxylamine stress.
The results indicate the presence of a robust oxidative and nitrosative stress defence network and identify NrfA as a multifunctional cytochrome implicated in both anaerobic respiration and periplasmic stress protection.

FTP56
Biochemical and functional characterization of a putative sensor kinase from *Methanosarcina acetivorans* C2A

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Methanosarcina acetivorans C2A is a methanogenic archaeon which relies on methanogenesis as the energy conserving mechanism. *M.acetivorans* is able to use acetate, methanol and other methylated compounds as growth substrates. In contrast to most other methanogenic archaea, it cannot reduce CO_2 with electrons from H_2. Only few methanogenic species were shown to use also CO as a substrate. These organisms oxidize 4 mol CO to CO_2 for every 1 mol of CO_2 reduced to CH_4 and produce H_2 during this process. *M.acetivorans* also grows with CO as the sole energy source. Despite the 4:1 ratio of CO to CH_4 and the production of H_2 in the other organisms, the major products of growth on CO in *M.acetivorans* are acetic acid, formic acid and methylated sulfides, besides methane, but not hydrogen. The latter is due to the lack of a functional hydrogenase system.
Recently, it was shown that three methyltransferase/corrinoid fusion proteins are required for generating dimethylsulfide (DMS) from CO and CH_4 from DMS [1]. These proteins are each differentially regulated by a downstream regulator protein [2]. MA4560, one of these regulators, is a putative response regulator of a two component regulatory system together with the sensor histidine kinase MA4561.
Different truncated variants of this sensor kinase were heterologously produced in *Escherichia coli* and purified using metal affinity chromatography. Full length protein as well as variant GAF2 were shown to contain a heme cofactor. The amount of bound heme in the recombinant protein could significantly be increased by addition of the heme precursor 5-aminolevulinic acid to the growth medium. The heme cofactor is tightly bound to the protein and cannot be removed using denaturing conditions pointing towards a covalent bond. Due to the involvement of the corresponding response regulator MA4560 in regulating gene expression in response to CO, a potential role of the sensor kinase MA4561 in CO-sensing via the heme cofactor is postulated.

[1] Oelschläger, E., and Rother, M., *Mol Microbiol (2009) 72(5), 1260–1272*
[2] Bose, A. *et al.*, *Mol Microbiol (2009) 74(1), 227-238*

FTP57
Immuno-localization of proteins in cells of Ignicoccus for electron microscopy

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The ultrastructure of the hyperthermophilic Archaeon *Ignicoccus hospitalis* is more complex than most other Archaea. It does not exhibit an S-layer, but has a vesicle-forming cytoplasmic membrane, a huge periplasmic space, and an outer membrane. In order to understand its ultrastructure, we have analyzed the subcellular distribution of a variety of proteins by immun-localization on ultrathin sections.
For best structural preservation, cells were cultivated in capillary tubes, immobilized by high-pressure freezing, freeze-substituted and embedded in Epon. Ultrathin sections were subsequently labeled with primary and secondary antibodies using established protocols; secondary antibodies were coupled to ultrasmall or 6 nm gold particles. In addition, membrane proteins were also localized by freeze-etching combined with immuno-labeling, or briefly freeze-fracture labeling.
So far, we were able to uniquely localize several proteins to the outer membrane: the Ihomp1 protein; the fiber protein; the sulfur reductase / hydrogenase complex; and the A1AO ATP synthase. The latter enzyme was also found to be located in low amounts in the cytoplasm; a possible location in the cytoplasmic membrane is yet unclear. Also, the fiber protein was detected in significant amounts in the cytoplasmic membrane. These findings show that outer membrane is far more complex than previously anticipated. Currently, we employ double labeling in order to investigate the spatial distribution of these protein complexes in more detail, and their possible interactions. Furthermore, we are in the course of raising additional antibodies against some abundant proteins, identified in our proteomic analysis of *I. hospitalis*.

FTP58
Regulation of the maltose ABC-transporter (MalE-FGK_2) by enzyme IIA^{Glc}

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Enzyme IIA^{Glc} ($EIIA^{Glc}$) is a component of the phosphoenolpyruvate-dependent glucose phosphotransferase system (PTS), which is essential for the coupled transport and phosphorylation of various types of carbohydrates. Furthermore, it is involved in the global control of carbon catabolism in *Escherichia coli* and *Salmonella enterica* serovar Typhimurium. The availability of glucose, the preferred carbon source, leads predominantly to the unphosphorylated state of $EIIA^{Glc}$. Thus, it is able to inhibit several permeases allosterically, including the maltose ABC-Importer MalE-FGK_2. The process of inhibition is termed "inducer exclusion" and is mediated by direct interaction of unphosphorylated $EIIA^{Glc}$ and $MalK_2$, the nucleotide binding subunit of the transporter.
Pep scan analysis revealed the presence of two sites on $EIIA^{Glc}$ that interact with MalK [1]. Here, we have investigated the putative contact sites on $MalK_2$ by homobifunctional cross-linking. To this end, mono-cysteine variants of both $EIIA^{Glc}$ and the reconstituted transporter were analyzed for the formation of cross-linked products under various conditions. Our results suggest that site I of $EIIA^{Glc}$ is close to an N-terminal region of one MalK subunit while site II is in cross-linking distance to the C-terminal domain of the opposing MalK monomer. Moreover, strongest cross-links were obtained in the post-hydrolysis (ADP-bound) state of the transporter. Thus, we propose that inhibition of the ATPase activity of the transport complex is due to preventing its return to the resting state after one step of hydrolysis.

[1] Blüschke, B, Volkmer-Engert, R, and Schneider, E, (2006) J. Biol. Chem. 281, 12833.

FTP59
Construction and characterization of a *Magnetospirillum gryphiswaldense recA* deletion mutant

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The most intriguing feature of magnetotactic bacteria (MTB) is their ability to produce species-specific membrane enclosed crystals of a magnetic iron material, the so called magnetosomes. Their uniform sizes (30-120 nm), shapes, and unique magnetic properties make them highly attractive for biotechnological applications, which have been hampered by the difficult cultivation of most MTB and poor understanding of genetic mechanisms governing magnetosome formation. In the model organism *M. gryphiswaldense*, most of the genes controlling magnetosome formation have been identified within the 130 kb genomic magnetosome island (MAI). The MAI is genetically instable due to frequent rearrangements and spontaneous deletions caused by homologous recombination between numerous direct repeats.
In order to increase genetic stability and improve expression of homologous and heterologous magnetosome gene clusters, a *ΔrecA* strain of *M. gryphiswaldense* was constructed by cre-lox based deletion mutagenesis, which is the first documented *recA* mutant in a magnetotactic bacterium. The *ΔrecA* strain of *M. gryphiswaldense* displays typical characteristics of the general $RecA^-$ phenotype observed in other bacteria, such as *E. coli*. For example, it shows a 17-fold increased UV sensitivity and is severely reduced by 98% in homologous recombination efficiency. Furthermore, viability and growth under aerobic conditions are significantly impaired. The *ΔrecA* strain is currently analyzed as a putative host for functional expression of large magnetosome clusters from cultivated and uncultivated MTB. Although first experiments suggest that the observed instability of large plasmids harboring entire magnetosome operons is not exclusively caused by homologous recombination, its increased genetic stability may render a *ΔrecA* strain superior for protein expression and production of bacterial magnetosome particles.

FTP60
Insights into chromosome segregation in staphylococci

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Accurate chromosome organization and segregation are essential for all living cells. Astonishingly, *Staphylococcus aureus* smc (structure maintenance of chromosome) insertion mutants showed neither growth defect nor aberrant chromosome organization although chromosome segregation was considerably affected. SpoIIIE protein is known to rescue septum-entrapped chromosomes. The *S. aureus* smc/spoIIIE double mutants showed a more severe phenotype than the single mutants, but were still viable in contrast to previous studies from rod-shaped bacteria.

FTP61
Loss of the actin-like MamK protein has pleiotropic effects on magnetosome chain formation in *Magnetospirillum gryphiswaldense*

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Magnetic orientation in magnetotactic bacteria (MTB) is based on organelles, the magnetosomes (MMs), which are intracellular membrane enveloped magnetites. Ultrastructural and genetic analysis of magnetosome chains in two related MTB revealed a cytoskeletal structure, the magnetosome filament, presumably the actin-like MamK protein, to which MM particles are connected via the acidic repeat protein MamJ. According to this model, deletion of either of them was expected to result in similar phenotypes. However, a *mamK* mutant in *M. magneticum* AMB-1 displayed a different phenotype (scattered MMs) than a *mamJ* mutant of *M. gryph.* MSR-1 (clustered MMs). To verify the differences of both mutants a Δ*mamK* strain of MSR-1 was generated.
Surprisingly, the phenotype of the $\Delta mamK_{M.\ gry}$ did neither resemble the $\Delta mamK_{M.\ mag}$ mutant nor the Δ*mamJ* phenotype in MSR-1. Consistent with $\Delta mamK_{M.\ mag}$ filamentous structures were absent in tomograms of a $\Delta mamK_{M.\ gry}$ recorded by cryo-electron tomography (CET). Magnetosome filaments could be restored by transcomplementation with MamK genes from either MSR-1 or AMB-1, suggesting their functional equivalence. Filaments in MSR-1 wildtype extend to the cell poles in CET and consistent to that cell spanning filaments were observed in eGFP fusions of MamK expressed in $\Delta mamK_{M.gry}$. Unlike the AMB-1 mutant, the $\Delta mamK_{M.\ gry}$ still formed magnetosome chains but fragmented and spaced by large gaps. Subchains were shorter, displaced from their usual midcell position to polar localization. Unexpectedly, iron accumulation decreased to 54 % of wildtype MM crystals.
These results are only partially consistent with previous experiments and the assigned function of MamK has to be revised in terms of a more complex and potentially dynamic role in magnetosome chain formation and positioning as well as in magnetite biomineralisation.

GIP01
Novel interactions of the Cag Type IV Secretion system of *Helicobacter pylori* with the Integrin β1 subunit

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Trägerin des Doktorandenpreises

Helicobacter pylori is a Gram negative bacterium, the presence of which in the human stomach is associated with gastric pathologies, including gastric cancer. The Cag Type IV Secretion System (Cag T4SS) and the Vacuolating cytotoxin (VacA) are part of its virulence mechanisms. The Cag system translocates the CagA protein into the host cells inducing changes in different pathways of the cell. The Cag T4SS is encoded in a pathogenicity island (PAI) which contains around 30 genes. The functionality of the Cag apparatus has been associated not only with the ability to translocate the CagA, but as well with its capacity to induce IL-8 production in human cells. Previously it has been proposed that the Cag apparatus uses integrin α5β1 through interactions of apparatus components with the region of natural ligand interaction on the integrin β1. While we have found as well the integrin β1 being essential for CagA translocation, we have discovered that the interaction is limited to the integrin β1 subunit and it is independent of the RGD motif present on the CagL component. As major integrin β1 interacting components of the cag-T4SS we identified CagA, CagY and CagI. Here we present the data supporting a novel way of interaction between the Cag apparatus and the integrin β1

GIP02
Identification of surface structures common to Gram-negative bacteria which are suitable for vaccine development

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Infectious diseases caused by Gram-negative bacteria are problematic throughout the world. Especially in developing countries with limited access to therapeutics the mortalities due to those diseases are partly tremendous. A broad-range vaccine that could provide full or partial protection against a number of infectious diseases and that is effective for years would be a big improvement of the health care in those countries.
To identify broad-range vaccine candidates we initiated an interdisciplinary approach of bioinformatics and biochemistry. The basic idea is to identify all proteins in Gram-negative bacteria that are part of the cell surface using bioinformatics, and to find conserved peptide epitopes between them that could be used as vaccine candidates. Ideally, such epitopes would be present in large subsets of Gram-negative pathogens. To this end we created a database of proteins of all Gram-negative bacteria with available sequenced genomes. In this database, proteins were clustered according to their conservation, and were annotated according to their predicted subcellular location. We searched this database for peptide epitopes that are present in homologous (i.e., closely related) proteins from different pathogenic species, and that are localized on the bacterial surface. To ensure that targets which are identified from bacterial genomes are really expressed during an infection, we performed extensive proteomics studies of bacterial cell walls using membrane fractionation techniques combined with mass spectrometry.
By comparing bioinformatics and biochemical approaches we got a list of promising candidate epitopes suitable for further biochemical and immunological experiments, and later, for vaccine development. We focused mainly on the identification of broad-range vaccine candidates against diarrhea-causing species, but our setup has potential to be implicated in a number of other similar vaccine and diagnostic approaches.

GIP03
Mortality burden of *Clostridium difficile*-associated infection: an underrecognized crisis

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Objectives: In response to the increase of Clostridium difficile associated infection (CDI) and the emergence of Clostridium difficile ribotype 027 in Germany in 2007, the Robert Koch Institute (RKI) implemented nationwide notification of severe cases of CDI, starting from November 2007.
Methods: We used first years surveillance data to assess the population burden of CDI by calculating years of life lost (YLL) for infections with *Clostridium difficile*. In addition, data from the national surveillance system were used to assess YLL for the most common gastrointestinal pathogens which are notifiable in Germany, i.e., *Campylobacter spp*., *Salmonella spp*., rotavirus, and norovirus. All fatal cases associated with these pathogens from 2004-2007 were included. For CDI, we included fatal cases from 01/01/2008-31/12/2008. WHO life expectancy table (West Label 26) without discount and age-weighting was used to calculate sex-weighted YLL for 19 age groups with a cut-off at age 85+ years.
Results: A total of 419 cases of severe CDI, including 224 fatalities, were reported to the RKI. Age dependent YLL are shown in figure 1. Overall, CDI constitutes 66% of all YLL among the included gastrointestinal diseases.
Conclusions: Substantial underreporting of severe and fatal CDI should be expected, since reporting is physician-based so far. With steeply increasing case numbers in Germany , our result is alarming. YLL from CDI is an important measure of population burden and should be considers when allocating public health resources. To determine trends in occurrence of more toxigenic strains and their impact in the community and in the hospital setting, continued surveillance for CDI is compulsory on an international scale.

GIP04
Single locus sequence typing of *Clostridium difficile* infections using surface layer protein A (slpA)

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Clostridium difficile infection and associated diarrhoea (CDAD) has attracted notice to the community due to the worldwide spreading of potentially highly virulent strains, as e.g. ribotype 027. 027 strains are characterized by unregulated toxin production due to mutations of a potential toxin repressor gene (tcdC), but also due to high antibiotic resistance against macrolides and fluorochinolones. However, the severity of disease varies between patients infected with the same clonal 027 strain and severe disease is also caused by various other strains as e.g. endemic 001 strains. Although the German reporting system requires only reports of cases with severe or recurrent 027 infections (§7 IfSG) the detection of 027 alone (e.g. by PCR) seems to be not sufficient for characterization of most nosocomial outbreaks. In contrast, *C. difficile* genotyping is more flexible for identification of outbreaks with various genotypes which seems to be important in times of changing *C. difficile* epidemiology with potentially new outbreak strains (e.g. 078). In the present single centre study we continued to characterize clinical *C. difficile* isolates of patients with CDAD by single locus sequence typing using the surface layer proteine A gene (slpAST). SlpAST proved to be a highly reproducible, easy applicable method for C. difficile typing. By using slpAST the most prevalent ribotypes can be easily identified by databank search (BLAST); however, the more rare sequence types are in part still missing in the databases which impedes the unrestricted use of slpAST in laboratories with less experience. Therefore we are constructing now an own extended database by including databank sequences, reference strain sequence and own sequence data of rare clinical isolates. This databank will facilitate identification of most *C. difficile* strains by single locus sequence typing which is important for easy and more general use of *C. difficile* genotyping by slpAST.

GIP05
Isolation of vibrio and other related organisms from Nworie river

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Water samples collected from Nworie River were evaluated for the presence of Vibrio species and related organisms. Media used for the isolation included nutrient agar, MacConkey agar and Thiosulphate Citrate Bile Salt Sucrose Agar (TCBS). Results obtained indicated that nutrient agar gave counts of 2.18X108 cfu/ml which were 1 and 4 log cycles higher than counts obtained from MacConkey (1.12X107 cfu/ml) and TCBS agar (3.8X104 cfu/ml) respectively. Most of the bacterial isolates were members of the entereobacteriacae family which are known to inhibits aquatic environments and they included *Escherichia coli*, Entereobacter species, *Klebsiella sp*., *Citrobacter sp*., *Shigella sp*., and *Proteus sp*; Others which included *Hafnia sp*., and *Salmonella sp*; The Vibrio group of organisms and Aeromonas sp., were isolated using MacConkey and TCBS agar respectively. This indicates that the study area is not good enough for domestic purposes unless concerted efforts are made to stop the pollution of the environment using mortuary effluents. The study was aimed at determining enteric gram negative aerobes from Nworie River. From the result they were all isolated and identified by some biochemical tests. It goes on to show that the site of study is highly polluted with these and many more organisms.

GIP06
Identification and characterization of human pathogenic *Escherichia coli* isolates from Brazilian patients

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Intestinal pathogenic *Escherichia* coli are important etiological agents worldwide and can cause a broad spectrum of diseases. Especially in developing countries, diarrhoea represents a major public health problem, mainly affecting children under the age of five. Diarrhoeagenic *E. coli* can be classified into seven major pathotypes due to their virulence factor profiles and pathogenesis, including enteropathogenic *E. coli* (EPEC), atypical enteropathogenic *E. coli* (ATEC), enterohaemorrhagic *E. coli* (EHEC), Shiga toxin-producing *E. coli* (STEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), and enteroaggregative *E. coli* (EAEC).
We have developed a specific one-step multiplex PCR (MPCR) for the differential identification of intestinal pathogenic *E. coli* isolates. This technique has proven to be a reliable tool to examine virulence factor profiles of potentially diarrhoeagenic *E. coli* strains isolated from patients living in São Paulo, Brazil.
In total, 702 isolates of 304 patients were investigated via MPCR. The majority of strains harbouring virulence factor-encoding genes can be assigned to the EAEC pathotype. In addition, we identified members of almost all other pathogroups. All these isolates are currently characterized further in terms of their virulence factor expression profiles, their effect on tissue culture cells with focus on the actin cytoskeleton, invasiveness, and secretion of locus of enterocyte effacement (LEE) effector proteins.

GIP07
Receptor mediated endocytosis of *Helicobacter pylori* VacA into human T lymphocytes

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Helicobacter pylori colonizes about half of the world's human population. *H. pylori* is a spiral – shaped, microaerophilic, Gram negative bacterium that colonizes the human stomach and is associated with gastric diseases including cancer. As part of its virulence mechanisms, it possess the Type IV Secretion System (T4SS) protein CagA and the Type V autotransporter Vacuolating Cytotoxin A (VacA). The mature form of VacA (95 kDa) toxin assembles in a flower shaped dodecameric form (>900kDa). The mature VacA toxin shows sequence variations on the N-terminal signal sequence (s1a, s1b, s1c, s2), in its middle region (m1, m2) and in a recently identified i-region (i1, i2). VacA induces multiple structural and functional alterations in various mammalian cell types. VacA was found to induce the formation of large intracellular acidic vacuoles in eukaryotic cells. Furthermore, VacA interferes with activation and proliferation of T lymphocytes by alteration of the IL-2 signaling pathway. In order to achieve this effect, VacA binds to its receptor on T-cells, the Integrin beta 2 (CD18), and it is internalized. Once inside, VacA reaches to the late endosomal compartment. This pathway has been well characterized in epithelial cells. Our work here describes part of the internalization pathway in T lymphocytes. Here we show that the cytoplasmic domain of CD18 plays an important role in the uptake process. Distinct members of the Protein Kinase C (PKC) family of proteins are essential for VacA uptake in $CD4^+$ T cells. Knowing the exact pathway which induces entry of VacA into immune cells, we hope to achieve a better understanding of the immunomodulatory function of VacA.

GIP08
Effects of antimicrobial agents on the release of Shiga toxin 2e by porcine *Escherichia coli* strains *in vitro*

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Edema disease (ED) is a life-threatening enterotoxaemia of weaned piglets caused by Shiga toxin-producing *E. coli* strains encoding the genes for Shiga toxin 2e (Stx2e) and F18 fimbriae (EDEC). The production of Stx2e is crucial in the pathogenesis and clinical outcome of ED. Antimicrobial agents (AM) are commonly used in an effort to eradicate EDEC from the intestine of infected piglets. However, several AM have been reported to enforce the release of Stx from enterohemorrhagic *E. coli* associated with human disease. In this study we assessed the effect of six conventional AM (amoxicillin, colistin, enrofloxacin, erythromycin, neomycin and tetracycline) on Stx2e release by 30 porcine STEC strains *in vitro* in comparison to the well characterized bacteriophage inducers Mitomycin C (MMC) and UV irradiation (UV).
Minimum inhibitory concentrations (MICs) for all AM were determined by the broth microdilution method. Isolates were grown to a defined culture density and exposed to MMC, UV and to the six AM (100 % MIC, 5 h) while a control culture was not treated. Subsequently, the amounts of released Stx2e were quantified in supernatants of treated and untreated cultures by ELISA.
Upon exposure to AM, 7 of 30 (23.3 %) strains responded with an increase (1.2-16.9-fold) of released Stx2e amounts. Enrofloxacin had the greatest impact on the Stx2e release. Stx2e release was induced by enrofloxacin, neomycin and amoxicillin in 6, 2 and 1 strains, respectively. When strains had been exposed to MMC or UV, the culture supernatants of 6 of 30 (20 %) strains contained up to 50.8-fold more Stx2e than those from untreated control cultures. Interestingly, 5 of 7 strains which responded to one or more AM did neither respond to treatment with MMC nor UV.
We conclude that Stx2e release of some porcine STEC can be enhanced by commonly applied AM *in vivo* and therefore may trigger the clinical manifestation of ED during treatment of infected animals with subinhibitory doses of AM.

GIP09
H. pylori outer membrane protein HopQ is a putative virulence factor essential for TFSS pathogenesis

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Helicobacter pylori infection induces several gastrointestinal diseases. A number of virulence factors of *H. pylori*, e.g. cagPAI, CagA, VacA, and some adhesins, were identified and described in association with the higher risk of peptic ulcer disease and gastric adenocarcinoma. *H. pylori* expresses more than thirty genes, which encode outer membrane proteins (OMPs) including Hop (Helicobacter outer membrane encoding proteins) and Hor (Hop-related) protein groups. Some of these autotransporter-like OMPs were identified as adhesins, yet, the role of several others is not fully understood. Two alleles of genes encoding HopQ were found; the type I allele was commonly detected in *H. pylori* strains carrying cagA, whereas the type II allele was associated with *H. pylori* strains lacking cagA gene.
In our work we observed that knockout of HopQ protein in the type I *H. pylori* P12 strain does not significantly affect bacterial adherence as determined by bacterial plating and immunofluorescence analysis. However, HopQ-defective mutant of *H. pylori* P12 is incapable to translocate CagA into gastric epithelial cells. Furthermore, we observed that signal transduction pathways dependent on the type IV secretion system were only partially activated in AGS cells after infection with the *H. pylori* mutant. On the basis of our data we hypothesize that deletion of HopQ protein causes a malfunction of the type IV secretion system and leads to failure of CagA translocation.

GIP10

Will not be presented and published

GIV01

Deciphering the nutritional requirements of *Campylobacter jejuni* during persistence in a murine colonization model

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Campylobacter jejuni is a major cause of food-borne disease in industrialized countries. We found recently that the highly pathogenic strain *C. jejuni* 81-176 has an extended capacity to metabolize amino acids in comparison to the *C. jejuni* reference strain NCTC 11168. The amino acids aspartate, glutamate, proline and serine are growth promoting substrates for both isolates, but only *C. jejuni* 81-176 possesses the capability to utilize additionally asparagine and glutamine / glutathione. We identified two enzymes in *C. jejuni* 81-176, a secreted asparaginase and a gamma-glutamyltranspeptidase, that are responsible for the growth with asparagine and glutamine / glutathione, respectively. These properties are missing in NCTC 11168 and several other tested *C. jejuni* isolates. Strikingly, the additional glutamine / glutathione metabolism of *C. jejuni* 81-176 contributed to its enhanced capability to colonize the intestine in a mouse colonization model, whereas the asparagine utilization resulted in a more efficient persistence of *C. jejuni* 81-176 in the liver of infected mice.
We have continued to investigate the contribution of amino-acid-driven growth on the persistence of *C. jejuni* 81-176 in mice by using co-infection experiments with wild-type and isogenic *C. jejuni* 81-176 mutants that are unable to import proline. In particular, we examined the importance of proline utilization for the infection process of *C. jejuni*, as we have shown that growth with proline is a conserved metabolic trait in all *C. jejuni* strains tested thus far. To complement these studies we tested the impact of serine and glutamate / aspartate metabolism on the tissue tropism of *C. jejuni 81-176* in this murine infection model. With this approach we discovered distinct correlations between the metabolism of tested amino acids and the persistence of *C. jejuni* in different tissues.

GIV02

Early Events in *Aeromonas hydrophila* Infection

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Aeromonads cause a variety of infectious diseases, including gastroenteritis, peritonitis, sepsis, and further wound infections, like cellulitis or necrotizing fasciitis. Although pathogenesis of *Aeromonas hydrophila* and its enterotoxin aerolysin has been described in many studies, the mechanisms of the epithelial barrier dysfunction and necrotization are not completely understood.
Human HT-29/B6 epithelial cell monolayers were apically inoculated with clinical isolates of *A. hydrophila* or by the secreted toxin aerolysin. Epithelial resistance and macromolecule fluxes were determined in Ussing chambers. The subcellular distribution of tight junction (TJ) and cytoskeleton proteins was analyzed by confocal laser-scanning microscopy. The ability of wound healing was quantified in infected epithelial cells by induction of single cell lesions and measuring of recovery time.
The main paracellular part of epithelial dysfunction was not considered yet, since aerolysin is only known to induce active chloride secretion with a concomitant resistance effect. Here we show that infection of HT-29/B6 cells increased paracellular permeability to fluorescein and to dextran-4 kDa. As structural correlate, TJ proteins were re-distributed off the TJ by actomyosin contraction. Moreover, we measured a delayed epithelial wound closure through disturbance of cytoskeletal and TJ components. All effects could be abolished by pre-treatment with zinc, which inhibits aerolysin channel formation in the host cell membrane. The significant inhibition by zinc parallels the efficacy of zinc therapy of epithelial lesions and diarrhea. In conclusion, the pore-forming toxin of *A. hydrophila* induces epithelial barrier dysfunction along the paracellular route via TJ disruption (leak flux) and concomitant delayed wound closure as early events in infection which contributes to our understanding of the pathomechanisms of hemolysin-producing bacteria.

GIV03

Characterization of secreted components of the HHGI1 type VI secretion system in *Helicobacter hepaticus*

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Type VI secretion systems (T6SS) were recognized as novel and complex secretory pathways of Gram-negative bacteria only in 2005. Since then, they have been detected in about 100 genomes of pathogenic as well as non-pathogenic bacteria. One such bacterial species is *Helicobacter hepaticus*, a murine intestinal pathogen of the enterohepatic group of *Helicobacter* spp.. *H. hepaticus* possesses a genomic island, HHGI1, comprising T6SS genes. HHGI1 was shown to be associated with the induction of severe colitis in *H. hepaticus*-infected IL-10-/- mice. However, the functionality of single T6SS components and their role in virulence in these bacteria has not been revealed yet.
The aim of our current investigation is the molecular characterization of the *H. hepaticus* HHGI1 T6SS with regard to its surface subunits, their structure and function, and possible role as effectors. Initially, we focussed on the homologs of the known transported T6SS components: VgrG, encoded on the HHGI1 by three different genes, vgrG1, vgrG2, vgrG3, and Hcp. We performed expression and localization studies of selected T6SS components (RNA and protein) and tested the functionality of the secretion system.
H. hepaticus T6SS components proved to be expressed both *in vitro* and *in vivo* during murine infections. Their transcript patterns in mice differed from expression *in vitro*. Detection of some gene transcripts *in vivo* increased over the duration of the infection. The Hcp and VgrG expression *in vitro* was not suppressed by defined mutations in other genes of the HHGI1. After subcellular fractionation, Hcp was prevalently present in the soluble fraction, while VgrG presented predominantly as an insoluble protein. Secretion of both VgrG1 and Hcp was observed and was dependent on other HHGI1 genes. Finally. the effects of VgrG and Hcp in eukaryotic cells were investigated and will be further discussed.

GIV04

Comparative analysis of *Helicobacter pylori* genome sequences reveals mechanisms of horizontal gene transfer and genome evolution

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The human gastric pathogen *Helicobacter pylori* is known as a species with an enormous sequence diversity in housekeeping genes. Although it is a highly adapted species with a comparably reduced genome size, previous data suggest that a considerable variability exists in gene content as well, and that adaptive evolution may lead to strain divergence. In many bacterial species, different isolates were found to contain a surprising extent of genome variability, and the respective core genomes often represent only a small fraction of the entire genetic repertoire. Comparative genomic approaches are therefore required to obtain a full view of host adaptation capabilities.
We have determined the complete genome sequence of *H. pylori* strain P12 that was isolated from a duodenal ulcer patient, and we have compared it with other complete *H. pylori* genome sequences with respect to isolate-specific genes and their putative horizontal transfer mechanisms. This analysis revealed that two individual *H. pylori* strains may differ in up to 12% of their gene content, and the amount of genes specific to each isolate indicated that *H. pylori* possesses an open pan-genome. Many strain-specific genes were found to be located at potential genome rearrangement sites or in genome plasticity zones. In contrast to other strains, the P12 genome contains three different plasticity zones, two of which are likely to represent genomic islands that have been integrated into restriction/modification pseudogenes. Moreover, both islands encode complete type IV secretion systems that are related but clearly distinct. Together with the cag pathogenicity island and the ComB system, P12 thus contains four different type IV secretion systems. We demonstrate that one of these genomic islands is capable of self-excision and horizontal transfer by a conjugative process.

We also show that excision of this genomic island is mediated by a member of the XerD rather than the IntG family of tyrosine recombinases.

GIV05
Characterization of DNA import events in NER-deficient *Helicobacter pylori* mutant strains

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Extensive genetic diversity and rapid allelic diversification are characteristic of the human gastric pathogen, *H. pylori*, and are believed to contribute to its ability to cause chronic infections. Both, a high mutation rate and frequent imports of short (~400 bp) fragments of exogenous *H. pylori* DNA during mixed infections play important roles in generating allelic diversity. We have previously developed an *in vitro* transformation protocol that permits to study the role of individual recombination or repair genes in determining import length and frequency. In this study, we have analyzed the influence of the nucleotide excision repair (NER) pathway on mutation and recombination frequencies, and on the lengths of the DNA fragments integrated after recombination. The NER system controls the elimination of a variety of bulky lesions produced by chemical agents and UV-light. In *E. coli*, this process includes the recognition of the lesion by UvrAB complex, incision at both sides of the damage catalyzed by UvrC, removal of the excised segment by UvrD and gap repair by DNA synthesis and ligation. We constructed mutants in the NER genes, uvrA, uvrB, uvrC and uvrD, and tested these mutants in the transformation model. Inactivation of uvrA or uvrB significantly reduced (uvrA) or abolished (uvrB) the generation of recombinants, while uvrD mutants exhibited a hyperrecombinational phenotype. Inactivation of uvrA and uvrD did not affect import length. Strikingly, we found that uvrC mutants incorporated longer fragments of donor DNA than wild type bacteria (2.2-fold increase). The data suggest that the UvrC component of the *H. pylori* NER pathway is involved in the control of the length of imported DNA after natural transformation. Possible mechanisms explaining this unexpected role of UvrC will be discussed.

GIV06
Prostaglandin E2 prevents Helicobacter-induced gastric preneoplasia and facilitates persistent infection in a mouse model

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Persistent infection with the human pathogen *Helicobacter pylori* is closely associated with an elevated gastric cancer risk. We aimed to elucidate the role of cyclooxygenase-2 (COX-2) and its main product, prostaglandin E2 (PGE2), in the development of gastric cancer precursor lesions. We utilized mouse models of Helicobacter-induced gastric preneoplasia and vaccine-induced protection to study the effects of COX-2 inhibition and PGE2 treatment on the induction of Helicobacter-specific immune responses and gastric premalignant immunopathology.
COX-2 and PGE2 were found to be up-regulated upon Helicobacter infection in cultured gastric epithelial cells and in the mucosa of infected mice. Inhibition of COX-2 enzymatic activity significantly accelerated early preneoplasia; conversely, systemic administration of synthetic PGE2 in different genetic backgrounds prevented the formation of premalignant pathology and completely reversed pre-existing lesions by suppressing IFN-γ production in the infected stomachs. The protective effect of PGE2 was accompanied by increased Helicobacter colonization in all models, including the vaccine-induced protection model. All *in vivo* effects could be attributed to a profound immunosuppressive effect of PGE2 on CD4+ Th1-polarized effector T-cells, which fail to migrate, proliferate and secrete cytokines when exposed to PGE2 *in vitro* and *in vivo*. T-cell inhibition was found to be due to silencing of IL-2 gene transcription, and could be overcome by supplementation with recombinant IL-2 *in vitro* and *in vivo*.
We show that COX-2/PGE2-dependent pathways have an important immunomodulating role in the Helicobacter-host interaction, preventing excessive local immune responses and the associated immunopathology by inhibiting the effector functions of pathogenic T-cells.

GRP01
Pleiotropic roles of a transcriptional regulator in *Ralstonia eutropha* H16

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Recently obtained proteome data of *Ralstonia eutropha* H16 revealed a two-component system that is upregulated under lithotrophic growth conditions [1]. Sequence analysis offered high similarity to the BhpP/Q two-component system of *Acidovorax* sp. KKS102 that is discussed to contribute to the catabolite control of the *bph* operon encoding the enzymatic repertoire for PCB/biphenyl degradation.
The β-proteobacterium *R. eutropha* has a remarkably versatile metabolism. It is capable of oxidizing hydrogen or formate as energy source while assimilating carbon dioxide into cell material. For heterotrophic growth, this bacterium utilizes numerous carbon sources including sugar, sugar acids and amino acids. Succinate and other dicarboxylates are the preferred carbon and energy sources. Despite the fact that a wealth of information is known about the degratative pathways in *R. eutropha*, the global regulatory circuits remain obscure. In this context, the properties of the lithotrophically upregulated two-component system were explored by characterizing a deletion mutant lacking the dedicated response regulator gene.
Growth experiments revealed a pleiotropic phenotype affected in growth on various substrates including fructose and other less favoured energy sources. Subsequent transcriptional analyses via RT-PCR of a set of degradative genes revealed a complete or partial inhibition of the expression in the response regulator mutant.
These results indicated that the response regulator likely acts as a transcriptional activator of a number of metabolic systems that code for consumption of fructose and alternative poor energy sources. This leads to the conclusion that the two-component system is an additional player in global metabolic regulation in *R. eutropha* and possibly other bacteria.

[1] E. Schwartz, *et al.*, Proteomics, 2009 Oct; 9(22):5132-5142

GRP02
Regulation of *leuO* in *Escherichia coli*

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The LysR-type transcription factor LeuO exists in several enterobacterial species and functions as a global transcriptional regulator in stress response and pathogenicity. In *Escherichia coli*, it affects transcription of a number of genes, such as the *bgl* operon, the *cadCBA* operon, the small ncRNA *dsrA*, and the *yjjQ-bglJ* operon. Moreover, a genome-wide SELEX experiment revealed that LeuO binds to regions of the *E. coli* chromosome encompassing genes for sulfa-drug efflux. In *Salmonella*, LeuO regulates expression of the pathogenicity determinant *ompS1*. In *Vibrio cholerae*, *leuO* is required for biofilm formation. At several loci, LeuO antagonizes the silencing effect of the nucleoid-associated protein and global repressor H-NS.
The *leuO* gene is located in divergent orientation to the *leuABCD* operon encoding enzymes for leucine synthesis. Previously, it was shown that *leuO* expression is repressed by H-NS and slightly increased under conditions of leucine limitation and upon entry into early stationary growth phase. A complex model for control of *leuO* was proposed which includes autoregulation and DNA looping by LeuO as well as transcription-induced changes in DNA supercoiling.
We investigated regulation of Escherichia coli K-12 *leuO* using a *leuO* promoter-*lacZ* fusion as reporter system and real-time PCR for expression analysis in the natural context. In addition, we performed a transposon mutagenesis to screen for mutations which activate *leuO* transcription. Our data confirm that *leuO* transcription is repressed by H-NS. Additionally, we found that StpA (the H-NS analogue) represses *leuO* independently of H-NS, and that the LeuO protein negatively auto-regulates expression of *leuO*. Furthermore, *leuO* expression is strongly activated by LuxR-type transcription factors. These and further data suggest that the membrane stress sensory phosphorelay system Rcs is involved in activation of *leuO* expression.

GRP03
Topology of functional sites of the anaerobic fumarate/succinate antiporter DcuB of *E. coli*

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The fumarate/succinate antiporter DcuB is the most important carrier for C_4-dicarboxylates during growth by fumarate respiration. Expression of *dcuB* is stimulated under anaerobic conditions by the C_4-dicarboxylates-sensing two-component system DcuSR in the presence of C_4-dicarboxylates [1, 2]. The carrier additionally affects the function of the DcuSR system and acts as a second sensor for anaerobic C_4-dicarboxylates uptake: Deletion of the antiporter causes constitutive expression of the DcuSR-dependent genes in the absence of C_4-dicarboxylates.
By random and site-directed mutagenesis amino acid residues of DcuB were identified which are essential for transport or for regulation. The regulatory site is located close to the C-terminal end of the protein and separated from transport relevant amino acid residues. Therefore DcuB is a bifunctional protein with independent sites for transport and regulation [3].
The topology of the regulatory and of the transport domains was determined by dcuB-phoA/-lacZ fusions and by mapping with MeO-PEG-MAL.

[1] Zientz *et al.* (1998) J. Bacteriol. 180:5421-5425
[2] Golby *et al.* (1999) J. Bacteriol. 181:1238-1248
[3] Kleefeld *et al.* (2009) J. Biol. Chem. 284:265-275

GRP04
Deglycating enzymes in *Bacillus subtilis*: Examination of gene expression and the physiological function

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Amadori products represent the first stable intermediates of the Maillard reaction; they result from glycation of amino acids or proteins with reducing sugars. The latter react non-enzymatically with amino groups to initially form a Schiff base that can rearrange to a ketoamine adduct.
Amadori products are found in foods that have been baked, roasted, or stored for longer periods of time at room temperature. Thus, autoclaved complex media with sugar and proteinacous ingredients also contain such products. Furthermore patients suffering from diabetes have a higher blood plasma concentration of Amadori products with implications for hyperglycemia-induced diabetes complications.
Two classes of enzymes are capable of deglycating Amadori products: fructosyl-amino acid oxidases (also known as „Amadoriases") and fructosamine kinases. Amadoriases deglycate their substrates by oxidation, generating the respective amino acid, glucosone and H_2O_2.
Fructosamine kinases phosphorylate the fructosyl-amino acid in a first step prior to cleavage. Mammals possess fructosamine kinases that phosphorylate at the third carbon atom; the product of the reaction subsequently undergoes an autocatalytic degredation. *Escherichia coli* and *Bacillus subtilis* express kinases phosphorylating at C6. For further cleavage of the intermediate compound a second „deglycase" enzyme is necessary.
In *B. subtilis* – as in *E. coli* – genes encoding the kinase and deglycase (frlD and frlB) are organized in an operon. However, other than their enzymatic function little is known about these two genes.
The global transcription regulator CodY has been shown to act as a repressor of the frl-genes in *B. subtilis*. Currently no further information about the gene expression control of this operon is known. This study will attempt to address the physiological function of these enzymes and their gene regulation.

GRP05
Steroid dependent gene regulation in *Comamonas testosteroni* and the TeiR protein

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Endocrine disrupting compounds such as steroids are contaminants of the environment and hormonally active at low concentrations. Steroids are also able to affect fitness levels of different aquatic species including fish.
The soil bacterium *Comamonas testosteroni* ATCC11996 is able to use the steroid testosterone as carbon and energy source. Previously, it was shown that the TeiR (testosterone inducible regulator) protein is necessary for regulation of complete testosterone degradation. Mutants of *C. testosteroni* disrupted in *teiR* no longer responded to any steroid compound by reporter-gene expression. Furthermore, mutant cells were devoid of steroid-dependent chemotaxis. Currently, however, the molecular mechanism of TeiR within the regulation process still remains obscure.
We found that the N-terminus of TeiR facilitates membrane localization and the central part of TeiR is responsible for steroid binding. The C-terminus ensures signal transducing acitivity in *in vitro* assay´s. Using 32P-labeled nucleotides we were able to demonstrate that TeiR possesses histidine-kinase activity, probably mediated by its C-terminus. Recent results indicate that activity of TeiR is linked to a second regulatory factor which is also involved in the steroid dependent signal transduction pathway. Overall, for regulation of steroid degradation the TeiR protein seems to be just the tip of the iceberg.

GRP06
Thermodynamic and molecular analysis of the AbrB binding sites within the *phyC* promoter region of *Bacillus amyloliquefaciens* FZB45

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We have previously identified the global transition state regulator AbrB as a repressor of *phyC* transcription during the growth phase in *Bacillus amyloliquefaciens* FZB45. AbrB represses the gene expression by cooperative binding at two regions distantly located in the *phyC* promoter region. Here, we used surface plasmon resonance (SPR) and circular dichroism (CD) to study the binding reactions between AbrB and single ds-DNA oligonucleotides, 40 bps in size, which were derived from both binding sites of the *phyC* promoter region. All the oligonucleotides containing parts of the *phyC* proximal binding site 2 were shown to form a stable complex with the entire AbrB protein, which upon binding underwent a conformational change. Surprisingly, only two oligonucleotides covering the central part of the *phyC* distal binding site 1, behaved similarly, whilst the other oligonucleotides were only loosely bound and did not induce any conformational changes in the full-length AbrB protein. Furthermore, comparing full-length AbrB and C-terminaly truncated AbrB55, revealed the importance for the C-terminal domain on DNA binding. Finally, we present a model describing our current understanding of AbrB binding at the *phyC* promoter region.

GRP07
The transcriptional regulator Cg0146 in *Corynebacterium glutamicum* is involved in regulation of mannitol metabolism

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Corynebacterium glutamicum is a Gram-positive organism that grows on various carbon sources, such as sugars, sugar alcohols and organic acids. The Cg0146 protein of *C. glutamicum* is a DeoR-type transcriptional regulator and represses the alcohol dehydrogenase gene *adhA* in this organism. To test whether further genes of *C. glutamicum* are under control of Cg0146, comparative two-dimensional gel electrophoreses of extracts from glucose- and acetate-grown cells from *C. glutamicum* wildtype and from *C. glutamicum Δcg0146* were performed. The protein patterns revealed one protein to be present throughout in high amounts in the mutant and to be absent in the wildtype strain. By mass spectrometry and peptide mass fingerprinting, we identified this protein as product of the *cg0143* gene, which previously has been annotated as *mtlD*, encoding a mannitol dehydrogenase. Comparative growth experiments showed that the *Δcg0146* mutant is able to grow in minimal medium containing mannitol as sole carbon and energy source, whereas the wildtype showed no growth on this substrate. A fructose accumulation of 10 mM in the extracellular supernatant of mannitol-grown mutant cells suggests the conversion of mannitol to fructose by the enzyme mannitol 2-dehydrogenase in this strain. In accordance, cell extracts of the *cg0146*-mutant showed high specific activities of mannitol 2-dehydrogenase. Taken together, our results indicate that the DeoR-type transcriptional regulator Cg0146 is involved in regulation of mannitol metabolism of *C. glutamicum* by repression of the mannitol 2-dehydrogenase gene *mtlD*.

GRP08

The putative ferric uptake regulator (Fur) from the magnetotactic bacterium *Magnetospirillum gryphiswaldense* MSR-1 is not involved in magnetite biomineralization

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The biomineralization of magnetosomes, which are membrane-enclosed crystals of the ferrimagnetic mineral magnetite (Fe_3O_4), involves the uptake of large amounts of iron, its intracellular sequestration and crystallization. Due to iron toxicity there is a strong need for magnetotactic bacteria to sustain a strict iron homeostasis. However, it is not clear how iron biomineralization is regulated and integrated with the biochemical iron requirement. Therefore we started to investigate components of general iron metabolism and their contribution to magnetite biomineralization in the magnetic model organism *M. gryphiswaldense* MSR-1.
Genome analyses revealed five ORFs which code for proteins belonging to different subfamilies of the Fur superfamily, the best characterized and most abundant metal-responsive regulator in bacteria. While three proteins are related to the Irr subfamily, only one protein belongs to the Zur and Fur/Mur subfamily (Mgr1314), respectively. Mgr1314 complemented an *Escherichia coli fur* mutant, suggesting equivalent functions in *M. gryphiswaldense*. Using the Fur titration assay we identified putative Fur binding sites in the promoter region of the iron regulated *mamGFDC* and *mms6* operons, whose gene products are involved in magnetite biosynthesis.
We constructed an unmarked, in-frame deletion mutant of *mgr1314*. The deletion strain shows a delayed growth and a 50 % reduced iron accumulation compared to the wildtype. Mössbauer spectroscopic analysis revealed an increased pool of iron bound to a ferritin-like component. However, magnetosome formation is only slightly delayed. Immunochemical detection of magnetosome proteins showed that Fur is not involved in their regulation. From that we conclude that general iron metabolism and magnetosome formation compete for cytosolic iron. Further analyses will include transcriptional reporter gene fusions as well as proteomic approaches.

GRP09

Cross-regulation in the β-ketoadipate pathway of *Acinetobacter baylyi* is mediated by two LysR-type transcriptional regulators, BenM and CatM

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The soil bacterium *Acinetobacter baylyi* selectively degrades components in carbon source mixtures; individual compounds are consumed in a precise order. This regulated hierarchy occurs in aromatic compound degradation via the β-ketoadipate pathway. In this branched pathway, diverse aromatic compounds are converted to catechol or protocatechuate, substrates of ring-cleaving enzymes. Then, parallel routes feed the metabolites into the tricarboxylic acid cycle. Here we studied the degradation of compounds by the protocatechuate branch of the pathway in the presence of benzoate, a preferred growth substrate of the catechol branch. Fusions to the *Photinus pyralis luc* gene were used to assess the expression of genes needed to degrade vanillate (*van*), hydroxycinnamates (*hca*), and dicarboxylates (*dca*). Whereas vanillate and hydroxycinnamates (such as caffeate, chlorogenate, coumarate and ferulate) are degraded via protocatechuate, the dicarboxylates are degraded through β-ketoadipyl-CoA but do not form catechol or protocatechuate as intermediates. Benzoate was found to prevent the *van*, *hca*, and *dca* genes from being induced by their cognate transcriptional regulators and inducers. These observations suggested possible roles for LysR-type regulators known to activate genes for benzoate degradation. To explore this possibility, their genes, *benM* and *catM*, were inactivated in strains with *luc*-gene reporter fusions. Without BenM and CatM, benzoate failed to prevent induction of the *van* or *hca* genes. In contrast, inhibition of the *dca* genes remained. The direct repression by BenM and CatM of the *hca* and *van* genes, but not the *dca* genes, was confirmed by studying protein-DNA interactions. Electrophoretic mobility shift assays demonstrated that purified BenM and CatM bind to sites that appear to control the expression of *van* and *hca* genes. These binding sites reveal the molecular basis of cross-regulation between the branches of the β-ketoadipate pathway in *A. baylyi*.

GRP10

The synthesis of important virulence factors in *Yersinia pseudotuberculosis* underlies the control of a complex regulatory network during the infection process

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The enteropathogen *Yersinia pseudotuberculosis* causes mainly gut-associated diseases. After passing the intestinal tract the bacteria reach the ileum and enter specialized cells of the host immune system in the epithelium, the M-cells. During this early phase of infection, the bacteria synthesize the outer membrane protein invasin for efficient translocation to the underlying tissues, the Peyer´s patches. *Invasin* expression occurs in response to environmental cues, in particular temperature, mediated by the global activator RovA. *rovA* expression is controlled by the Carbon storage regulator (Csr) system consisting of the RNA-binding protein CsrA and the regulatory RNA CsrC. The nucleoid-associated protein YmoA supports the synthesis of CsrC, which leads to the sequestration of CsrA. This allows *rovA* transcription and thus invasin synthesis.
When *Y. pseudotuberculosis* reaches the Peyer´s patches, *invasin* expression is turned off because of the degradation of RovA and YmoA by ATP-dependent Clp/Lon proteases at higher temperatures. Instead, the bacteria produce the Yersinia adhesin A (YadA) that is activated by the AraC-like activator LcrF. YmoA no longer represses transcription of the *virGlcrF* operon. LcrF translation is regulated by a FourU thermometer, in which the LcrF Shine-Dalgarno region basepairs with four Uracils within the 5´-untranslated region. At 37°C, this secondary structure linearizes and allows binding of ribosomes to synthesize LcrF. Besides *yadA* transcription, LcrF activates the synthesis of the Yersinia outer proteins (Yops). These virulence factors help *Y. pseudotuberculosis* to adhere to cells of deeper tissues and to counteract the host immune system during this late phase of infection.

GRP11

Deep sequencing-based discovery of the *Chlamydia trachomatis* transcriptome

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Chlamydia trachomatis is an obligate intracellular pathogenic bacterium that has been refractory to genetic manipulations. Although the genomes of several strains have been sequenced, very little information is available on the gene structure of these bacteria. We used deep sequencing to define the transcriptome of purified elementary bodies (EB) and reticulate bodies (RB) of *C. trachomatis* L2b, respectively. Using an RNA-seq approach, we have mapped 363 transcriptional start sites (TSS) of annotated genes. Semi-quantitative analysis of mapped cDNA reads revealed differences in the RNA levels of 84 genes isolated from EB and RB, respectively. We have identified and in part confirmed 42 genome- and 1 plasmid-derived novel non-coding RNAs. The genome encoded non-coding RNA, ctrR0332 was one of the most abundantly and differentially expressed RNA in EB and RB, implying an important role in the developmental cycle of *C. trachomatis*. The detailed map of TSS in a thus far unprecedented resolution as a complement to the genome sequence will help to understand the organization, control and function of genes of this important pathogen.

GRP12
Stringent response and persister cell formation in *Pseudomonas aeruginosa*

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The bacterial stringent response is a cellular adaptation mechanism to nutrient limitation such as amino acid or carbon starvation, mediated by the guanosine nucleotide ppGpp. In the opportunistic human pathogen *Pseudomonas aeruginosa* proteins RelA and SpoT control the cellular level of ppGpp. In order to investigate the stringent response, we constructed a *P. aeruginosa* PAO1 *relA spoT* double mutant strain, determined conditions suitable for induction of a RelA- or SpoT-dependent stringent response and identified ppGpp-regulated promotors by microarray analysis. Furthermore, we carried out an extensive phenotypic characterization of the *relA spoT* mutant strain, which displayed a severe defect during anaerobic biofilm growth on nitrate.
The *rmf* gene, encoding the ribosome modulation factor (RMF), was highly upregulated via the stringent response. *Escherichia coli* RMF was shown to mediate dimerization of 70S ribosomes and increase tolerance to various stress conditions. We investigated if the Rmf protein also contributes to antibiotic tolerance of *P. aeruginosa* and if this tolerance is limited to specific ribosome-targeted antibiotics or covers other antibiotic classes, indicating a role of RMF in persister cell formation. Persisters are a small subpopulation of cells tolerant to antibiotic treatment even at high concentrations. In survival assays with various antibiotics we could show that overexpression of the *rmf* gene does indeed promote increased tolerance of *P. aeruginosa*. More importantly, this effect was not restricted to ribosome-targeted antibiotics, suggesting a role of Rmf protein in the formation of *P. aeruginosa* persister cells. Currently, we are verifying these effects with a transcriptional *rmf-gfp* fusion and real-time PCR, and also investigate the role of other candidate genes which might contribute to a persister phenotype.

GRP13
Functional analyses of small non-coding RNAs of *Methanosarcina mazei* Gö1

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In recent years the global impact of small non-coding RNAs (sRNAs) in all domains of life comes more and more obvious. As still little is known on regulatory roles of sRNAs in the domain of Archaea, we recently performed a genome-wide RNA-seq approach, resulting in the discovery of 250 sRNAs in *Methanosarcina mazei* strain Gö1. The archaeal model organism *M. mazei* is a representative methylotrophic archaeon of significant ecological importance due to its role in biogenic methane production in various anaerobic habitats on Earth and is able to fix molecular nitrogen. Here we present the characterization of selected sRNAs using biochemical and genetic approaches. The respective results will be discussed in order to elucidate potential roles of those selected sRNAs in the regulatory network of *M. mazei*.

GRP14
The transcriptional regulation of the succinyl-CoA:acetate CoA-transferase Cat of *Corynebacterium glutamicum*

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The utilization of acetate had been studied intensively in *Corynebacterium glutamicum*. Growth on acetate as a sole carbon source requires activation by phosphotransacetylase (PTA) and acetate kinase (AK) in *C. glutamicum* [2]. During growth on acetate-glucose mixtures, acetate may be converted to acetyl-CoA by CoA transferase (encoded by cat) [5]. During growth on propionate-containing media, propionate is converted to propionyl-CoA by AK and PTA or, when glucose is present, also by Cat. While the transcriptional regulation of the pta-ack operon has been studied to some detail and involves the transcriptional regulators RamA, RamB and RipA, less is known about regulation of cat expression.
Concerning regulation of cat, a RamB binding site was identified by sequence comparison [3] and the promoter region of *cat* was shown to be bound by the transcriptional regulator GlxR [4]. Here, we used transcriptional fusion analysis and gel retardation experiments to determine the role(s) of GlxR, RamA and/or RamB in control of cat expression.

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GRP15
Combination of oligonucleotide-based recombineering and induced double-strand break for scarless mutagenesis in *Salmonella enterica*

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The generation of marker-less in-frame deletions is still the best approach to circumvent unwanted polar effects on adjacent genes. Established recombinase-based methods have several disadvantages regarding selection of successful recombinants or the insertion of a 27-codon "scar" sequence that may interfere with target function in an unpredictable manner. Here we describe an improved approach using the I-*Sce*I meganuclease as a counter-selection tool combined with Red recombinase-mediated recombination of short synthetic DNA molecules for generation of scarless in-frame deletions in the *Salmonella* chromosome. This two-step approach involves (I) the insertion of an antibiotic resistance cassette together with the I-*Sce*I restriction recognition site. (II) The homologous recombination of an 80mer oligonucleotide deletes the antibiotic resistance cassette, and successful recombinants are subsequently selected by induction of I-*Sce*I expression using anhydrotetracycline.
To test the approach, we selected the gene *phoQ* which encodes for the sensor histidine kinase of the two-component system PhoPQ controlling the expression of many virulence-associated genes in *S. enterica*. We could show successful deletion of *phoQ* as well as introduction of the constitutive active T48I mutation (*pho-24*).
To conclude, we developed a tool greatly facilitating chromosomal manipulation of *S. enterica* and other Gram (-) bacteria amenable for the Red recombinase system. This technique not only enables introduction of marker-less in-frame deletions but can also be used for precise site-directed mutagenesis or generation of N- and C-terminal fusion proteins.

GRP16
The LiaFSR Three-Component System of *Bacillus subtilis*: Mechanism of Stimulus Perception and Signal Transduction

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The cell envelope stress response in *B. subtilis* is orchestrated by extracytoplasmic function sigma factors and two-component signal transducing systems (TCS) [1, 2]. The LiaSR TCS is one such system which strongly responds to the presence of cell wall antibiotics that interfere with the undecaprenol cycle, but also to detergents, organic solvents, secretion stress and alkaline shock [3]. Moreover, it is induced without external stimuli at the transition to stationary phase [4]. LiaF is functionally connected with the LiaSR TCS and was identified as its inhibitor. The *liaF* gene is conserved in the *Firmicutes* group and located directly upstream of *liaS*, encoding the histidine kinase. Deletion of *liaF* results in a strong, stimulus-independent activity of the response regulator LiaR. Therefore, LiaF and LiaSR constitute a three-component system (3CS) [5]. The membrane protein LiaF contains a highly conserved and functionally important C-terminus which is located in the cytoplasm. LiaS is an intramembrane-sensing histidine kinase: its N-terminus consists of two transmembrane helices but lacks an extracytoplasmic input domain [6]. In contrast to the robust behaviour usually attributed to TCS-dependent regulation, the LiaFSR 3CS is extremely sensitive towards perturbations of its stoichiometry. Here, we present new results on the mechanism of stimulus perception and signal transduction by LiaFSR, based on mutagenesis/complementation and biochemical studies.

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GRP17
Sensing hydrogen gas in the facultative H_2 oxidizer *Ralstonia eutropha*: signal transduction via a multi component system

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Ralstonia eutropha H16 is a β-proteobacterium capable of using H_2 as a facultative energy source by means of two distinct, energy-conserving [NiFe]-hydrogenases. The detection of external H_2 is accomplished by a cofactor-containing sensory unit which is part of a histidyl-aspartyl phosphorelay comprising a heterodimeric [NiFe]-hydrogenase (RH), a histidine protein kinase (HoxJ) and a response regulator (HoxA) [1,2]. This regulatory system controls transcription of hydrogenase and its corresponding maturation genes in response to the availability of H_2.
Previous investigations showed complex formation between the RH protein and the histidine kinase HoxJ [3]. More recently a supercomplex comprising the RH-HoxJ sensor and the response regulator HoxA was isolated.
Unlike orthodox histidyl-aspartyl phosphorelays the response regulator HoxA is inactive in its phosphorylated form and becomes activated upon stimulus perception [1]. Specific phosphorylation of HoxJ bound to the sensory complex and subsequent phosphotransfer to HoxA was demonstrated in response to H_2 by using γ-^{32}P ATP as substrate. Mutants with alterations in conserved residues in the RH, HoxJ and HoxA are integrated in this study to get further insights into the mechanism of H_2 sensing.

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GRP18
CsgD is an atypical response regulator that controls biofilm formation in *Salmonella enterica* serovar Typhimurium

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The transcriptional activator CsgD of *Salmonella enterica* serovar Typhimurium is a major regulator of biofilm formation required for the expression of *csgBA* coding for the minor and major subunit of curli fimbriae and *adrA* coding for a diguanylate cyclase. CsgD is a response regulator with a C-terminal LuxR like helix-turn-helix DNA binding motif, but the mechanisms of target gene activation remained unclear. To study the DNA binding properties of CsgD, we over expressed CsgD and purified it as a histidine-tagged fusion protein. Using electrophoretic mobility shift assays (EMSA) and DNase I footprint analysis, we show that CsgD-His_6 binds specifically to the *csgBA* and *adrA* promoter regions. *In vitro* transcription analysis revealed that CsgD-His_6 is crucial for the expression of *csgBA* and *adrA*. CsgD contains a conserved aspartate (D59) in its N-terminal receiver domain which is generally the target site for phosphorylation of response regulators. We found that CsgD-His_6 is phosphorylated by acetyl phosphate *in vitro*, in which reduced its DNA-binding properties. Replacement of the conserved aspartate by alternative amino acids *in vivo* reduced CsgD functionality and protein stability. In addition, we also found novel targets for CsgD.

GRP19
Regulation of the nitrogen metabolism in *Streptomyces coelicolor* M145

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The antibiotic producing Gram-positive actinomycete *Streptomyces coelicolor* M145 competes with permanent nutrient limitations in soil especially for nitrogen. *S. coelicolor* has evolved a complex regulatory network to control nitrogen assimilation and metabolism.
In the genome of *S. coelicolor* two genes *glnA* and *glnII* encoding functional glutamine synthetases (GS) and three additional genes *glnA2*, *glnA3* and *glnA4*, encoding putative glutamine synthetases were identified. The *glnA* gene encodes a glutamine synthetase (GSI) with high sequence similarity to other prokaryotic GS enzymes. Like most glutamine synthetases the activity of GSI is regulated via adenylylation by the adenylyl transferase GlnE. Unlike the regulation in *Enterobacteriaceae* the regulator of GlnE in *S. coelicolor* is not known so far. In order to identify possible regulators and interaction partners of GlnE, a bacterial two-hybrid system has been used. With this system, an interaction between GlnE and GlnA2 has been observed.
To investigate the expression profile of *glnE* and *glnA2*, the luciferase reporter gene system will be applied. The pRLux86 plasmid contains the *luxAB* operon coding for the luciferase without a promoter region. The promotor regions of *glnE* and *glnA2* will be cloned into pRLux86 in front of the *luxAB* operon and then transferred into *S. coelicolor*. These strains will be used for expression analysis with several nitrogen sources and under different conditions.

GRP20
Regulation of *glmS* expression by a hierarchically acting regulatory cascade composed of two small RNAs GlmY and GlmZ

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Glucosamine-6-Phosphate (GlcN6P), an essential precursor for the biosynthesis of the cell wall is synthesized by the GlmS enzyme. The expression of *glmS* is regulated by the two structurally homologous small RNAs GlmY and GlmZ which act in a hierarchical manner. Upon depletion of external aminosugars GlmY accumulates and inhibits processing of GlmZ. Base pairing of full-length GlmZ with *glmS* mRNA activates *glmS* expression [1, 2, 3]. In the presence of external aminosugars GlmZ is processed and therefore marked for degradation.

The intracellular GlcN6P concentration might be sensed upstream of GlmY or by GlmY itself. Upstream of the *glmY*-gene perfectly overlapping σ54- and σ70- dependent promoters are present. The σ70-dependent promoter contributes to *glmY* expression during exponential growth while the GlrK/GlrR two component system stimulates *glmY* expression during transition to the stationary growth phase by activation of the σ54-dependent promoter [4]. YhbJ, a protein engaged in this regulatory cascade might be involved in sensing of GlcN6P and GlmZ-processing since full-length GlmZ accumulates in ΔyhbJ-mutants leading to chronic activation of *glmS*. Here we address the regulatory network governing *glmY* expression. In addition, we present data concerning the role of YhbJ in the GlmYZ regulatory cascade.

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GRP21
Pilin regulation in the *pilT* mutant of *Neisseria gonorrhoeae* strain MS11

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Neisseria gonorrhoeae expresses type IV pili (Tfp) which play an important role in a number of processes, e.g. DNA transformation, twitching motility and attachment to host cells. An important feature of Tfp is their ability to retract, a process that is dependent on the ATP binding protein PilT. Retraction of a single gonococcal pilus can exert forces up to 100 pN on its substrate. By the formation of Tfp bundles the achieved force is even 10 times higher. It has been shown that pilus retraction influences epithelial cell gene expression through stimulation of mechanosensitive pathways.
To find out whether a *pilT* mutation and lack of Tfp retraction also leads to gene regulation in bacteria we performed microarrays comparing the transcriptional profiles of the *N. gonorrhoeae* wild-type (wt) strain MS11 and its isogenic *pilT* mutant during growth on agar plates. A loss-of-function-mutation in *pilT* led to altered transcript levels of 63 open reading frames. Level of *pilE* transcripts and its deduced protein, the major Tfp subunit pilin, were increased most markedly by a mutation in *pilT*. Further studies revealed that *pilE* expression was also controlled by two other genes encoding Tfp biogenesis proteins, *pilD* and *pilF*. Interestingly, regulation of PilE was also observed during growth in liquid cultures without attachment to a solid surface, suggesting that this regulation is due to intrinsic differences between wt and *pil* mutants. In contrast to *pilE*, other *pilT*-responsive genes were not regulated during growth in liquid culture. This implies that expression of these genes is regulated by pilus retraction and retraction force which is exerted and experienced by the bacteria during growth on solid surfaces, such as on infected human epithelial cells or agar plates.

GRP22
Impact of point mutations in *marA* on expression of genes encoding type 1 fimbriae and multiple drug resistance efflux pump AcrAB-TolC

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High level fluoroquinolone resistant mutants MIII and MIVa selected *in vitro* from an *E. coli* wildtype (WT) isolate have shown to carry mutations in genes *gyrA, parC* and *marR*. The latter mutation is associated with a multiple-antibiotic resistance (mar) phenotype due to a *marA*-mediated upregulation of multiple drug resistance (mdr) efflux pump AcrAB-TolC. Furthermore, the expression of type 1 fimbriae (t1f) and growth rate are reduced. Growing MIII and MIVa over 300 generations in the absence of selective pressure yielded mutants MIII-300-S16 and MIVa-300-S32 characterized by a restored expression of t1f and a partial loss of the *mar* phenotype. DNA sequence analysis revealed a specific *marA* point mutation in each of these mutants. This study aimed at investigating the impact of these *marA* mutations on the expression of t1f and mdr efflux pump AcrAB.
The respective *marA* point mutation of MIII-300-S16 and MIVa-300-S32 was individually introduced into the chromosome of parent strains WT and its isogenic *marR* deletion derivative WTIII by site-directed mutagenesis to yield WT-S16, WT-S32, WTIII-S16 and WTIII-S32, respectively. Expression rates of genes *marA* and *acrA* were determined by qRT-PCR. T1f expression was investigated genotypically by PCR and phenotypically by mannose-sensitive agglutination of *Saccharomyces cerevisiae* cells.
Compared to WT qRT-PCR analysis revealed an 40-, 35- and 20fold overexpression of *marA* in WTIII, WTIII-S16 and WTIII-S32, respectively, while *acrA* was overexpressed only in WTIII (fourfold). In WTIII-S16 and WTIII-S32 *acrA* expression was comparable to the level determined for WT. These data support the idea of a *marA*point mutation compensating the loss of *marR* function in mutants MIII-300-S16 and MIVa-300-S32. No alteration in the expression of t1f was detected in both *marA* and *marR* mutants of WT arguing against an involvement of *mar* in the regulation of type 1 fimbriae expression.

GRP23
Transcriptional response of the photoheterotrophic marine bacterium *Dinoroseobacter shibae* to light exposure

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Objective: The *Roseobacter* lineage is a physiological heterogeneous group of *Alphaproteobacteria* with a high abundance in marine ecosystems and plays a major role in global carbon and sulphur cycle. Our model organism *Dinoroseobacter shibae* has been isolated from the surface of the dinoflagellate *Prorocentrum lima* and is thought to live in close symbiosis with its host. The genome of D. shibae has been sequenced recently. *D. shibae* is capable of aerobic anoxygenic photosynthesis (AAP), i.e. the gain of energy through photophosphorylation, and shows increased biomass formation when exposed to light. The photosynthetic apparatus is built up only in the dark, light stops the production of pigments and photosystems. This behaviour seems at first glance not to be logical, but is thought as a strategy to minimize the formation of singulett-O2 by the photo pigment bacteriochlorophyll a in the light. The regulatory mechanisms controlling AAP have not been clarified yet.
Results: We used continuous cultivation and time resolved microarray analysis to identify regulons responding to a change of illumination. 447 out of 4106 genes present on the custom made Agilent microarray showed a change in expression >1.7 fold when a dark grown culture was exposed to light. 35 down regulated genes are located in the photosynthesis gene cluster and encode pigment biosynthesis and photosystem structure proteins. Analysis of the kinetic of repression highlights differences in the regulation of genes encoding enzymes responsible for pigment biosynthesis, structural genes of the central reaction center and genes of the light harvesting complexes. The upregulation of genes relevant for cell-cell-adhesion and symbiosis points to light as a putative signal for the attachment of *D. shibae* to it's host.
Conclusion: The combination of chemostat continuous cultivation and microarray analysis leads to clear and reproducible results.

GRP24
Differential expression of the global virulence regulator SlyA as a consequence of sigma factor RpoS heterogeneity in non-pathogenic and clinical isolates of enteric *Escherichia coli*

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The key virulence genes of enteric *Escherichia coli* associated with intestinal diseases are controlled by complex networks implicating multiple regulatory factors.
MarR-type transcriptional regulators such as SlyA have been shown to control the expression of metabolism, stress adaptation and virulence genes in response to environmental signals, facilitating the adaptation of the pathogen to changing conditions throughout the infection process.

We report that *slyA* expression varies significantly in non-pathogenic and clinical isolates of *E. coli*. SlyA levels are generally high in *E. coli* isolated from intestinal infections, but low in non-pathogenic *E. coli* K-12 strains. The expression of the *slyA* gene is temperature-, growth phase- and growth medium-dependent, suggesting an adaptive function in response to environmental factors.
We further show that differential *slyA* expression is a consequence of sigma factor RpoS heterogeneity among enteric *E. coli* strains. RpoS controls *slyA* transcription negatively, most likely by interfering with regulatory factors involved in the environmental control of SlyA. Confirming a negative correlation of RpoS and SlyA levels, we observed the loss, reduced expression or truncation of the *rpoS* gene in various clinical isolates, which resulted in elevated *slyA* expression levels in the respective *rpoS*-deficient strains.
We further found that the EvgAS two-component system known to activate drug efflux pumps and acid resistance genes is controlled by SlyA. As a result, *rpoS*-deficient pathogenic *E. coli* strains, which are generally more stress sensitive, are able to compensate the loss of RpoS-mediated acidic stress resistance by consequently elevating SlyA levels, conferring selective advantages during the infection process.

GRP25
Stimulus perception by the sensor kinase KdpD of *Escherichia coli*

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The sensor kinase/response regulator system KdpD/ KdpE regulates the expression of the *kdpFABC* operon encoding the high affinity potassium uptake system KdpFABC in *Escherichia coli*. Under potassium limitation in the medium the membrane-bound histidine sensor kinase KdpD undergoes autophosphorylation at a conserved histidine residue (H673) and subsequently, the phosphoryl group is transferred to the cytoplasmic response regulator KdpE at a conserved aspartate residue (D52). Phosphorylated and dimerized KdpE exhibits an increased affinity for a 23 bp sequence immediately upstream of the canonical -35 an -10 regions of the *kdpFABC* promoter. Consequently, the phosphorylated KdpE triggers *kdpFABC* transcription in response to the stimulus.
The stimulus that is sensed by KdpD is controversially discussed (for more details see Hamann *et al*., 2008). Therefore, KdpD mutants impaired for stimulus perception were analysed by genetical and biochemical approaches. Candidates for this study are two aspartate residues D424 and D474, wich are located in the polar loop regions facing the periplasm. Furthermore, strains with complete chromosomal *kdpD*-deletions have been constructed to test the physiological consequences on growth behaviour under different conditions and on *kdpFABC*-expression. First results led to the suggestion that unknown interaction partner(s) play a role in the KdpD/E signal transduction cascade.

[1] Hamann, K., Zimmann, P., & Altendorf, K., Reduction of turgor is not the stimulus for the sensor kinase KdpD of *Escherichia coli*. J Bacteriol 190 (7), 2360-2367 (2008)

GRP26
Analysis of GvpD-mediated reduction of the transcription activator GvpE in *Halobacterium salinarum* using a GFP reporter system

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14 genes are involved in gas vesicle formation in halophilic archaea. The gene products of *gvpE* and *gvpD* have been identified as regulatory proteins with GvpE acting as transcription activator and GvpD counteracting the GvpE effect. Both proteins interact with each other and this interaction might lead to a reduced amount of GvpE. To investigate whether this reduction is due to proteolytic degradation a GvpE-GFP fusion protein was constructed and the fluorescence levels were analyzed in presence or absence of GvpD wild-type or of GvpD mutant proteins with loss of function ($GvpD_{Mut6}$) or a superrepressor ($GvpD_{3\text{-}AAA}$). The N-terminal fusion to GFP did not negatively affect the GvpE-induced activity of the corresponding pA promoter in comparison to wild-type. Transformants harbouring GvpE-GFP and GvpD wild-type showed a significant reduction (45%) of fluorescence. For the combinations of GvpE-GFP with the superrepressor $GvpD_{3\text{-}AAA}$ or $GvpD_{Mut6}$ a reduction of 60% or 20%, respectively, was obtained. These results demonstrate that GFP-fusion proteins can be used to study the regulatory interactions of GvpE and GvpD *in vivo*.

GRP27
Small RNAs in the Bradyrhizobium / Rhodopseudomonas lineage

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The important function of small RNAs (sRNAs) in the regulation of many processes in bacterial cells was recognised during the last years. Despite their importance, the genes for sRNAs are still not annotated in most bacterial genomes. With exception of the highly conserved tmRNA, 6S RNA and SRP RNA [1, 2], no small RNAs are known in representatives of the family Bradyrhizobiaceae. We used the bioinformatic approach published by Voss et al. [3] to predict sRNAs in the genera Rhodopseudomonas and Bradyrhizobium. The expression of eight novel sRNAs in *Bradyrhizobium japonicum* 110 was confirmed by Northern blot analysis. We present data on the expression of these sRNAs under different stress conditions and at different growth phases. Additionally, predicted secondary structures will be shown and potential targets will be discussed.

[1] Ebeling S, Kündig C, Hennecke H. (1991) Discovery of a rhizobial RNA that is essential for symbiotic root nodule development. J. Bacteriol. 1991 Oct;173(20):6373-82.
[2] Voss B, Hölscher M, Baumgarth B, Kalbfleisch A, Kaya C, Hess WR, Becker A, Evguenieva-Hackenberg E. (2009) Expression of small RNAs in Rhizobiales and protection of a small RNA and its degradation products by Hfq in Sinorhizobium meliloti. Biochem. Biophys. Res. Commun. 2009 Dec 11;390(2):331-6.
[3] Voss, B., J. Georg, V. Schon, S. Ude, W.R. Hess, (2009) Biocomputational prediction of non-coding RNAs in model cyanobacteria, BMC Genomics 10 123.

GRP28
Functional analysis of the ribonucleases E and J in *Sinorhizobium meliloti*

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The mechanisms of RNA processing and degradation in bacteria were intensely studied using the gram-negative E. coli and the gram-positive B. subtilis as model organisms. Based on these studies, it was proposed that different RNA degradation pathways operate in these bacteria. The principle endoribonuclease in *E. coli,* RNase E, participates in rRNA maturation, organises a protein complex, the degradosome, and is responsible for the decay of mRNAs and small non-coding RNAs. The subsequent exoribonucleolytic degradation of RNA fragments proceeds exclusively in 3'-5' direction in *E. coli*. *B. subtilis* harbours no RNase E, but two proteins with similar endonucleolytic properties called RNase J1 and RNase J2. RNase J1 is involved in processing and degradation of mRNA and rRNA, and it has a dual function: in addition to its endoribonuclease activity it exhibits a 5'-3' exoribonuclease activity. RNase J is widely spread in prokaryotes, but nothing is known about the RNA degradation mechanisms in organisms harbouring genes for RNase E and RNase J. We studied the role of RNase E and RNase J in maturation and degradation of ribosomal RNAs, mRNAs and small RNAs in *Sinorhizobium meliloti*, and show that the two RNases have distinct and overlapping functions in this organism.

[1] Condon C. (2007) Maturation and degradation of RNA in bacteria. Curr Opin Microbiol. 10:271-278.
[2] Madhugiri R, Evguenieva-Hackenberg E. (2009) RNase J is involved in the 5′-end maturation of 16S rRNA and 23S rRNA in Sinorhizobium meliloti. FEBS Lett. 2009 Jul 21;583(14):2339-42.
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GRP29
LsdRS-AB and BceRS-AB detoxification modules: sensing peptide antibiotics in *Bacillus subtilis*

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Two-component signal transducing systems (TCS) consist of a histidine kinase (HK) that senses a specific environmental stimulus, and a cognate response regulator that mediates the cellular response. The HKs of the cell envelope stress response in *Bacillus subtilis* are characterized by a very short N-terminal input domain, consisting of two membrane-spanning helices but lacking a discernable extracellular domain. Because of this architecture, they were termed intramembrane-sensing (IM-)HK [1]. BceS-like HK represent the largest conserved group of IM-HK. The corresponding TCS are functionally linked to ABC transporters encoded by neighboring operons. Such units represent antibiotic-specific detoxification modules widely distributed in Firmicutes bacteria [2]. Importantly, these transporters are crucial both for sensing the antibiotic and mediating resistance against them.
Two closely related detoxification modules are encoded in the genome of *B. subtilis*, BceRS-AB and LsdRS-AB. These systems sense bacitracin [3] and cationic lipid II-binding lantibiotics (such as nisin and gallidermin), respectively. Here, results on the similarities and differences in mechanism of stimulus perception and signal transduction of these two systems will be presented.

[1] Mascher *et al.* (2003), Mol. Microbiol. 50:1591-1604
[2] Mascher et al. (2006), FEMS Microbiol. Lett. 264:133-144
[3] Rietkötter *et al.* (2008), Mol.Microbiol. 68:768-785

GRP30
The Expression of *aprBi* and *gseBi* genes under Control of different DNA-binding Proteins in *Bacillus intermedius*

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To adapt to drastic variations of environmental conditions bacilli have developed a highly sophisticated regulatory network orchestrating the transcriptional modulation of large sets of genes. The regulatory pathways include two-component histidine kinase/response regulator proteins that are highly interconnected. Their activity leads to induction of genes encoding stress response proteins, including the extracellular degradation enzymes.
Bacillus intermedius produces the extracellular proteases after the end of the exponential growth phase, and among those enzymes, the alkaline proteases encoded by *aprBi* and *gseBi* are the major ones [1,2]. It was shown that the regulation of expressing these protease genes in *B. intermedius* was controlled by two two-component signal systems, DegS-DegU, KinA/Spo0F/Spo0A, the pleiotropic regulator CcpA and probably AbrB. The regulatory regions of *gseBi* and *aprBi* genes contain specific target sequences for binding with these proteins. But both the organization of promoters of these genes and their regulation of expression occurs differently: the expression of these genes is distinguished in regulation at the different stages of bacterial growth. So far, no study directly addressed the contribution of all these systems in the control of protease gene expression. The goal of this study is to analyze the mechanism of DNA-protein interaction between the promoters upstream of genes encoding serine proteases and their regulatory proteins.

[1] Sharipova M. *et al.* (2008), Microbiological research. 163:39-50
[2] Sharipova M.R. *et al.* (2007), Mol. Biology. 34:79-87

GRP31
RT-PCR based transcription analysis of toxin production by *Clostridium difficile* strains with variant *tcdC*-subtypes

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Hallmarks of epidemic, hypervirulent *Clostridium difficile* Ribotype 027 are mutations in the negative regulator of toxin production *tcdC*. These mutations result in severely truncated TcdC and thus are thought to lead to an alteration of the kinetics and extent of toxin gene expression.
In order to investigate the effect of different *tcdC* subtypes on toxin gene transcription a quantitative real time PCR was established. Primers were set to target variant *tcdC* alleles, *tcdR* and *tcdA*. *rpoA* was used as a means of expression normalization. For transcription analysis the growth kinetics of four strains grown in broth media were determined. Samples at defined time points were drawn and analyzed by RT-PCR.
All tested strains showed comparable growth kinetics. High levels of *tcdA* transcripts were detected at the onset of the stationary phase regardless of the respective *tcdC* variation. However, differences in absolute amounts of *tcdA* transcripts were observed.
The presented method enables transcriptional analysis of toxin gene expression in *tcdC*-variant *Clostridium difficile* strains. An alteration of transcription kinetics of *tcdA* due to variant TcdC could not be observed so far. Differences in the level of *tcdA* transcription may correlate with increased *in vitro* toxin production seen with strains like Ribotype 027.

GRP32
Evidence for a Timing Mechanism in *Prochlorococcus*

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Cyanobacteria are the simplest known cellular systems that regulate their biological activities into daily cycles using an internal circadian clock. Only three proteins are required to generate robust 24-hour oscillations: KaiC, the principal protein of this biochemical oscillator, undergoes rhythmic autophosphorylation and dephosphorylation modulated by KaiA and KaiB.
Interestingly, the marine *Prochlorococcus*, probably the most abundant photosynthetic organisms on Earth, lost the *kaiA* gene by genome reduction during evolution. We showed that the two remaining clock proteins exhibit their known biochemical function, although the influence of *Prochlorococcus* KaiB on *Prochlorococcus* KaiC phosphorylation is different. The deletion of KaiA seems to be compensated by the enhanced autophosphorylation of *Prochlorococcus* KaiC.
This leads to a reduced hourglass-like timing mechanism: Although cultures display robust 24-h rhythms in gene expression and cell cycle phases, when they are grown under alternating 12-h-light and 12-h-dark periods, under continuous light both cell cycle and gene expression rhythms damped very rapidly.
Our results might have further implications for the analysis of a possible timing mechanism in other bacterial species, such as purplebacteria that encode KaiB and KaiC homologs but lack the KaiA component.

GRP33
An Hfq-dependent small RNA directly activates Cfa synthesis in *Salmonella*

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Small noncoding RNAs (sRNA) are a steadily growing class of post-transcriptional regulators which often exert their function through direct pairing with target mRNAs. While commonly down-regulating gene expression, bacterial sRNAs have also been shown to activate their targets by a variety of mechanisms [1]. Many sRNAs associate with and function through the RNA chaperone, Hfq.
RydC, a ~65 nt sRNA that was initially discovered in *E. coli* [2], is conserved among several γ-proteobacteria, and associates with the Hfq protein of *Salmonella* [3]. Intriguingly, RydC folds into a peculiar structure marked by a 3' pseudo-knot (including the terminator) and a single-stranded 5' end [4].

We have characterized the role of RydC in the pathogen *Salmonella* Typhimurium, and have identified the *cfa* mRNA encoding a cyclopropane fatty acids synthase as a target that is directly activated by RydC. The *cfa* gene is transcribed from two independent promoters [5], a distal σ70-dependent promoter and a proximal σS promoter. Our *in vitro* and *in vivo* analyses have shown that RydC specifically activates the longer mRNA transcribed from the distal promoter, whose translational initiation region is normally sequestered by internal secondary structure. Compensatory base-pair exchanges corroborate a model in which the 5'end of RydC base-pairs with, and disrupts the inhibitory structure of *cfa* mRNA, to increase its translation.

[1] Fröhlich KS and Vogel J 2009
[2] Zhang A *et al.* 2003
[3] Sittka A *et al.* 2008
[4] Antal M *et al.* 2005
[5] Wang AY and Cronan JE Jr 1994

GRP34
Expression of *Staphylococcus aureus* superantigens (SAgs) in the authentic human nasal environment

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The nasopharynx represents the main ecological niche of the human pathogen *Staphylococcus aureus*. Although colonization of the nares is asymptomatic, nasal carriage is a known risk factor for endogenous staphylococcal infection. *S. aureus* superantigens (SAgs) are highly potent T cell mitogens. Antibodies against non-egc SAgs are common in the healthy population, whereas neutralizing antibodies against *egc* SAgs are very rare, even in *S. aureus* carriers of egc-positive strains. This might be explained by their differential regulation. While non-*egc* SAgs are secreted during post-exponential growth *in vitro*, egc SAgs are produced during exponential growth phase. However, only little is known about their expression and regulation *in vivo.* Our aim was to analyze the transcriptional level of *S. aureus* SAgs, especially *egc* SAgs, during nasal colonization in humans.
Six healthy persistent *S. aureus* carriers were included in this study. After evaluation of the SAg gene pattern of the respective nose isolates, the transcription profile during colonization was compared to that of the *in vitro* grown isolates using quantitative real-time PCR. Additionally, serum samples were analyzed for their SAg-neutralizing capacity.
Direct transcript analysis from nose swabs revealed a weak *in vivo* transcription of sec and an intermediate transcription of sea. Surprisingly, we observed a high transcription of the *egc* SAg seo during colonization which even exceeds transcription *in vitro*. In contrast, neutralizing antibodies against SEC and SEA but not against SEO were detected in the serum samples. Thus, transcription of seo in the nose seems not to elicit an antibody response in humans. For the first time, we could demonstrate the *in vivo* transcription of SAgs, which differed clearly from *in vitro* regulation.

GRP35
ArgR is a specific regulator of the arginine deiminase system of *Streptococcus suis*

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Streptococcus (S.) suis is a very important porcine pathogen which is responsible for high economical losses worldwide. As a zoonotic pathogen it is a causative agent of meningitis and septicaemia in humans. Only little is known about pathogenesis and virulence factors of *S. suis*. The arginine deiminase system (ADS) of *S. suis* which converts arginine to ornithine, ammonia, carbon dioxide and ATP, increases bacterial survival under acidic conditions. The system is regulated by arginine, oxygen tension and carbon catabolite repression. In this study we analysed the role of a transcription factor called arginine repressor (ArgR), which has been shown to positively and negatively regulate the arginine catabolism and anabolism, respectively, in other bacteria.
We have previously defined the ADS in *S. suis* as a gene cluster which is confined by the regulator genes *flp*S and *arg*R. In this study we analysed the role of ArgR in *arc*ABC regulation. To elucidate if ArgR is associated with the arginine dependent *arc*ABC activity we constructed a *arg*R deficient knock out strain (Δ*arg*R). In contrast to the wildtype the *arc*ABC expression was abolished in Δ*arg*R. Interestingly, Δ*arg*R showed reduced growth kinetics comparable to that of a Δ*arc*ABC mutant and it was unable to elevate extracellular pH value due to diminished *arc*ABC activity. Comparative RNA microarray analysis of the wild type and Δ*arg*R grown either to the exponential or the stationary growth phase revealed the positive almost local regulatory effect of ArgR on the arginine catabolism (ADS). By northern blot analysis we could exclude any effect on arginine biosynthesis (*arg*HG). *Gfp*-reporter studies revealed that the Δ*arg*R strain could not induce *arc*ABC promoter activity. *In vitro* binding assays confirmed binding of recombinant ArgR to a specific *arc*ABC promoter region. Finally, specific binding of ArgR *in vivo* to this region was shown by chromatin immunoprecipitation. Taken together, our results demonstrate that ArgR is a specific local activator for the arginine catabolism in *S. suis*.

GRP36
Characterization of the transcription factor LysR in *Comamonas testosteroni*

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Comamonas testosteroni 3α-hydroxysteroid dehydrogenase/carbonyl reductase (3α-HSD/CR) is a key enzyme in the degradation of steroid compounds and polycyclic aromatic hydrocarbons in soil, and may therefore play a significant role in the bioremediation of harmful compounds in the environment. We previously reported the cloning and isolation of two repressors and an activator which regulate the gene expression of 3α-HSD/CR in *C. testosteroni*. After steroid induction 3α-HSD/CR gene expression increased. In the present study, we have identified the *lysR* gene as another and new activator gene for 3α-HSD/CR gene regulation. *LysR* was isolated from the chromosomal DNA of *C. testosteroni*. The *lysR* gene consists of 912 bp and locates 3.360 kb downstream from the 3a-HSD/CR gene. To produce purified LysR protein, the *lysR* gene was cloned into plasmid pET-15b and the overexpressed protein purified by its His-tag sequence on metal chelate chromatography. SDS PAGE revealed a molecular mass of the LysR protein of 33.6 kDa (303 aa). Antibodies against LysR were prepared and an ELISA was established for LysR quantification. Five truncated His-tag LysR proteins were prepared for functional studies. We then produced knock-out mutants of the *lysR* gene in *C. testosteroni*. As expected, these knock-out mutants expressed low levels of 3α-HSD/CR, even after testosterone induction. Several experiments revealed that LysR is a membrane protein. LysR binds also to testosterone. Electrophoresis mobility shift assays (EMSA) showed that LysR could bind to the promoter domain of the hsdA gene. From these results we conclude that LysR is an important player in the regulation of the steroid degradation pathway in *C. testosteroni*. The substrate binding domain, transmembrane domain and DNA binding domain of LysR protein and LysR foot printing is in progress.

GRP37
Translation on demand: A simple cyanobacterial RNA-based thermosensor is critical for stress management

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Structured RNA regions are important gene control elements in pro- and eukaryotes. Here, we show that the mRNA of a cyanobacterial heat shock gene contains a built-in thermosensor critical for photosynthetic activity under stress conditions. The exceptionally short 44-nucleotide 5'-untranslated region is comprised of a single hairpin with an internal asymmetric loop. It inhibits translation of the *Synechocystis hsp17* transcript at normal growth conditions, permits translation initiation under stress conditions and shuts down Hsp17 production in the recovery phase. Point mutations that stabilized or destabilized the RNA structure deregulated reporter gene expression *in vivo* and ribosome binding *in vitro*. Introduction of such point mutations into the *Synechocystis* genome produced severe phenotypic defects under heat shock and high light conditions. The proper on- and off-function of the thermosensor was beneficial for viability of *Synechocystis*, the integrity of its photosynthetic apparatus and the oxygen evolution rate. Interestingly, constitutive production of Hsp17 in the derepressed thermometer mutant was detrimental in the recovery phase when the stress declined. Our results suggest that shutting-off heat shock protein production is an important, previously unrecognized function of RNA thermometers. In summary, we discovered a simple biosensor that strictly adjusts the cellular level of a molecular chaperone to the physiological need.

GRP38
Aerobic photosynthesis: boon and bane for *Roseobacter*

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Simultaneous exposure to oxygen and light generates singlet oxygen ($^1O_2$) when photosensitizers are present. Since bacteriochlorophylls can act as photosensitizers, every photosynthetic bacterium has to deal with $^1O_2$ stress. Aerobic anoxygenic phototrophs, like e.g. *Roseobacter* species, express a functional photosynthetic apparatus exclusively under aerobic conditions [1]. Therefore it is believed that they exhibit efficient stress defense systems. Here we report on the $^1O_2$ stress response of *Roseobacter denitrificans*. The stressed proteome was investigated by 2D gelelectrophoresis and upregulated proteins were identified by MALDI-TOF analysis. Following qRT-PCR experiments of corresponding mRNAs implied posttranscriptional regulation by e.g. small RNAs [3]. Induction of the stress related alternative sigma factors RpoE and $RpoH_{II}$ [2] was also observed for *R. denitrificans* and it was further demonstrated that heterologous expression of RpoE of *R. denitrificans* in an *rpoE* deletion mutant of *Rhodobacter sphaeroides* can partially restore the wild type phenotype. The observation that light promotes growth of *R. denitrificans*, even when $^1O_2$ is generated, indicates that stress is encountered efficiently whereby oxygen and light can promote growth without restriction.

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[3] Berghoff, B. A., J. Glaeser, C. M. Sharma, J. Vogel & G. Klug (2009) Photooxidative stress induced and abundant small RNAs in *Rhodobacter sphaeroides*. Mol Microbiol. (Ahead of print)

GRP39
Characterization of blue light photoreceptor mutants in the phototropic bacterium *Rhodobacter sphaeroides* - regulatory roles of small RNAs

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Small RNAs (sRNAs) play a regulatory role in the metabolism of various organisms and are currently of high research interest. The identification of multiple sRNAs in the phototrophic bacterium *Rhodobacter sphaeroides* increased the interest into this research field further [1]. Here we present the expression of sRNAs in the *R. sphaeroides* wild type (WT) and in two blue light photoreceptors mutants, the CryptochromeB mutant *R. sphaeroides* Δ*cryB* and the LOV-domain mutant Δ*lov*. Via Northern Blot analyses after oxygen shift from aerobic to semi aerobic conditions and after blue light shifts we could identify one sRNA (sRNA 2430) which showed an increased expression during the oxygen shift and a decrease of expression after blue light irradiation in the WT.
It was shown before that oxygen has an influence on the formation of the photosynthetic apparatus in *R. sphaeroides*. Under high oxygen levels the formation is repressed by the AppA/PpsR system [2]. We also tested if there is a repression in the two mutants Δ*cryB* and Δ*lov*. Therefore, we examined the expression of the *puc*-operon, which encodes structural proteins of the photosynthetic apparatus, after oxygen shift from aerobic to semi aerobic conditions. Interestingly we can detect lower expression levels in the two mutants compared to the WT.
Furthermore, we suppose that the blue light perception occurs at the outer membrane of *R. sphaeroides*. Therefore, we try to show, if there are varieties in the composition of the membrane proteins between the WT and the cryptochrome blue light photoreceptor mutant Δ*cryB*.

[1] Berghoff et al. (2009), Photooxidative stress induced and abundant small RNAs in *Rhodobacter sphaeroides*. Mol.Microbiol. (ahead of print)
[2] Braatsch et al. (2002), A single flavoprotein, AppA, integrates both redox and light signals in *Rhodobacter sphaeroides*. Mol. Microbiol. 45 (3):827-36

GRP40
Photooxidative stress affected sRNAs in *Rhodobacter sphaeroides*

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The facultative phototrophic proteobacterium *Rhodobacter sphaeroides* performs anoxygenic photosynthesis under anaerobic conditions and in the presence of light. The simultaneous presence of oxygen and light might cause oxidative stress by the bacteriochlorophyll mediated formation of singlet oxygen ($^1O_2$). We recently identified 20 sRNAs in *R. sphaeroides* by 454 pyrosequencing and Northern blot analysis [1]. Four of these new sRNAs appear to be involved in the oxidative stress response, and two are specific for photooxidative stress. RSs0019 is specifically induced by $^1O_2$ in an RpoE-dependent manner. A prolonged exposure to $^1O_2$ leads to processing of RSs0682 which in turn leads to an accumulation of a smaller fragment. The RNA chaperone Hfq is likely to mediate this processing because the smaller fragment of RSs0682 is not seen in an *hfq* deletion strain. Whether there is a direct interaction of one of these fragments with a FLAG-tagged Hfq was elucidated by co-immunoprecipitation. Forced overexpression of RSs0019 from a constitutive promoter increases the sensitivity of *Rhodobacter* towards $^1O_2$, as we have shown by inhibition zone assays. Using 2D gelelectrophoresis, overexpression of RSs0019 under control of its own promoter was shown to alter the abundance of several yet to be identified proteins.

[1] Berghoff, B. A., J. Glaeser, C. M. Sharma, J. Vogel & G. Klug (2009) Photooxidative stress induced and abundant small RNAs in *Rhodobacter sphaeroides*. Mol Microbiol. (Ahead of print)

GRP41
Identification of an interaction partner for a single domain response regulator controlling the *Myxococcus xanthus* developmental program

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Myxococcus xanthus is a Gram negative and soil inhabiting bacterium with a complex life cycle and is a model for prokaryotic multicellular behavior. Under nutrient limited conditions, a developmental program is initiated in which cells first aggregate into mounds and then, within these mounds, differentiate into environmentally resistant spores. Progression through this developmental program is coordinated by a complex "four-component" histidine-aspartate phosphorelay system that appears to be necessary to allow a controlled, gradual accumulation of MrpC, a transcriptional regulator that plays a major role in controlling the developmental program. It is unknown how the Red system exactly influences MrpC accumulation, but it has been previously demonstrated that MrpC is subject to multiple levels of regulation including transcriptional activation by a proteolytic processing event and prevention of this processing by phosphorylation via the Pkn8/Pkn14 serine/threonine protein kinase cascade. MrpC is also a positive regulator of its own transcription.

The output of the Red signalling system is RedF, a single receiver protein with no associated output domain. To determine how RedF could mediate a signal output, we used yeast two hybrid analysis to screen a *M. xanthus* genomic library for RedF interaction partners. This approach identified KapC, a protein previously proposed to interact with the serine/threonine kinase Pkn8, as a specific interaction partner for RedF. These results suggest a model in which RedF interacts with KapC to stimulate the Pkn8/Pkn14 kinase cascade such that MrpC is phosphorylated and is prevented from stimulating its own transcription. This model suggests a complex signaling system where two-component signal transduction systems converge with eukaryotic like serine/threonine protein kinase cascades. We are currently testing this model using both genetic and *in vivo* interaction analyses.

GRP42
Nitrogen control in *Mycobacterium smegmatis*

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Based on sequence analyses and studies of a deletion mutant, the OmpR-type regulator GlnR was identified as the regulator of nitrogen metabolism in *Mycobacterium smegmatis* [1,2]. While binding to its target genes *amtB* and *glnA*, as well as a lack of their transcription in a *glnR* deletion mutant could be shown [2], little was known about the activation of GlnR. GlnR is a typical response regulator of a two component signal transduction system. Therefore, it is expected to be phosphorylated by a histidine kinase.
In fact, we could show phosphorylation of GlnR by *M. smegmatis* cell extract. In a bioinformatical approach we found two putative sensor histidine kinases, msmeg_1918 and msmeg_5241. Unfortunately, a deletion of these genes had no influence on nitrogen metabolism, as shown by growth and RNA hybridization experiments. The kinases were purified and used for further phosphorylation experiments. They were not able to phosphorylate GlnR *in vitro*, although a strong autophosphorylation was observed. In the future more experiments are planned to study the role of msmeg_1918 and msmeg_5241, as well as to find the activator of GlnR.
In a second approach, the GlnR regulon was further characterized. Performing gel retardation experiments we observed binding of GlnR to new target genes such as *amtA*, *amt1* (ammonium transport systems)
or *nirB* (nitrite reductase). These results were confirmed by RNA hybridization experiments.

[1] Amon, J., Titgemeyer, F. & Burkovski, A. (2008). A Genomic View on Nitrogen Metabolism and Nitrogen Control in Mycobacteria. J. Mol. Microbiol. Biotechnol.
[2] Amon, J., Bräu, T., Grimrath, A., Hänßler, E., Hasselt, K., Höller, M., Jeßberger, N., Ott, L., Szököl, J., Titgemeyer, F. & Burkovski, A. (2008). Nitrogen control in *Mycobacterium smegmatis*: Nitrogen-dependent expression of ammonium transport and assimilation proteins depends on OmpR-type regulator GlnR. J. Bacteriol.

GRP43
Impact of different electron acceptors on the tetrachloroethene reductive dehalogenase gene expression in *Sulfurospirillum multivorans*

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Organohalide respiration with tetrachloroethene (PCE) as terminal electron acceptor is used by the anaerobe *Sulfurospirillum multivorans* to drive ATP synthesis. Previous investigations on the transcriptional regulation of the PCE reductive dehalogenase genes revealed a long-term decrease of the *pce* transcript level when the cells were grown in the absence of PCE. The expression of the *pce* genes was recovered by the addition of PCE [1].
In this study we showed that *S. multivorans* is able to grow under microaerobic conditions using oxygen as terminal electron acceptor. To elucidate the effect of oxygen on the PCE reductive dehalogenase gene expression, we tested the *pce* transcript level via RT-PCR and the formation of the enzyme via activity measurements and immunoblot analyses. We compared the results with cells cultivated on nitrate, fumarate, or PCE. In addition to the impact on the PCE reductive dehalogenase, we proved the effect on other oxidoreductases like fumarate reductase or nitrate reductase. Furthermore, we compared the influence of different electron acceptors on the composition of the *S. multivorans* membrane proteom.

[1] John, M., Rubick, R., Schmitz, R.P., Rakoczy, J., Schubert, T., and Diekert, G. (2009) Retentive memory of bacteria: Long-term regulation of dehalorespiration in *Sulfurospirillum multivorans*. *J Bacteriol* 91:1650-5

GRP44
Different substrate specificities of the conserved RNA binding subunits Rrp4 and Csl4 of the exosome

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The archaeal exosome is an RNA processing protein complex containing a phosphorolytically active hexamer of Rrp41 and Rrp42, and a trimeric cap of Rrp4 and/or Csl4. The RNA binding proteins Rrp4 and Csl4 are found in archaeal exosomes as well as in the exosomes of yeast, protozoa and human. *In vitro*, the archaeal Rrp4 and Csl4 proteins stimulate degradation of RNA by the exosome. So far nothing was known about the substrate specificity of the exosome. Using recombinant protein complexes of the hyperthermophilic and acidophilic archaeon *Sulfolobus solfataricus*, we found that C-rich and U-rich sequences negatively influence RNA degradation and RNA tailing. The Rrp4-exosome shows a strong preference for poly(A)-RNA longer than 25 nt and, in contrast to the Csl4-exosome, it degrades heteropolymeric RNA with poly(A)-tail or with physiologically relevant heteropolymeric tail more efficiently than non-tailed RNA. The Csl4-exosome degrades heteropolymeric, structured RNA and polyurydylated RNA with higher efficiency than the Rrp4-exosome. In conclusion, the sequence of the RNA substrate influences its processing by the exosome. Moreover, the different specificities of Rrp4 and Csl4 explain their high conservation during evolution.

GRP45
Regulation of the malic enzyme gene *malE* by the transcriptional regulator MalR in *Corynebacterium glutamicum*

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Corynebacterium glutamicum is a Gram positive nonpathogenic bacterium that is used for the biotechnological production of 1.500.000 t L-glutamate and 750.000 t L-lysine annually. Here, we investigated the transcriptional control of *malE* encoding for malic enzyme (MalE) in *C. glutamicum* ATCC13032. MalE operates at the phosphoenolpyruvate (PEP)-pyruvate-oxaloacetate node, a central point of carbon metabolism, by catalysing the strictly $NADP^+$ dependent decarboxylation of L-malate to pyruvate. DNA-affinity purification identified proteins binding to *malE* promoter DNA. Besides the master regulator of nitrogen metabolism AmtR (Jakoby *et al*. 2000) a protein annotated as transcriptional regulator bound to the promoter DNA of *malE* and was designated as MalR. Deletion of *malR* revealed that this regulatory protein is dispensable for growth of *C. glutamicum* in minimal media with all carbon substrates tested. *C. glutamicum* cells overexpressing *malR* showed reduced malic enzyme activities when grown in LB complex media or minimal media with acetate, glucose, pyruvate or citrate. On the other hand, deletion of *malR* positively affected malic enzyme activities during growth in LB and minimal media with pyruvate, glucose or the TCA-cycle dicarboxylates L-malate, succinate and fumarate as substrates. These results suggested that MalR acts as a repressor of *malE*. For *in vitro* analysis, MalR was purified from recombinant *E. coli* as His-tagged protein and shown to bind *malE* promoter DNA in gel shift experiments. Two MalR binding sites in the -10 and -35 region of the *malE* promoter were detected and shown to be required for binding of MalR by mutational analysis. In addition, further gel shift experiments using purified regulators RamA and RamB revealed a complex transcriptional control of *malE* by MalR, RamA, RamB and as previously reported by AmtR (Buchinger *et al*. 2009).

GRP46
A large number of small non-coding RNAs are controlled by the two-component regulatory system CiaRH in streptococci

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The two-component regulatory system CiaRH of *S. pneumoniae* controls besides 19 protein encoding genes the expression of five small non-coding RNAs, designated csRNAs (cia-dependent small RNA). These csRNAs show a high degree of similarity to each other. Their secondary structures were predicted to contain two stem-loop structures flanking a conserved unpaired region with an anti-AUG and anti-Shine-Dalgarno sequence motif (UCUCC(C)U) suggesting a regulatory effect on translation initiation by blocking the ribosomal binding site of mRNAs. The response regulator CiaR is well conserved among streptococci. Therefore, the presence of the direct repeat, TTTAAG-N5-TTTAAG, shown to be essential for CiaR-mediated regulation in *S. pneumoniae* should also be indicative of CiaR-dependent promoters in these organisms. To determine, if csRNAs are also present in streptococci other than *S. pneumoniae*, genomic searches were performed with the CiaR-related repeat to detect CiaR-activated promoters in intergenic regions, which are followed by a transcriptional terminator.
By this approach, a large number of putative csRNA candidate genes have been detected for those streptococcal species, whose genome sequences were available. Between two and six csRNA genes were found in individual streptococcal strains. Secondary structure predictions revealed structures similar to the csRNAs of *S. pneumoniae*, but also new structures.
The existance of these csRNAs encoded in *Streptococcus mitis*, *Streptococcus oralis*, *Streptococcus sanguinis* and on a *Streptococcus thermophilus* plasmid was tested by northern blot experiments with probes for individual csRNAs. All predicted csRNAs could be verified demonstrating that a valuable csRNA prediction had been performed. In conclusion, the results of our study strongly suggest that genes for small non-coding RNAs belong to the CiaR regulon in all streptococci.

GRP47
Role of the two-component system AfsQ1-Q2 in the regulation of primary and secondary metabolism in *Streptomyces coelicolor*

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AfsQ1/Q2, a two-component system (TCS) in *Streptomyces coelicolor*, was identified previously for its ability to stimulate actinorhodin (ACT) and undecylprodigiosin (RED) production in Streptomyces lividans. However, disruption of either afsQ1 or afsQ2 in *S. coelicolor* did not result in any detectable changes in secondary metabolite formation nor morphogenesis.
In our studies, it was found that AfsQ1/Q2 and SigQ, a putative sigma factor located divergently upstream of afsQ1/Q2, have a markedly effect on antibiotic production (ACT, RED and calcium-dependent antibiotic (CDA)) on minimal medium containing glutamate as the sole nitrogen source. AfsQ1/Q2-SigQ influences antibiotic production through direct and indirect positive control of pathway-specific activator gene expression as shown by electrophoretic mobility shift assays (EMSA) and reverse transcription analyses. Furthermore, AfsQ1 binds to the intergenic region between afsQ1 and sigQ and regulates both genes by inducing sigQ transcription, while repressing afsQ1/Q2 transcription. Microarray data revealed that the transcription of several important nitrogen metabolism genes is reduced or enhanced significantly in the afsQ1/Q2 mutant. It indicates that AfsQ1/Q2 plays an important role on nitrogen metabolism. Altogether the studies suggest the pleiotropic regulatory function of AfsQ1/Q2 involved in primary and secondary metabolism in S.coelicolor.

GRP48
In vitro characteristics with RNase J from *Rhodobacter sphaeroides* 2.4.1

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A few years ago a new player occurred on the wide field of RNA metabolism, RNase J. In *Bacillus subtilis* RNase J1, one of two paralogues in this organism, is essential and involved in different aspects of RNA turnover as there are rRNA maturation, mRNA processing and degradation. Interestingly a 5´-to-3´ exo- or an endoribonucleolytic activity are responsible for these processes, both present in one enzyme. Thereby the 5´-end phosphorylation state of the respective RNA seems to be an important determinant for switching between one of these two cleavage modes. The endonucleolytic activity in some characteristics resembles that of RNase E, which is lacking in *Bacillus subtilis*.
We are investigating RNase J from the α-proteobacterium *Rhodobacter sphaeroides* 2.4.1. Its genome encodes homologues of both, RNase E and RNase J. *In vitro* RNA degradation analyses with recombinant wild type protein as well as variants with amino acid exchanges were performed to analyse the 5´-end depending cleavage as well as the direction of processivity.

[1] Even et al., 2005, *NAR* 33 (7): 2141-2152
[2] Mathy et al., 2007, *Cell* 129: 681-692
[3] Deikus et al., 2008, *JBC* 283 (25): 17158-17167

GRP49
Adaptation of the thermoalkaliphilic bacterium *Caldalkalibacillus thermarum* strain TA2.A1 to high temperature

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Caldalkalibacillus thermarum strain TA2.A1 is a thermoalkaliphilic bacterium isolated from a thermal alkaline spring at Mt. Te Aroha, New Zealand at a source temperature of 77°C and pH of 9.2. Strain TA2.A1 is part of a basal lineage of *Bacillaceae* with *Bacillus smithii* and *Bacillus horti* as its closest relatives. As a strictly aerobic thermoalkaliphile, strain TA2.A1 is adapted to two different extremes, high temperature and high pH, with growth optima of 65-70°C and pH 9.5 respectively. *C. thermarum* grows at temperatures ranging from 45°C to 75°C, but the biochemical and molecular mechanisms responsible for the ability of strain TA2.A1 to adapt to wide fluctuations in temperature have not been investigated.
In this study we used a proteomic approach, supported by transcriptional analyses, to study this phenomenon in cultures exposed to 70°C for 24 hours in comparison to cultures grown at 60°C. In addition, we compared long-term adaptation to temperature with a short-term heat shock treatment of 82°C for 1 min. Neither conditions impair the viability of TA2.A1, and growth at 70°C does not differ significantly from growth at 60°C. In these experiments, we identified more than 50 proteins with a significantly altered expression profile at 70-82°C compared to 60°C. Our results indicate that the heat shock chaperone GroEL plays a crucial role in short and long term adaptation to temperature. In addition, myo-Inositol-1-phosphate synthase, an enzyme that catalyzes the first committed step in the production of all inositol-containing compound like phospholipids, was strongly increased at high temperature. This enzyme is present in a wide range of eukaryotes where its products often act as secondary messengers, but has rarely been reported in bacteria.

GRP50
Genetic analysis of *tisAB/istr-1*, a multidrug tolerance locus in *Escherichia coli*

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We have recently shown that DNA damaging agents induce the formation of antibiotic tolerant persister cells in *Escherichia coli* through the SOS-inducible *tisB* gene product. At least one antisense RNA, *istr-1* controls expression of *tisB* and a knockout of this RNA locus leads to an increase in persister levels. To study what other factors are involved in *tisB* regulation we tested knockouts of different genes and regions implicated in *tisB* regulation for persister levels in response to ciprofloxacin.
A knockout of all antisense RNAs hypothesized to regulate *tisB* (*istr-1, uxaA, dinD*) led to the same increase in persister levels as that of the *istr-1* knockout alone, showing that the other two RNAs do not influence TisB production in persister cells. A knockout of the promoter region of *istr-2*, a small RNA containing the entire *istr-1* sequence, had a low persister phenotype indicating that this region is important for TisB production. Further, a knockout of the lexbox[*tisAB*] had a low persister phenotype when replaced with a chloramphenicol resistance cassette and a high persister phenotype when replaced with short FRT sequences. This high persister phenotype of Δlexbox[*tisAB*]::FRT is consistent with expected constitutive upregulation of *tisB* in the absence of the binding site for the LexA repressor. Replacement of the lexbox with the large chloramphenicol cassette separates the *istr-2* promoter region and the *tisAB* promoter region by 1.4 kb. The low persister phenotype of Δlexbox[*tisAB*]::CAT therefore indicates that proximity between the *istr-2* and *tisAB* promoter regions is essential for TisB production in persister cells.
We thus conclude that the promoter region of *istr-2* contains the binding site for a positive transcriptional regulator essential for TisB production in persister cells.

GRP51
Thiol redox profiling for exploring redox signaling networks in *Rhodospirillum rubrum*

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Rhodospirillum rubrum serves as an important model system for photosynthetic conversion of light energy to chemical energy with the unique property that the availability of light is not a prerequisite for the expression of the photosynthetic system, and maximal levels of intracellular photosynthetic membranes (PM) can be obtained under micro-aerophilic conditions in the dark.
Current hypotheses suggest that the biosynthesis of PM in purple bacteria is under control of a complex interplay of transcriptional regulators in response to metabolic redox signals. The ubiquinone pool has been suggested to play a major role in activating membrane-bound sensor kinases of the RegB type in combination with redox-sensitive cysteine residues. In addition, the activity of the cytosolic repressor PpsR is dependent on a disulfide-thiol switch. Recently, we found highly elevated levels of PM in response to external application of glutathione (GSH) to cultures of *R. rubrum*. The effect indicates an additional regulatory layer involved in the cellular control of PM expression and raises the question about the cellular targets of GSH. This compound was reported previously not to penetrate cell membranes, hence not being capable of directly interacting with cytosolic Cysteine residues.
In the present study we applied fluorescence labeling of accessible protein thiol groups with monobromobimane for identifying proteins that specifically interact with applied GSH in different compartments (periplasm and membrane). The labeled proteins were separated by SDS-PAGE and fluorescent bands were subsequently analyzed by mass-spectrometry.
The results will be used for setting-up a putative redox signaling network of interacting redox compartments as a basis for a systems level analysis using computational models. In addition, we determined intracellular GSH levels in *R. rubrum* and provide experimental evidence that in contrast to previous reports, GSH can actively be incorporated in the cytosol of *R. rubrum* cells.

GRP52
The contribution of charged amino acid residues to the signaling of the sensor kinase KdpD in *Escherichia coli*

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Potassium is the major intracellular cation in prokaryotes and eukaryotes. *Escherichia coli* contains three different K^+ uptake systems that vary in kinetics, energy coupling, and regulation. Under K^+-limiting conditions and in response to salt stress the high affinity K^+ uptake system KdpFABC is produced. This system restores the internal K^+ concentration or accumulates K^+ to adjust the turgor.
The *kdpFABC* operon is under the control of the histidine kinase/response regulator system KdpD/KdpE. KdpD specifically phosphorylates and dephosphorylates KdpE. Phosphorylated KdpE binds to the *kdpFABC* promoter and triggers *kdpFABC* expression. KdpD is anchored in the cytoplasmic membrane with four transmembrane helices and consists of large N- and C-terminal cytoplasmic domains. It has been proposed that KdpD sensing/signalling involves alterations of electrostatic interactions between the N- and C-terminal domains.
Homology modelling of two subdomains within the N-terminal domain, "KdpD" and Usp, suggests that the surface of the "KdpD" subdomain is negatively charged while the surface of the Usp subdomain is positively charged. Manipulation of these charged surfaces by single or multiple amino acid replacements resulted in KdpD variants with altered signaling properties. Inversion of negatively to positively charged amino acid residues in the "KdpD" subdomain and vice versa positively to negatively charged amino acid residues in the Usp subdomain produced KdpD variants that were unable to activate *kdpFABC* expression in response to salt stress and required stronger external inputs in response to the external K^+ concentration. Our results underline the idea of an electrostatic switch mechanism within the protein that modulates the activities of KdpD.

GRP53
A highly conserved gene of unknown function is involved in carbon storage accumulation of *Synechocystis* sp. PCC 6803

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Nitrogen frequently is a limiting nutrient in natural habitats. Therefore, cyanobacteria as well as other autotrophic organisms have developed multiple strategies to adapt to nitrogen deficiency. Transcriptomic analyses of the strain *Synechocystis* PCC 6803 under nitrogen-deficient conditions revealed a highly induced gene (*sll0783*), which is annotated as conserved protein with unknown function. This gene is part of a cluster with seven genes and in the upstream region lies a predicted NtcA-binding site. Homologues of this cluster occur in some unicellular, non-diazotrophic cyanobacteria, in several beta- and gamma-proteobacteria as well as in some gram-positives. The common link between the heterotrophic bacteria seems to be the ability of nitrogen fixation and production of PHB, whereas in the cyanobacteria only *Synechocystis* PCC 6803 can accumulate PHB.
In this presentation a knockout mutant of this gene in *Synechocystis* PCC 6803 is characterized. This mutant is unable to accumulate polyhydroxybutyrate (PHB), a carbon and energy storage compound. Since the levels of precursor metabolites such as glycogen and acetyl-CoA were not reduced, a direct effect on the PHB synthase was implied. Indeed, the mutant exhibits impaired PHB synthase activity during prolonged nitrogen starvation, although the expression of genes encoding PHB synthase was not strongly affected. PHB synthase activity in *Synechocystis* appears to depend on a so far unidentified activation process, in which the *sll0783* product may be involved.

GRP54
Regulon and Operator Specificity of the Bacillus subtilis Response Regulators LiaR and YhcZ

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Monitoring the cell envelope integrity is critical for survival. The LiaRS two-component system (TCS) is part of the cell envelope stress response in *Bacillus subtilis* [1]. It is induced by lipid II-interacting antibiotics and to a weaker degree also by detergents, organic solvents, secretion stress and alkaline shock [1,2]. This two-component system consists of an intramembrane-sensing histidine kinase, LiaS, and a cytoplasmic response regulator, LiaR. A third component, the membrane protein LiaF, acts as a strong inhibitor of LiaRS function. Together, these proteins form a three-component system that is highly conserved in Firmicutes bacteria [3]. In-depth transcriptional profiling identified three targets of LiaR-dependent gene expression. The primary target of LiaR is the *liaI* promoter, resulting in a strong induction of the *liaIH* operon and the complete *lia* operon [2]. Moreover, two additional loci (*yhcYZ-yhdA* and *ydhE*) are activated by LiaR. Strikingly, the *yhcYZ-yhdA* operon encodes another TCS that is highly homologous to LiaRS, especially with regard to the output domains of the NarX/FixJ-like response regulators, LiaR and YhcZ. We therefore performed detailed studies on binding site specificity and regulatory overlap between LiaR and YhcZ, using chimeric response regulators and in-depth mutagenesis of the *liaI* and *yhcY* promoter regions.

[1] Jordan *et al.* (2008), FEMS Microbiol. Rev. 32:107-146
[2] Mascher *et al.* (2004), Antimicrob. Agents Chemother. 48:2888-2896
[3] Jordan *et al.* (2006), J. Bacteriol. 188:5153-5166

GRP55
Regulation of terminal oxidases in *Ralstonia eutropha* H16

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The strictly respiratory β-Proteobacterium *Ralstonia eutropha* strain H16 can draw on an extensive inventory of electron transport chain components, including genes for eight distinct terminal oxidases for aerobic respiration [1]. Under anoxic condition *R. eutropha* forms a complete denitrification pathway. Transcript analysis by Real Time qPCR revealed that the expression of a putative high affinity cbb_3-type oxidase was increased approx. 500-fold under oxygen limited conditions. The role of a FNR-type regulator (Fnr3) on the expression of this oxidase was analysed. The inactivation of the *fnr3* gene led to a 30-fold lower expression of the cbb_3 oxidase. Furthermore the Fnr3 negative mutant showed poor growth and an accumulation of nitrite under denitrifying conditions. This underlines the importance of both proteins upon shifting from aerobic to anaerobic growth conditions. In addition the influence of the RegAB regulatory system on the expression of the terminal oxidases was investigated. Two oxidases, namely a *bd*-type oxidase and aa_3-type oxidase, turned out to be upregulated in the RegAB mutant.

[1] Cramm, R. 2009. Genomic view of energy metabolism in Ralstonia eutropha H16. J Mol Microbiol Biotechnol 16: 38-52.

GRP56
Involvement of Hsp100/Clp proteases in the motility of *Bacillus subtilis*

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Clp/Hsp100 proteases serve various functions in the bacterial cell. They are involved in protein quality control by removing misfolded and aggregated proteins from the cell. At the same time, they fulfill important functions in cellular regulation by specifically degrading regulator proteins such as transcription factors. For example, *Bacillus subtilis* ClpCP, together with specific adaptor proteins, controls competence development by proteolysis of the competence master regulator, ComK and affects endospore formation by degradation of the anti-sigma factor SpoIIAB.
We identified Hag (flagellin) as potential ClpCP substrate and are therefore currently investigating the involvement of the protease complexes ClpCP and ClpXP in the motility development of *B. subtilis*.
Motility genes and their expression in *B. subtilis* are organized in a different way compared to Gram-negative bacteria. The central transcriptional regulator FlhDC is missing in *B. subtilis* and other Gram-positive organisms. Also, the genes encoding components of the hook-basal body, the flagellar export machinery and chemotaxis genes are all organized in the large *fla/che* operon. The *sigD* gene, which encodes the alternative sigma factor σ^D, is located near the 3'-end of this operon. σ^D controls the expression of the late motility genes, such as *hag*, encoding flagellin.
Here we present *in vivo* and *in vitro* experiments suggesting that the expression of Hag is influenced on different regulatory levels by the presence of ClpCP and ClpXP.

GRP57
In search of the role of the alternative sigma factor SigH in *Staphylococcus aureus*

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The human pathogen *S. aureus* expresses a plethora of virulence factors. Most of them are part of regulatory networks and therefore are tightly regulated. For many bacteria, a common mechanism to regulate a certain set of genes in dependence of changing environmental conditions is the expression of alternative sigma factors. In *S. aureus*, only three alternative sigma factors have been identified so far. The best studied is SigB, which was shown to regulate not only virulence and resistance genes, but also global regulators. For SigS, which has recently been identified, a role in virulence was demonstrated.
SigH, finally, is a homologue of the alternative sigma factors SigH of *B. subtilis* and ComX of *S. pneumoniae*, which both are involved in regulating genetic competence and stress response. So far only two operons, SA1374-SA1371 and SA1418-SA1416, have been shown to be controlled by SigH of *S. aureus*. These genes are homologues of *B. subtilis* competence genes, which are involved in DNA-binding and -uptake.

All studies performed on SigH in *S. aureus* showed *sigH* being expressed on a very low level, and no growth conditions that activate SigH have been identified yet. In *B. subtilis*, a role of SigH besides genetic competence was seen in biofilm formation, nutrient depletion, sporulation and transition from exponential to stationary growth phase. Therefore, SigH also might be active under so far undetected growth conditions in *S. aureus*. To analyze this question in further detail, SA1418 promoter-*gfp* reporter gene fusions and a plasmid which allows inducible overexpression of *sigH* were constructed. These tools now allow to determine the influence of the alternative sigma factor SigH with the help of fluorescence assays and realtime RT-PCR under different conditions like late stationary growth phase and nutrient depletion, respectively.

GRP58
Interaction of SaeQ and SaeP with the two-component system SaeRS of *Staphylococcus aureus*

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In *Staphylococcus aureus* the regulatory *saePQRS* operon controls the expression of major virulence genes such as *hla* (α-hemolysin), *hlb* (β-hemolysin) and *coa* (coagulase). SaeR and SaeS show strong sequence homology to response regulators and histidin kinases of bacterial two-component systems, respectively. *saeP* (coding for a lipoprotein) and *saeQ* (coding for a membrane protein) are located upstream of *saeRS* and presumably are important for the functionality of the *sae* operon. To gain more insights into the function of SaeP and SaeQ we have cloned different components of the *saePQRS* operon into a tetracyline-inducible vector. Expression of *saeQ* in strains lacking the histidin kinase SaeS revealed that SaeQ can replace SaeS with regard to SaeR mediated gene activation. Additionally, induction of *saeQ* mediated expression of some target genes was shown to be independent of SaeR phosphorylation. To analyse the function of SaeP, a multicopy plasmid containing *saeP* with its native promotor and a downstream terminator sequence was introduced into *S. aureus* strain Newman. In this strain, SaeP resulted in severe inhibition of the *saePQRS* system: transcription of the native *sae* operon as well as of all of the target genes was abolished. Similar results were obtained when the whole *saePQRS* operon was cloned in a multicopy vector and introduced into strain Newman. Expression of a construct containing *saeQRS* only had no effect. In summary, the *saePQRS* operon seems to constitute a complex four component regulatory system.

GRP59
Functional characterization of the transcriptional regulator AlsR of *Bacillus subtilis*

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Acetoin is formed by *Bacillus subtilis* under anaerobic fermentative growth conditions and as part of the general overflow metabolism. It requires acetolactate synthase and -decarbobxylase encoded by the *alsSD* operon. The *alsSD* promoter is activated in response to acetate accumulation, low pH and the aerobic stationary phase. The transcriptional regulator AlsR is essential for *alsS-lacZ* reportergene expression under all growth conditions tested. AlsR belongs to the family of LysR-type transcriptional regulators (LTTR). Common for these regulators is a two domain structure composed of an N-terminal DNA binding HTH motif and a C-terminal regulatory domain. Binding of an inducer to the regulatory domain activates the transcription factors. For AlsR acetate is postulated as effector molecule. We are able to produce and purify the AlsR protein as Trx/Strep-AlsR fusion protein and after cleavage with the HRV-3C protease we finally obtained AlsR protein.
AlsR, as other members of the LTTR family, tends to aggregate in solution and gets rapidly inactivated by precipitation. We used limited proteolysis with chymotrypsin to identify stable proteinfragments which are soluble and possibly can be used for crystallization attempts.
To learn more about functionally relevant amino acid residues and also to increase protein stability we mutagenized the *alsR* gene and established an *in vivo* complementation system to test the activity of AlsR mutantproteins. For this purpose the altered *alsR* genes were integrated into the *amyE* locus of a *B. subtilis alsR* knock out mutant strain and expressed under the control of the xylose-inducible *xylA* promoter. AlsR activity was monitored by β-galactosidase activities derived from an AlsR-dependent *alsS-lacZ* reportergenefusion.

GRP60
Promoter architecture of anaerobically induced genes of *Bacillus subtilis*

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Bacillus subtilis is able to grow in the absence of oxygen using nitrate respiration and fermentative processes. The two-component regulatory system ResDE and the redox regulator Fnr are the main anaerobic regulators. Under anaerobic conditions, Fnr directly induces transcription of *narGHJI* and *narK*, encoding the nitrate reductase and a nitrite extrusion protein, respectively, and *arfM*, encoding the transcriptional modulator ArfM via a cis-acting Fnr box in the corresponding promoter regions (group 1 genes). By regulation of nitrate reductase formation, Fnr indirectly mediates the repression of *alsSD*, *ldhlctP*, *ywcJ*, and *cydABCD* via YdiH (group 2 genes).
Common for Fnr-dependent promoters is the conserved Fnr-binding site TGTGA-N6-TCACA located at position -41,5 with respect to the transcriptional start point. Fnr binding to this sites were shown by DNaseI footprinting analysis. Surprisingly, also the promoters only indirectly regulated by Fnr does contain highly conserved Fnr-binding sites but not at the appropriate position in the promoter. Nevertheless, *in vitro* Fnr is also able to bind to the almost conserved Fnr binding site located in the *alsS, ywcJ* and *ldh* promoter. Thus, the question rises: how is Fnr able to discriminate between functional and non-functional binding sites *in vivo*. Therefore, we altered the Fnr binding site position of the *narK* promoter to -29 as found in the *ywcJ* promoter, *narK* was no longer activated by Fnr. When we moved the Fnr binding site of the *ywcJ* promoter to position -41,5 we could partly gain Fnr-dependent expression of the *ywcJ-lacZ* fusion. Thus, position of the Fnr binding site is important for Fnr transcriptional activity. Using chromatin immune precipitation experiments (CHIP) we analyzed *in vivo* binding of FNR to the *narG, narK, arfM, alsS, ywcJ* and *ldh* promoter.

GRP61
Oxygen induced stress in the methanogenic archaeon *Methanosarcina mazei* Gö1

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Methanogenic archaea belonging to the phylum Euryarchaeota are restricted to anaerobic conditions and redox potentials below -300 mV to gain energy via methanogenesis. In previous studies it has been shown that methanogenic archaea are able to survive longer periods of aeration and resume growth after a shift to anaerobic conditions (Kiener & Leisinger, 1983). Recently it has been demonstrated that a member of the family *Methanobacteriales* is still able to perform methanogenesis under low oxygen conditions (Tholen *et al*., 2007). Since discovering that anaerobic bacteria contain oxygen detoxifying systems an increasing number of enzymes of methanogenic archaea were characterized that showed oxygen detoxifying activity on enzymatic level, however the regulatory network of oxygen stress in methanogenic archaea has not yet been investigated in detail.
Our model system *Methanosarcina mazei* strain Gö1 of the family *Methanosarcinaceae* is able to use a versatile spectrum of substrates. Here we demonstrate that *M. mazei* can not only survive but continues to grow under low oxygen conditions. Aiming to identify the respective regulatory network and to detect members of a regulatory cascade regarding oxygen stress response in *M. mazei*, genome-wide transcriptional analysis using DNA-microarrays and qRT-PCRs were performed after a shift to low oxygen conditions. This genome-wide analysis demonstrated an elevated transcriptional level of 113 ORFs including genes annotated as chaperones (4), heat shock proteins (3) and transcriptional regulators of unknown function (9). The involvement in the oxygen stress response of the transcriptional regulators was further analyzed and will be discussed.

[1] Kiener A. and Leisinger T. (1983), *Syst Appl Microbiol* 4:305–312
[2] Tholen A., Pester M. and Brune A. (2007), *FEMS Microbiol Ecol* 62:303–312

GRP62

Qdr, a transcriptional regulator of quinaldine degradation operons in *Arthrobacter nitroguajacolicus* Rü61a

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Gene products of two upper pathway operons on the 113 kbp linear plasmid pAL1 confer to *Arthrobacter nitroguajacolicus* Rü61a the ability to convert quinaldine to anthranilate. Anthranilate is presumed to be utilized via two different pathways, i.e., catechol and *ortho*-cleavage, and reactions involving CoA-thioester intermediates. Three genes presumably involved in anthranoyl-CoA metabolism are clustered in a third catabolic operon on pAL1 [1].
The gene product of *qdr* of pAL1 exhibits up to 26% identity to PaaX repressors involved in transcriptional regulation of the phenylacetate catabolon of *E. coli* and *Pseudomonas* spp.. Recombinant Qdr was purified with a C-terminal Strep-tagII and an N-terminal fusion to the maltose binding protein to enhance solubility and stability. Size exclusion chromatography indicated a dimeric structure of the native protein. The purified protein binds specifically to all three promotors of the catabolic operons of pAL1 in a concentration dependent manner as determined by gel retardation assays. The DNA sequences recognized by Qdr will be identified by DNase I footprinting analysis.
Aromatic metabolites of the upper pathway of quinaldine degradation were found to induce transcription of the catabolic operons [1]; however, at physiological concentrations none of these compounds interferes with the DNA-binding activity of Qdr. Interestingly, anthranoyl-CoA has the ability to mediate dissociation of the Qdr-DNA complexes. A binding constant K_a of 45.3 ± 1.51 μM^{-1} and a reaction enthalpy ΔH of -64.8 ± 1.4 kJ/mol was determined by isothermal titration calorimetry for the complex of anthranoyl-CoA and Qdr with a binding stoichiometry of one ligand molecule per monomer.

[1] Parschat et al. (2007) J. Bacteriol. 189:3855-3867

GRP63

The cytoplasmic PAS domain of the histidine kinase DcuS and its role in signal transduction

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Bacteria contain membrane integral sensors for response to changing environmental conditions. Many of the sensors are two-component systems consisting of a sensor histidine kinase and a response regulator that triggers the cellular response [1]. DcuS, the C_4-dicarboxylate sensor of *E. coli* is a membrane integral periplasmic sensing histidine kinase [2]. DcuS is a multidomain protein consisting of a sensing periplasmic PAS_P (Per-Arnt-Sim) domain, two transmembrane helices, a cytoplasmic PAS_C and the C-terminal kinase domain. PAS domains are ubiquitous signalling modules found in all kingdoms of life. They can detect a vast quantity of stimuli including light, oxygen, redox potential, proton motive force and various small molecules as well as modulating protein-protein interactions. PAS domains are structurally homologous and characterised by a conserved α/β-fold. A large number of sensory PAS domains have been identified but many of the PAS_C domains contain no apparent cofactor and their function is unknown.
A combination of mutagenesis and solid-state NMR (ssNMR) experiments with structural modelling [4] was used to study the structure and function of a membrane embedded construct of DcuS and of the cytoplasmic PAS_C domain. The experiments show a role for PAS_C for signal transduction from the PAS_P to the C-terminal histidine kinase of DcuS.

[1] Mascher T., Helmann J. D., Unden G. (2006) Microbiol Mol Biol Rev 70: 910-938
[2] Zientz E., Bongaerts J., Unden G. (1998) J. Bacteriol 180: 5421-5425
[3] Taylor B. L., Zhulin I. B. (1999) Microbiol Mol Biol Rev 63: 479-506
[4] Etzkorn *et al.* (2008) Nature Struct & Mol Biol 15:1031-1039

GRP64

Lysine-dependent regulation of the membrane-integrated transcriptional activator CadC by an interplay with the lysine permease LysP in *Escherichia coli*

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The Cad system is involved in the acid stress response of *E. coli* and is composed of the lysine decarboxylase CadA, the lysine/cadaverine antiporter CadB and the membrane-integrated transcriptional activator CadC. The decarboxylation of lysine to cadaverine results in the consumption of a cytoplasmic proton and thereby in an increase of the pH. CadC regulates expression of the *cadBA* operon and induces transcription under conditions of low external pH and sufficient amounts of lysine. Whereas CadC senses the pH directly, lysine is sensed only indirectly via an interaction with the lysine permease LysP (Tetsch *et al*., 2008). LysP is a secondary transporter with high affinity for lysine and belongs to the amino acid transporter family within the amino acid/polyamine/organocation superfamily. It represses CadC activation in the absence of external lysine by direct protein-protein-interaction which is mediated via the transmembrane domain, specifically a cluster of aromatic amino acids, of CadC (Tetsch *et al*., 2008). In contrast, nothing is known about possible interaction sites in LysP. In order to find these interaction sites and to elucidate the connection between the two functions of LysP, regulation and lysine transport, random and directed mutagenesis was performed. Both methods generated several LysP variants which had lost the ability to inhibit CadC resulting in a lysine-independent *cadBA* expression. Transport function of the variants was investigated *in vivo* in an *E. coli* strain lacking all lysine transporters. These analyses revealed that several amino acids are important both for transport and regulation and that most of these residues cluster in the cytoplasmic loops of LysP. To make the picture more complex, the lysine-2,3-aminomutase Yjek, now designated CadS, was found to be involved in the activation of the Cad system. Deletion of *yjek* resulted in a mutant that was unable to activate *cadBA* expression when cultivated in minimal medium. It remains to be elucidated whether the activation of the Cad system is influenced by the conversion of lysine to β-lysine or by another function of CadS.

GRP65

The two-component system KdpD/KdpE is important for virulence in the insect pathogen *Photorhabdus luminescens*

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Photorhabdus luminescens is a Gram-negative luminescent enterobacterium which forms an entomopathogenic symbiosis with soil nematodes belonging to the species *Heterorhabditis bacteriophora*, and is highly pathogenic towards insects. The switch between the hosts, and therefore symbiosis and pathogenicity, must precisely be regulated by the bacteria. To sense and to monitor their environment, bacteria are equipped with two-component systems consisting of a sensor kinase and a cognate response regulator. One of the most distributed two-component systems among bacteria is KdpD/KdpE which regulates production of the high affinity K^+ uptake system KdpFABC under K^+ limitation. A *P. luminescens* Δ*kdpDE* mutant was attenuated in pathogenicity against the greater wax moth *Galleria mellonella*. Since insect hemolymph has a high K^+ content, it was puzzling how the Kdp-system can be activated when *P. luminescens* resides in the insect host. Therefore, we compared KdpD/KdpE of *P. luminescens* with the homologous and well-studied system of *E. coli*. As a first step, we measured *kdpFABC* expression mediated by $KdpD_{Plu}/KdpE_{Plu}$ in an *E. coli kdp*-reporter strain. In response to external K^+ $KdpD_{Plu}/KdpE_{Plu}$ induced *kdpFABC* expression in a comparable pattern like $KdpD_{Ec}/KdpE_{Ec}$. Under osmotic stress $KdpD_{Plu}/KdpE_{Plu}$ induced *kdpFABC* expression much stronger than the *E. coli* homologue. Furthermore, *kdp*-promoter activity was significantly elevated in the *P. luminescens* Δ*kdpDE* mutant compared to the wild-type. In contrast, an *E. coli* Δ*kdpDE* strain shows no *kdpFABC* expression under any condition. These results suggest a different activation mechanism of the KdpD/KdpE system in *P. luminescens*. The role of K^+ and the question how the Kdp-system contributes to virulence remains unclear.

GRP66
Target genes of the YehU/YehT sensor kinase/response regulator system in *Escherichia coli*

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Two-component systems (TCS) are the predominant signal transduction systems in prokaryotes and consist of at least two components: a membrane-integrated sensor kinase (SK) which senses a stimulus and transduces it in a cellular signal by autophosphorylation, and a response regulator (RR) with DNA-binding activity. Whereas most TCS in *Escherichia coli* are well characterized, little is known about the YehU/YehT system. The membrane-integrated SK YehU has a GAF-domain, and the highly conserved input domain is structurally similar to the input domain of LytS, a potential sensor for murein subunits in Gram-positive bacteria. YehT posseses a CheY-like receiver domain and a LytTR DNA-binding domain. The structure of AgrA, a RR with a LytTR DNA-binding domain, represents a novel DNA-binding type.
Comparative proteomics of *E. coli* MG1655 and several *yehUT* and *yehT* mutants identified a set of proteins regulated by the SK/RR system YehU/YehT. Transcription of the corresponding genes was analyzed by Northern blot hybridization. Gel retardation experiments demonstrated a specific DNA-YehT interaction. The YehT-binding site was determined using DNase I footprint experiments. Furthermore, transcription of neighbouring genes, genes with an ArgA-binding sequence and homologs of the target genes of the TCS LytS/LytR in *Staphylococcus aureus* were analyzed by Northern blot hybridization. According to our results it is suggested that the YehU/YehT-system plays a role in cell envelope biosynthesis.

GRP67
Interaction of the *Escherichia coli* C_4-dicarboxylate carrier DctA with the DcuSR two-component system

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E. coli can use various C_4-dicarboxylates as carbon and energy source for aerobic or anaerobic respiration. The two component system DcuSR activates the transcription of *dctA* (succinate import), *dcuB* (fumarate-succinate antiport), *fumB* (fumarase) and *frdABCD* (fumarate reductase) in the presence of C_4-dicarboxylates. DcuSR consists of the membrane integral sensor kinase DcuS and the cytoplasmic response regulator DcuR.
Under anaerobic conditions the main transport proteins for C_4-dicarboxylates are DcuA, DcuB and DcuC (1). DcuA and DcuB import fumarate into the cell. Fumarate is reduced to succinate which is exported by DcuB and DcuC.
DctA is the main transport protein for C_4-dicarboxylates under aerobic conditions. It mediates the uptake of succinate and similar C_4-dicarboxylates in symport with protons. The expression of *dctA* is activated by DcuSR in the presence of C_4-dicarboxylates (2). Deletion of *dctA* has an effect on the expression of a *dctA'-'lacZ* fusion indicating a mechanism of self regulation (3). Interaction between the integral membrane proteins DcuS and DctA was analysed *in vivo* using FRET-measurements and a bacterial two-hybrid system. In the FRET measurements DctA and DcuS were expressed as GFP and GFP-derivative fusion proteins (4). For the use in the bacterial two hybrid system, DctA and DcuS were each fused to one part of the *Bordetella pertussis* adenylate cyclase (5). Direct interaction between DcuS and DctA was detected with both systems.

[1] Six *et al.* (1994) J. Bacteriol. 176: 6470-6478
[2] Davies *et al.* (1999) J. Bacteriol 181: 5624-5635
[3] Golby *et al.* (1999) J. Bacteriol 181: 1238-1248

GRP68
The fumarate sensor DcuS of *Geobacillus kaustophilus*

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The DcuSR two-component system of *Escherichia coli* consists of the sensor histidine kinase DcuS and the response regulator DcuR. It controls the expression of genes for fumarate respiration and of the aerobic C_4-dicarboxylate transporter DctA (1).

The thermophilic bacterium *Geobacillus kaustophilus* (2) consists of a sensor kinase showing homology to $DcuS_{Ec}$. The protein has a similar domain structure composed of two transmembrane helices, a periplasmic sensor domain, a cytosolic PAS- and kinase domain (3). $DcuS_{Gk}$ was expressed and purified in *E. coli*. The sensor kinase $DcuS_{Gk}$ was able to complement the DcuS function of an *E. coli* strain lacking $dcuS_{Ec}$ under anaerobic conditions. Furthermore, $DcuS_{Gk}$ stimulated the aerobic growth of the $dcuS_{Ec}$ mutant in the presence of the C_4-dicarboxylates fumarate and L-malate.

1) Zientz *et al*., 1998 J. Bacteriol. 180 (2): 5421-5425
2) Nazina *et al*., 2001 Int. J. Syst. Evol. Microbiol. 51 (Pt 2): 433-446
3) Etzkorn *et al*., 2008 Nat. Struct. Mol. Biol. 15 (10): 1031-1039

GRP69
Carbon storage regulator A (CsrA) of *Clostridium acetobutylicum* is essential for motility

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The Gram-positive, spore-forming anaerobe *C. acetobutylicum* is characterized by the Acetone-Butanol-Ethanol (ABE) fermentation. *C. acetobutylicum* showed some interesting metabolic and morphological differentiations during the transition phase and formed swollen and cigar shaped cells, the so called clostridal stages. In parallel, carbohydrates in form of granulose granules are accumulated. These macromolecules are expected to serve as energy- und carbon storage and are potentially important for initiation of sporulation. So far, the regulation of this morphological switch is unknown. Concerning to this, the genome of *C. acetobutylicum* (Nölling *et al.*, 2001) revealed an interesting open reading frame (*csrA*, carbon storage regulator A) which was expected to play a role in the metabolism of granulose. This kind of regulator directly controls the carbon metabolism in *Escherichia coli* (Timmermans *et al*., 2009). However, due to the location of the *csrA* (*cac2209*) gene it was conjecturable that this open readin frame could also be involved in motility. For detailed analyses, we created a *csrA* knock-out mutant of *C. acetobutylicum* ATCC 824 by the integration of group II intron (LlltrB) using the ClosTron system (Heap et al., 2007). Its analyses revealed a lack of motility whereas neither granulose formation nor sporulation seemed to be influenced.

[1] Nölling, J. *et al*., 2001. Genome sequence and comparative analysis of the solvent-producing bacterium *Clostridium acetobutylicum*. J. Bacteriol. 183: 4823-4838.
[2] Timmermans, J., and Van Melderen, L. 2009. Conditional Essentiality of the *csrA* Gene in *Escherichia coli*. J. Bacteriol. 190: 1722-1724.
[3] Heap, J.T., *et al*., 2007. The ClosTron: a universal gene knock-out system for the genus Clostridium. J. Microbiol. Methods 70: 452-464.

GRP70
Systems biology of *Clostridium acetobutylicum* - regulation of solventogenesis in an important butanol producer

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Microbially produced biofuels gain increasing importance due to limited resources of fossil fuels and CO_2 increase by their combustion. Butanol proved to be a superior biofuel than ethanol, because of its higher energy content, lower water absorption, better blending ability, and use of existing infrastructure and conventional engines. An important microbial butanol producer is *Clostridium acetobutylicum*.
The clostridial acetone-butanol production starts with the typical butyric acid pathway and then switches to production of acetone and butanol. Although the biochemical pathways leading to solvent formation in *C. acetobutylicum* are known, the regulation of these processes is only poorly understood. Objective of this work is to gain insight into the key regulatory events that occur during the transition between acidogenic, vegetative growth and the onset of solvent production and sporulation.
Besides the global transcriptional regulator Spo0A, other DNA-binding proteins, namely AdcR and CodY, seem to be involved in the regulation of the transcription of the *sol* operon. AdcR, a novel transcriptional regulator binds to the promoter region of the *adc* gene. CodY, a multifunctional, pleiotropic regulator in many other bacteria binds in the intergenic region of the *sol* operon.

Continuous culture experiments with *C. acetobutylicum* were accomplished under acidogenic and solventogenic conditions to get a deeper insight into regulation of solvent formation. At pH 6.5, acids were formed, at pH 4.5 mainly solvents were produced. Transcriptome analyses from cells taken at different pH values during the shift were performed. Genes were identified, which are up- and downregulated during the metabolic switch. Genes regulated in an identical pattern as the *adc* gene, being the first to be induced at the onset of solventogenesis, might be involved in regulation of solvent production. They are checked by mutant analyses.

GRP71
Structure, function and regulation of the glucose-PTS in *Escherichia coli*

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E.coli strain K-12 has at least five different permeases for glucose, including the major uptake system $IICB^{Glc}$ (PtsG), which belongs to the PEP-dependent carbohydrate phosphotransferase system (PTS). We have investigated different aspects of this important protein: The structure, the regulation of *ptsG* expression as well as the $IICB^{Glc}$ activity, and its function within the metabolism.
Structure: $IICB^{Glc}$ consists of two domains, the membrane-bound C-domain and the cytoplasmic B-domain. The C-domain contains the carbohydrate binding site, the B-domain the phosphorylation site C241. Whereas the structure of the B-domain was solved by NMR- and X-ray crystallography, the structure of the hydrophobic C-domain is still a matter of discussion.
We perform the cysteine-scanning method in order to get a better understanding of the structure. This knowledge will, in turn, help us to explain the way glucose is transported, phosphorylated and how the *ptsG* regulation might function.
Regulation: Due to its important function within the cell the *ptsG* gene is regulated in a sophisticated manner. The expression of *ptsG* involves various transcription and sigma factors, auxiliary proteins, a small regulatory RNA, and a small regulatory peptide. We performed different experiments to gain further insight into this regulatory network. We are concentrating our work on the SgrRST system and the repressor Mlc.
Metabolism: $IICB^{Glc}$ plays a central role, not only for the uptake and concomitant phosphorylation of D-glucose, but also as a glucose sensor causing an accurate response in the regulation of carbon catabolite fluxes.
Regulatory activity varies with the degree of phosphorylation of the PTS enzymes, which, in turn, varies with the ratio of sugar dependent dephosphorylation. Results from simulations and wet-lab experiments indicated that small changes in the amounts of $IICB^{Glc}$ cause severe changes in the phosphorylation level of $EIIA^{Glc}$.

GRP72
Molybdenum homeostasis in *Rhodobacter capsulatus*

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The phototrophic purple bacterium *R. capsulatus* synthesizes a molybdenum-dependent nitrogenase
(Mo-nitrogenase) and a Mo-free iron-only nitrogenase (Fe-nitrogenase), both catalyzing the reduction of dinitrogen to ammonia. At nanomolar Mo concentrations in the environment, activity of Mo-nitrogenase depends on a high-affinity Mo uptake system, ModABC. At micromolar Mo-concentrations, either of two related regulators, MopA and MopB, is sufficient to repress *modABC* and *anfA*, which codes for the activator of Fe-nitrogenase genes. In addition, MopA activates transcription of *mop* coding for a Mo-binding protein specifically interacting with MopB.
To examine discrimination of the Mo-repressed *anfA* and the Mo-activated *mop* gene, site-directed mutations were introduced into conserved promoter elements called Mo-boxes. The effects of these mutations on regulator binding and gene expression were determined by DNA mobility shift assays and *lacZ* reporter fusions. Mo-boxes were found to be both essential and sufficient for regulator binding. Several single-base-substitutions increased binding of the regulators suggesting that Mo-boxes have not been evolved for maximal binding. Both Mo-boxes tolerated numerous point mutations indicating high flexibility of these cis-regulatory elements.
In search for a transporter substituting for ModABC at high Mo concentrations, transposon Tn5 mutants were screened for growth at millimolar Mo concentrations. Six clones carrying Tn5 at different sites within *rc1997* were identified. Rc1997 is predicted to encompass twelve transmembrane domains and exhibits similarity to members of the ArsB-NhaD permease superfamily. As compared to the wild-type, *rc1997* mutants accumulated much lower amounts of molybdenum. Mo repression of *anfA* was greatly relieved in a *modABC-rc1997* double mutant. These findings strongly suggest that Rc1997 is involved in Mo-uptake in the micromolar range.

GRP73
Identification of mechanisms in a cryptochrome-dependent signalling pathway in *Rhodobacter sphaeroides*

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Rhodobacter sphaeroides is a phototrophic α-proteobacterium. To prevent the production of harmful reactive oxygen species, the formation of photosynthetic apparatus has to be tightly regulated. Blue light photoreceptors belonging to the cryptochrome/photolyase family are widely distributed throughout all kingdoms of life. We recently presented the identification of a cryptochrome-like protein (CryB) that shows a blue light dependent photocycle, binds single stranded DNA with high affinity and alters the composition of photosynthetic complexes (Hendrischk *et al*., 2009). Further experiments to reveal the signalling downstream of CryB are carried out now.
We observed significantly different halflifes of *puc*- and *puf*-mRNA (encoding proteins that build the photosynthetic complexes) between the wildtype and the *cryB* deletion mutant. Interestingly, an interaction with AppA, another major player in the light-dependent regulation of photosynthesis gene expression, was proven by pull down and Western blot experiments. AppA belongs to the BLUF domain containing blue light photoreceptors that acts as an antirepressor to PpsR, a repressor of photosynthesis gene expression. These findings suggest that CryB influences the expression of photosynthetic apparatus through the AppA/PpsR signalling pathway.
Proteome analysis of the *cryB* deletion mutant compared to the wildtype was performed by 2D gel electrophoresis and by the use of a yeast two hybrid system. Several possible interaction partners could be detected, already. Together with first results of a microarray transcriptome analysis we attempt to receive deeper insights into the complete signalling pathway mechanism of this prokaryotic cryptochrome.

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GRP74
Copper homeostasis in *Rhodobacter capsulatus*

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The phototrophic purple bacterium *R. capsulatus* synthesizes a multicopper oxidase, CutO, conferring copper tolerance. The *cutO* gene forms part of a tri-cistronic operon, *rc635-cutO-cutR*, as implied by reverse transcriptase PCR analysis. Expression of *cutO-lacZ* fusions strictly depended on the *rc635* promoter as shown by nested deletions. Remarkably, while *cutO* expression was regulated by copper, transcription starting at the *rc635* promoter was not affected by copper. Instead, copper regulation of *cutO* involved the 40-bp *rc635-cutO* intergenic region. This region is predicted to form a stem-loop structure which might prevent ribosome binding to the *cutO* Shine-Dalgarno sequence. In line with this assumption, deletion of the putative anti-Shine-Dalgarno sequence abolished translation inhibition in *Escherichia coli*.
To identify further copper homeostasis genes, *R. capsulatus* mutants generated by random transposon Tn5 mutagenesis were screened for copper sensitivity. Screening of more than 10,000 mutants led to identification of ten new copper tolerance genes. Correlation of copper sensitive phenotypes with Tn5 insertion sites was proven by mutant reconstruction. Based on database searches we suggest that three copper tolerance genes are involved in copper export (*rc87, rc1740, rc2710*), one gene mediates DNA repair (*rc186*), and one gene codes for a regulator (*rc1006*). Furthermore, one gene codes for the cytochrome-c oxidase-related *ccoH* gene (*rc5325*), while the function of the remaining four genes remains unknown (*rc356, rc1930, rc3935, rc6006*). In contrast to *cutO*, none of the ten copper tolerance genes was regulated by copper as shown by *lacZ* reporter fusions. To identify further copper-regulated genes transcriptome and proteome analyses will be carried out.

GRP75
Oligomerisation of the sensor histidine kinase DcuS in *E. coli*

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The two-component system DcuSR of *Escherichia coli* regulates gene expression of anaerobic fumarate respiration and aerobic C_4-dicarboxylate uptake [1]. The sensor histidine kinase DcuS responds by a periplasmic sensory domain to C_4-dicarboxylates and citrate [2]. The signal is transduced across the membrane by phosphorylation of DcuS and of the response regulator DcuR, resulting in activation of DcuR and transcription of the target genes.
Sensor histidine kinases are supposed to function in a preformed dimeric state in the membrane [3,4]. During trans-phosphorylation, the catalytic domain of one monomer phosphorylates the conserved histidine residue from the kinase domain in the second monomer by hydrolysis of ATP. In addition, signal transduction across the membrane and to the cytosolic kinase domain is supposed to be a mechanical process requiring protein dimers as well [5,6].
The oligomerisation of full-length DcuS was studied *in vivo* and *in vitro*. DcuS was genetically fused to derivatives of the green fluorescent protein (GFP), enabling fluorescence resonance energy transfer (FRET) measurements to detect protein-protein interactions *in vivo* [7]. FRET measurements were also performed with purified His_6-DcuS after labelling with fluorescent dyes and reconstitution into liposomes. *In vitro* and *in vivo* fluorescence resonance energy transfer showed the presence of permanent oligomeric DcuS in the membrane. Chemical crosslinking allowed direct evaluation of the oligomeric state of DcuS. Detergent-solubilised His_6-DcuS was mainly monomeric, whereas DcuS in proteoliposomes and in bacterial membranes was tetrameric.

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GRP76
Mutational inactivation of the citrate synthase gene *gltA* in *Corynebacterium glutamicum* caused by the absence of aconitase or isocitrate dehydrogenase: a selection to avoid high internal tricarboxylate concentrations

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The Gram-positive soil bacterium *Corynebacterium glutamicum* is used industrially for the production of amino acids such as L-glutamate and L-lysine. The tricarboxylic acid (TCA) cycle is a central metabolic pathway which, besides its function in energy generation, provides the biosynthetic precursors for glutamate and lysine, namely 2-oxoglutarate and oxaloacetate. In order to improve production strains of *C. glutamicum*, it is necessary to have a detailed knowledge of the enzymes and regulatory mechanisms involved in the TCA cycle [1-3]. During our studies of *acn* (aconitase) and *icd* (isocitrate dehydrogenase) deletion mutants we surprisingly discovered that these deletions were very often accompanied by secondary mutations leading to an inactive or missing citrate synthase (encoded by *gltA*). The *gltA* mutations included point mutations, nonsense mutations, partial duplications, partial deletions or transposon insertions. This is a strong indication that the accumulation of citrate or isocitrate in the cytoplasm is toxic for the cell probably due to complex formation with divalent cations such as Ca^{2+} and Mg^{2+}. These cations are essential for many enzymatic and regulatory functions which would be impaired. We are currently testing this hypothesis experimentally.

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GRP77
Regulation of autotrophic CO_2 fixation in the archaeon *Thermoproteus neutrophilus*

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Archaea contain many thermophilic chemolithoautotrophs such as *Thermoproteus neutrophilus*. This anaerobic crenarchaeon uses a novel autotrophic CO_2 fixation pathway, the dicarboxylate/4-hydroxybutyrate cycle [1, 2]. The organism is a facultative autotroph, which prefers as carbon sources organic acids that can easily feed into the metabolite pools of this cycle [3]. Addition of the preferred carbon sources acetate, pyruvate, succinate and 4-hydroxybutyrate to cultures resulted in a notable stimulation of the growth rate and a diauxic growth response. The characteristic enzyme activities of the carbon fixation cycle, fumarate hydratase, fumarate reductase, succinyl-CoA synthetase and enzymes catalyzing the conversion of succinyl-CoA to crotonyl-CoA, were differentially downregulated in the presence of acetate and to a lesser extent in the presence of other organic substrates. This regulation pattern of enzyme activities correlated well with the differential expression profile of the proteome and with the transcription of the encoding genes. The genes encoding PEP carboxylase, fumarate reductase and four enzymes catalyzing the conversion of succinyl-CoA to crotonyl-CoA were organized in a cluster [4]. Their cotranscription, transcription start sites and promoter structures as well as the participation of DNA-binding regulator proteins were analyzed. Altogether the observed regulation fully supports the functioning of the proposed cycle and designates *T. neutrophilus* as a good model for studying regulation of central carbon metabolism in Archaea.

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GRP78
Regulatory links between iron metabolism and oxidative stress in the alpha-proteobacterium *Rhodobacter sphaeroides*

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Iron (Fe) is an extremely important element in biology and the 4th most abundant metal on Earth. It is an integral part of heme and is used as a cofactor in iron-sulfur proteins. Among the most important cellular functions of iron proteins are protection from oxidative and nitrosative stresses, nitrogen fixation, hydrogen production and consumption, photosynthesis and methanogenesis. All forms of life from humans to bacteria require Fe but excessive Fe is toxic because of produced hydroxyl radicals in the Fenton reaction which damage DNA, lipids and other cell components. Thus a strict regulation of iron metabolism and its coupling to the oxidative stress defense is needed. Therefore we want to use *Rhodobacter sphaeroides*, as an established model organism, to elucidate the regulation of iron metabolism and its link to the oxidative stress defense which is well studied in that bacterium.
To investigate the effects of iron limitation we cultivated *R. sphaeroides* without added Fe(III)citrate but with the iron chelator 2,2'-dipyridyl. Under these iron limited conditions *R. sphaeroides* loses its typical dark red pigmentation and becomes nearly white. By using realtime RT-PCR the expression level of putative iron responsive genes was determined. This analysis revealed an induction of e.g. *hemP*, *bfd* and *exbB*. These findings were also supported by microarray analysis. A reduced expression was found for photosynthesis genes and genes of the chemotatic pathway. Because regulation can take place both on transcriptional and post transcriptional level the effects of iron are also investigated on proteome level. In addition we analyzed the response of small RNAs to iron and will further elucidate their role in iron metabolism. The aim of the project is to get new insights in the iron regulation pathway and its link to oxidative stress in alpha-proteobacteria.

GRP79
Characterization of activity and transcriptional regulation of the lysine-specific permease LysP of *Escherichia coli*

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The lysine-dependent regulation of *cadBA* expression is mediated by an interplay between the lysine permease LysP and the transcriptional activator CadC in *E. coli*. Under conditions of low pH and externally available lysine, CadC activates expression of the *cadBA* operon encoding the lysine decarboxylase CadA and the lysine/cadaverine antiporter CadB. The Cad system is part of the acid stress response of *E. coli*. We are interested in the molecular mechanisms of the transcriptional regulation of *lysP* expression and the interplay between the transporter LysP with the membrane-integrated sensor CadC. Reporter gene assays revealed that *lysP* expression is repressed by high lysine concentrations and that the transcriptional activator ArgP is required for *lysP* expression. In DNA-affinity purification assays a protein bound to the *lysP* promoter was identified as the leucine responsive regulatory protein Lrp, and an *lrp* mutant exhibited a reduced *lysP* expression in the absence of external lysine. According to electrophoretic mobility shift assays, Lrp binds to the *lysP* promoter with high affinity underlining the important role of Lrp in the regulation of *lysP* expression. A prerequesite for understanding the interplay between CadC and LysP is a detailled characterization of the LysP transport function which was analyzed by measuring ^{14}C-L-lysine uptake both *in vivo* and *in vitro* with purified LysP reconstituted in proteoliposomes. The transport mode was examined by imposing a membrane potential and different ion gradients across the proteoliposome membrane. The experiments revealed that LysP transports lysine specifically with a high affinity of 2 µM. Co-reconstitution of LysP and CadC did not alter the transport rates of LysP suggesting that CadC does not exert any effect on LysP activity.

GRP80
Identification of RNA Helicases in the Degradosome of *Bacillus subtilis*

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Regulating the stability of mRNAs enables bacterial cells to adapt quickly to challenging environmental conditions. The major players in the mRNA turnover are RNases. These proteins can act as single enzymes or in large multiprotein complexes called RNA-degradosome [1].
Recently, we identified a RNA degradosome-like complex in the Gram positive model organism *Bacillus subtilis*. This complex consists on the RNases Y, J1/J2, PNPase and the glycolytic enzymes enolase and phosphofructokinase. This combination of RNases and glycolytic proteins roughly resembles the composition of the *E. coli* degradosome with the exception that a RNA helicase was missing [2]. Therefore we readdressed this issue.
Bacillus subtilis encodes four different RNA helicases of the DEAD box family. Using the bacterial two-hybrid system and *in vivo* approaches we identified the protein CshA out of the four as the central RNA helicase in the complex. This helicase interacts with all members of the degradosome (except RNase J2). Furthermore we analyzed the domain structure of CshA. Investigating truncations of the protein we identified domains within the protein which are essential for binding to the complex. We even demonstrate that the carboxy-terminal domain of CshA is responsible for the specificity to the complex coordinating protein RNase Y by characterizing a chimera in which this domain is fused to the core of CshB, a DEAD box protein with no former binding capacity.

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GRP81
Characterization of RNAse mutants in the human pathogen *Staphylococcus aureus*

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Bacteria are able to cope with dramatic environmental changes by rapidly altering gene expression. In this regulation RNA metabolism plays an important role. It consists of different processes that can be grouped in RNA decay, RNA processing and RNA maturation. RNA decay is crucial in determining intracellular levels of RNA species. RNA processing takes place within complex operons and permits the tuning of protein ratios of the co-transcribed genes. Each of these processes requires the action of ribonucleases (RNases). The involved RNases were elucidated in *Escherichia coli* but in Gram-positive bacteria, RNA metabolism, so far best studied in *Bacillus subtilis*, seems to be different. The aim of our work is to discern this process in the human pathogen *Staphylococcus aureus* in order to understand its contribution to the virulence potential of the bacteria. We could identify in the genome of *S. aureus* homologues of most of the RNases described in *B. subtilis*. Using the pMUTIN vector, we constructed conditional mutants of three putative RNases in different *S. aureus* strains. With this system, mutants are obtained by integration of the vector upstream the target gene, which then falls under control of the IPTG inducible promoter Pspac. We could show that some RNases, known to be essential in *B. subtilis*, are not in *S. aureus*, indicating that these enzymes could have different roles in the two organisms. As a model for RNase action, we used the processing of the global virulence regulator *sae*. There are indications that the major transcript of the *sae* operon is originated from an endonucleolytic cleavage of the primary transcript. Here, we show in different backgrounds (MW2 and UAMS1) that conditional mutants of RNaseY have defects in *sae* cleavage, thus indicating that this RNase could be the key in this process.

GRV01
Response of the oxygen sensor NreB to air *in vivo* in aerobically and anaerobically growing *Staphylococcus carnosus*

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The NreBC two-component system is required for activation of anaerobic respiration in *Staphylococcus carnosus* [1]. The sensor kinase NreB contains an O_2 sensitive $[4Fe\text{-}4S]^{2+}$ cluster which is converted by $O_2$to a $[2Fe\text{-}2S]^{2+}$ cluster followed by complete degradation and formation of Fe-S-less apoNreB [2]. NreB contains four Cys residues which ligate the Fe-S cluster. The accessibility of the Cys residues to alkylating agents was tested and used to differentiate Fe-S-containing NreB and Fe-S-less apoNreB [3].
In anaerobic bacteria only a small portion of the Cys residues of NreB was accessible to alkylation, whereas most of the Cys residues from aerobic bacteria were accessible. The change in accessibility allowed determining the half-time for the conversion of $[4Fe\text{-}4S]^{2+}$-NreB to apoNreB after addition of air. Overall, in anaerobic bacteria most of the NreB exists as $[4Fe\text{-}4S]^{2+}$-NreB, whereas in aerobic bacteria apoNreB is predominant and represents the physiological form. The number of accessible Cys residues was confirmed by iodoacetate alkylation followed by mass spectrometry of Cys containing peptides [4].

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GRV02
Cell wall metabolism in *Bacillus subtilis*: a global analysis of gene expression in growing and phosphate limited cells

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The cell wall is the interface between the bacterium and its environment. It is a complex structure composed of peptidoglycan and anionic polymers that confers shape on the bacterium and resists internal turgor pressure. Exposure of *B. subtilis* to phosphate limitation has a profound effect on the metabolism of cell wall anionic polymers: the cell must affect a switch from synthesis of phosphate-rich teichoic acid to the non-phosphate containing teichuronic acid, a transition that is effected by the PhoPR two-component signal transduction system.

We sought to gain detailed expression profiles of genes encoding cell wall metabolic functions in *B. subtilis* during growth, phosphate limitation and the transition between these states. We constructed 140 transcriptional promoter fusions with a *gfp* reporter gene. Using high-throughput Live Cell Arrays, we established real time expression profiles, taking measurements every 10 minutes without interrupting the experiment.
Here we present analysis of the expression profiles during the different phases of growth. In exponentially growing cells four distinct profiles of expression were observed. These profiles differ in time and duration of expression. Upon entering of phosphate starvation a general decrease in gene expression was observed. In contrast the genes of the PhoPR regulon are highly induced. The results demonstrate a clear hierarchy of expression concerning the PhoPR regulated promoters. Injection of phosphate to the medium enabled us to study the exit of cell out of the phosphate starvation state. After injection both wild-type and a *phoPR* deletion strain start to grow immediately. Surprisingly, the *phoPR* mutant grows significantly faster. Analysis of the expression profiles of the cell wall promoters indicate that a different expr

GRV03
Studies of the two-component signal transduction system CopSR in *Corynebacterium glutamicum*

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The soil bacterium <i>Corynebacterium glutamicum serves as a model organism of the high-GC Gram-positive bacteria for systems biology. In order to understand the responses of the bacterium to certain environmental changes it is important to study the regulation systems of *C. glutamicum*. In the genome 13 two-component signal transduction systems are encoded [1]. One of these is the CopS/CopR system, previously named CgtS9/CgtR9. In silico analyses suggested that CopSR might be involved in heavy metal ion stress. A mutant in which the *copSR* genes are deleted (Δ*copSR*) showed an increased sensitivity towards copper ions which could be complemented by plasmid-encoded *copSR* genes. DNA microarrays gave a genome-wide view on the response of *C. glutamicum* wild type and of the Δ*copSR* mutant to increased copper concentrations. The results indicated that CopSR is the key regulatory system in *C. glutamicum* for sensing elevated copper ion concentrations and for induction of a set of genes capable of diminishing copper stress. CopSR consists of the membrane-bound sensor kinase CopS and the response regulator CopR. To answer the question for the stimulus of CopS and how it is sensed, the CopS protein was purified and reconstituted into proteoliposomes. The protein orientation was determined as inside-out. This system and the solubilised CopS protein were used for activity tests and interaction studies. The identification of amino acid residues that might be responsible for metal ion binding was another aim of this study. Growth experiments of strains containing mutated CopS proteins showed that His41 and His56 are important for sensing and might be involved in binding of copper ions. In additional studies, target genes of the response regulator CopR were identified by electrophoretic mobility shift assays.

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GRV04
The regulation of the two-component sensor kinase SenS by the extracellular heme-binding protein HbpS in *Streptomyces reticuli*

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In the cellulose degrader *Strepomyces reticuli* the two-component system SenS/SenR was identified [1]. Physiological studies revealed that the presence of SenS/SenR and the secreted heme-binding protein HbpS considerably enhances the resistance of *S. reticuli* against redox-active compounds. Further biochemical approaches demonstrated that HbpS interacts specifically with the sensorkinase SenS. We could also show that heme-free HbpS represses the autokinase activity of SenS; whereas hemin-treated HbpS considerably enhances SenS autophosphorylation under redox stressing conditions [2]. The previous reported high resolution crystal structures (2.2 Å and 1.6 Å) of HbpS crystallized in the presence and absence of heme demonstrate that iron ions bind to surface exposed lysine residues. Subsequent biochemical experiments show that HbpS possesses additionally heme degrading activity and that while the octomeric assembly state of HbpS is not essential for heme-degrading activity, full assembly of HbpS is required for interaction with the sensor kinase SenS and hence for its activation [3].

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GRV05
The two-component system CiaRH of *Streptococcus pneumoniae* – further insights into its complex phenotypes

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The CiaRH two-component system is involved in β-lactam resistance, genetic competence, maintenance of cell integrity, and virulence. It has been identified in a screen for spontaneous β-lactam resistant mutants of *Streptococcus pneumoniae* R6. A mutation in the histidine kinase gene *ciaH* led to decreased susceptibility to β-lactam antibiotics and to a block of spontaneous genetic competence. Characterization of several other *ciaH* mutations isolated by similar selection procedures substantiated these phenotypes. The consequence of all these *ciaH* mutations is enhanced regulation of the CiaR regulon, consisting of 24 genes organized in 15 transcriptional units. The genes of the regulon included *ciaRH*, loci involved in teichoic acids modification (*lic*), sugar metabolism (*mal, man*), stress response (*htrA*), chromosome segregation (*parB*), protease maturation (*ppmA*) and unknown functions. Remarkably, the five strongest CiaR-dependent promoters drive expression of small non-coding RNAs (csRNAs).
The link of CiaR-regulated genes to the complex CiaR-dependent phenotypes remained elusive. Therefore, some of these genes were inactivated and phenotypic analyses were performed. The results of these studies showed that the csRNAs are major effectors for the CiaR-related phenotypes. In strains with activated CiaR regulon the absence of csRNAs reduces β-lactam resistance and restores competence. The serine protease HtrA is additionally decreasing competence, but is not involved in β-lactam resistance. Thus, competence development is under multiple negative control by CiaR-dependent gene products. Their individual importance in this complex control circuit appear to vary under different growth conditions.
The identification of the small non-coding csRNAs and the protease HtrA as major regulators of competence in *Streptococcus pneumoniae* is a further important step to unravel the underlying molecular mechanisms of CiaR-related phenotypes.

GRV06
The hybrid sensor kinase SO0577 - representative of an evolutionary new branch of ArcB proteins in *Shewanellaceae*

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Two component signal transduction (TCS) enables microorganisms to sense environmental signals and allows adaptation to changing conditions, e.g. by regulating gene expression. The anoxic redox control (Arc) represents a key TCS system in numerous γ-proteobacteria for mediating the response to altered environmental oxygen levels. In the well-characterized Arc system of *E. coli,* the sensor kinase ArcB senses the redox state of the membrane quinone pool. Under anaerobic conditions, a phospho group is transferred to its cognate response regulator ArcA which in turn regulates expression of the target genes.

The facultative anaerobic γ-proteobacterium *Shewanella oneidensis* MR-1 and all other members of this genus possess an ArcA ortholog. However, sequence comparisons did not identify a full-length ArcB sensor kinase in *S. oneidensis* MR-1 or any other *Shewanella species* . Only a short protein consisting of a single phospho transfer domain, HptA, revealed significant homologies to the corresponding domain of ArcB. Interestingly, *hptA* resides in the same genetic context as *arcB* in *E. coli* , strongly suggesting a loss of the sensory and kinase domains of ArcB in *Shewanella* . Previous analyses on HptA and ArcA in *S. oneidensis*MR-1 indicate that both proteins interact but a corresponding sensor kinase for this system still remains unknown.
By a candidate approach and mutant analyses on *S. oneidensis* MR-1 we identified the hybrid sensor kinase SO_0577 as a potential sensor kinase for the *Shewanella* Arc system. The protein contains two PAS domains, one kinase and two receiver domains, but lacks a phosphotransfer (Hpt) domain. Phenotypic analyses of mutants carrying in-frame deletions in SO_0577, *hptA* and *arcA* as well as the corresponding double and triple knock-outs revealed similar phenotypes regarding growth, motility and gene expression of selected marker genes. Accordingly, Investigations on transcriptomic changes in an SO_0577 knock-out strain compared to the wild type exhibited striking similarities to those in an *arcA* mutant. *In vitro* interaction studies on the purified proteins demonstrated phosphotransfer between SO_0577, HptA and ArcA. Thus, we have unambiguously identified SO_0577, now designated ArcS, as cognate sensor kinase for an atypical Arc system in *S. oneidensis* MR-1.
ArcS does not display any striking homologies to any ArcB sensor kinases identified so far and forms a new phylogenetic branch of ArcB sensor kinases. Despite the striking differences in homology, *E. coli* ArcB provided in *trans* was able to fully complement the phenotypes of ArcS and ArcS/HptA in *S. oneidensis* MR-1. Taken together we suggest that a truncation of the sensory component ArcB in *S. oneidensis* MR-1 was bypassed by recruiting an alternative sensor kinase SO_0577 as exact functional replacement. Thus, ArcS, HptA and ArcA form a new functional Arc-TCS within the family of *Shewanellaceae* .

GRV07
Post-transcriptional up-regulation of the SacP phosphatase counteracts glucose-phosphate stress in enterobacteria

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In *Escherichia coli* the small regulatory RNA SgrS is well known to counteract phosphosugar stress, a process that involves the post-transcriptional targeting of the *ptsG* mRNA, coding for the major glucose transporter [1]. The study of the underlying interaction revealed duplex formation of the 3' end of SgrS with the 5' UTR of *ptsG* which facilitates RNaseE-dependent repression [2].
To investigate the role of SgrS in the model pathogen *Salmonella typhimurium* we made use of a pulse-expression approach that combines tightly controlled expression of an sRNA from an inducible promoter with whole genome microarrays analysis [3]. This analysis revealed an extended SgrS regulon, displaying a larger set of repressed mRNA targets, but also up-regulation of a single transcript, termed *sacP*.
Interestingly, *sacP* is the 2nd gene of a polycistronic messenger, however SgrS mediated gene activation is limited to *sacP* and does not render the expression of other members of this operon. Mechanistically, this up-regulation involves RNA duplex formation of SgrS with distal parts of the preceding *pldB* mRNA and requires the action of the RNA chaperone Hfq and the RNAse E ribonuclease.
Biocomputational and biochemical analysis have shown that SacP belongs to the group of HAD-phosphatases that display high affinity towards phosphorylated sugar substrates, including Glucose-6-phosphate [4]. Indeed, under phosphosugar stress conditions, post-transcriptional up-regulation of SacP by SgrS is critical for cellular replication, suggesting that SacP activation is required to decrease the intracellular amount of phosphorylated sugars.
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GRV08
Two highly homologous sRNAs in *Agrobacterium tumefaciens*

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In the past years small noncoding RNAs (sRNAs) have been found in a wide range of organisms and have received enormous attention as a new class of gene expression regulators. The largest and most extensively studied set of sRNAs act through base pairing with target RNAs, usually modulating the translation and stability of mRNAs [1].
We searched for sRNAs in the plant pathogen *Agrobacterium tumefaciens* using a comparative bioinformatic approach [2]. We identified diverse sRNAs which are highly conserved among many Rhizobiacea. Two tandem sRNAs (C2A and C2B) similar in structure and sequence are currently under investigation. Both sRNAs were also found in *Sinorhizobium meliloti* [3].
Proteome analyses with deletion mutants revealed at least two periplasmatic substrate binding proteins to be silenced by C2A and/or C2B. Northern blot studies and reporter gene fusions confirm these data.

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GRV09
Identification of a small noncoding RNA in *Clostridium acetobutylicum*

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Since 2001, small noncoding RNAs (ncRNA, sRNA) are in the focus of many research projects, as they play an important role in the regulation of numerous eukaryotic as well as prokaryotic processes. While, in the last years, research was very successful concerning sRNAs in Gram-negative bacteria (*Escherichia coli* and *Salmonella typhimurium*), still very little is known about sRNAs in Gram-positive bacteria. One well analyzed example is a sRNA in *Clostridium acetobutylicum* that is involved in nitrogen metabolism (Fierro-Monti et al., 1992, J. Bacteriol. 174, 7642-7647). This bacterium gained in importance over the last years, as it naturally produces butanol. Butanol represents a bulk chemical and, moreover, a biofuel and/or biofuel additive. Thus the interest in butanol fermentation using *C. acetobutylicum* is increasing, and it is expected that butanol production will be a major industry in the near future. Therefore, the most important task in research is to elucidate the regulation mechanisms involved in butanol production.
Recent data show the existence of a further sRNA in *C. acetobutylicum*, which is crucial for solvent production. In *C. acetobutylicum*, the *sol* operon is the essential transcription unit for the production of butanol, as it comprises nearly all necessary genes. Upstream of the *sol* operon, a gene for a sRNA, *solB*, could be identified. Overexpression of the *solB* gene in *C. acetobutylicum* using the natural promoter leads to a phenotype which is no longer able to produce solvents. This result clearly demonstrates the importance of SolB for the production of solvents and indicates SolB being a repressor of solvent production.

GRV10
Identification and characterization of small non-coding RNAs in the archaeal diazotroh *Methanosarcina mazei* Gö1

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Small non-coding RNAs (sRNAs) have been identified in all three domains of life. In Eukarya and Bacteria functions have been assigned for many sRNAs. To get a deeper insight into potential regulatory roles of sRNAs in Archaea, we studied the methane-producing archaeon *Methanosarcina mazei* strain Gö1, which is able to fix molecular nitrogen. Although many aspects of the organism's adaptation to different stress situations are currently under investigation, e.g. limiting carbon sources (Hovey *et al.* 2005), nitrogen limitation (Veit *et al.* 2006, Weidenbach *et al.* 2008) and osmotic stress (Pflüger *et al.* 2007, Spanheimer & Müller, 2008), still less is known about the overall transcriptional organization, particularly with regard to the non-coding regions representing 25 % of the genome.
Here we present genome-wide identification of sRNAs in *M. mazei*, with the main focus on those potentially involved in nitrogen metabolism. Total-RNA from cells grown with ammonium and molecular nitrogen as nitrogen-source was investigated by massive parallel sequencing technology, resulting in two libraries consisting of 228.000 cDNAs in total. The performed RNA-seq approach revealed the presence of approximately 250 small non-coding RNAs expressed from intergenic regions (IGRs). 76 of those were characterized via northern blot analysis, confirming the expression of 56 sRNAs, 18 of which are differentially transcribed in response to nitrogen availability. Selected (nitrogen regulated) sRNAs have been further analyzed using biochemical and genetic approaches.

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GRV11
The role of the CRISPR/Cas-system in *Thermoproteus tenax*

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Clusters of regularly interspaced short palindromic repeats (CRISPR) are a novel feature of prokaryotic organisms, present in nearly all archaeal and 50% of all bacterial genomes. These clusters consist of repeat sequences of 24 to 48 nt, separated by slightly longer spacer sequences [1]. Beside CRISPR sequences, the genomes contain a diversity of conserved *cas* (CRISPR-associated) genes [2], in which the encoded Cas proteins promote the formation of psiRNAs of transcribed CRISPR cluster and the interference reactions against foreign nucleic acids. It has been shown in *E. coli*, that the CRISPR interference guide antiviral defence in prokaryotes, with the specificity determined by sequence similarity between spacer and the phage genome [3]. But the high abundance of Cas proteins in different organisms result in complex molecular pathways, which are mainly not understood.
To get an insight into the function of the CRISPR/Cas-system in Archaea, the seven CRISPR clusters (TTX_1-7) and conserved cas genes of the hyperthermophilic Crenarchaeote *Thermoproteus tenax* were analysed. In Northern Blots small non-coding RNAs with a length of approx. 120-60 nt could be detected for 5 of the 7 CRISPR clusters. The *cas* genes located between CRISPR cluster TTX_4 and 5, are organised in two operons (*casa1*, *casa2*). Surprisingly, transcription of the cas genes was induced by abiotic stress (UV-light, high ionic strength), which raises the question about the functions of the system in Archaea. As shown by heterologous expression in *E. coli*, the recombinant proteins from both operons, form complexes with exhibiting RNA nuclease activity (CasA1) or RNA/DNA binding capacity (CasA2), respectively. These results emphasise the interaction and organisation of RNAs and protein complexes in the CRISPR/Cas system.

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GRV12
Membrane localization of the archaeal exosome

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Many macromolecular complexes and even RNA molecules previously thought to be distributed in the cytoplasm, were recently shown to have specific subcellular localization in bacteria [1, 2]. We show that the archaeal exosome, an RNA degrading and RNA-tailing protein complex [3], is localized at the membrane. Fractionation of cell-free extract of the hyperthermophilic and acidophilic archaeon *Sulfolobus solfataricus* through sucrose density gradients revealed that DnaG, an exosomal subunit of unknown function [4], and the vast majority of the active-site comprising subunit Rrp41 [5] sediment together with membranes, which contain surface layer (Sla) proteins. Membranes with Sla proteins and exosomes were enriched in a yellow colored ring in the bottom part of the gradient, implicating that the exosome of *S. solfataricus* is membrane-bound. This was confirmed by immunofluorescence detection of DnaG and Rrp41 at the periphery of the cells using thin layer cuts of *S. solfataricus*. Our findings underline the importance of the spatial organization of RNA processing and degradation in prokaryotic cells.

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GRV13
Structural insights into the pH-sensing mechanism of the membrane-integrated transcriptional activator CadC in *Escherichia coli*

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The Cad system is involved in the adaptation of *E. coli* to mild acidic stress and helps to maintain the cytosolic pH within the physiological range. The membrane-integrated transcriptional activator CadC initiates transcription of the *cadBA* operon at conditions of low external pH and concomitantly available elevated concentrations of external lysine. CadA converts lysine under consumption of a cytoplasmic proton into cadaverine and carbon dioxide. Cadaverine is subsequently excreted by the antiporter CadB.
We are interested in elucidating how the inner-membrane protein CadC is able to perceive and transduce these signals across the membrane and then to activate transcription of the *cadBA* operon. Earlier it was shown that the periplasmic domain of CadC is responsible for pH sensing, while lysine signaling is mediated by an interaction with the lysine permease LysP. To gain further insight into the pH-dependent activation, the crystal structure of the periplasmic domain of CadC ($CadC_{pd}$) was solved at 1.9 Å resolution. This is the first structure of a signal input domain of a ToxR-like membrane-integrated transcriptional activator. $CadC_{pd}$ consists of two subdomains: the N-terminal subdomain is a β-sheet in contact with α-helices whereas the C-terminal subdomain is a pure α-helical bundle.

A directed mutagenesis approach identified several amino acids to be involved in the detection of low external pH. Most of these amino acids form part of a negatively charged surface cluster in the C-terminal subdomain that contacts the N-terminal part. It is suggested that upon drop in external pH, protons are sensed via the negatively charged cluster, which in turn alters the relative position of the two subdomains to each other and transduces the signal to the transmembrane domain.

GRV14
Regulation of potassium transport by the "nitrogen PTS" in *Escherichia coli*

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The phosphotransferase system (PTS) plays a central role in sugar transport and carbon source-related signal transduction. The homologous nitrogen PTS is composed of protein EI^{Ntr}, the HPr homolog NPr and the final phosphate acceptor IIA^{Ntr}. This system works in parallel to the canonical PTS [1], but it is not involved in sugar transport. In contrast, recent work revealed that the nitrogen PTS controls potassium transport in *E. coli* [2, 3].
When potassium concentrations are limiting, K^+ is taken up by the high affinity K^+ transporter KdpFABC. Expression of the *kdp* genes is positively controlled by the corresponding two-component system (TCS) KdpD/KdpE in response to low K^+ concentrations [4]. We found that the unphosphorylated form of IIA^{Ntr} enhances expression of the *kdp* operon, resulting in higher KdpFABC amounts in the cell. This is achieved by stimulation of kinase activity of the sensor kinase KdpD by direct interaction with IIA^{Ntr} (3). Hence, IIA^{Ntr} modulates activity of the KdpD/KdpE-TCS and thereby *kdpFABC* expression. First evidence suggests that this regulatory device coordinates K^+ transport with carbohydrate utilization.

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[3] Lüttmann *et al.*, 2009
[4] Heermann *et al.*, 2003

GRV15
Structural and mechanistic insights into *Helicobacter pylori* NikR activation

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Since metals are concurrently essential cofactors and dangerous free-radical initiators, transition metal homeostasis is tightly regulated. This is mostly performed by regulators that react to changes in the intracellular metal concentration. NikR is such a transcription factor that plays a central role in the mandatory response to acidity of *Helicobacter pylori*. This metalloregulator is activated under nickel surcharge by Ni(II) binding to high-affinity (HA) and possibly external (X) sites; binds therefore specifically to different promoters and controls the expression level of genes involved in diverse pathways such as metal import and metabolism, stress response and respiratory process. Although the mechanism of target discrimination is still debated, it is widely accepted that the activation upon Ni(II) binding is mandatory for all targeted genes. The present work provides some insights into the activation process of NikR. The biochemical characterisation of HpNikR mutants shows that the HA sites are essential but not sufficient for DNA binding while the X sites interfere with the protein-DNA affinity. We show that a second metal is, in some cases, necessary for HpNikR DNA binding. Small angle X-ray scattering shows that apo-NikR adopts a cis-like conformation which is compatible with DNA binding, and which is not significantly affected by nickel addition. This study unravels key structural features from which we derive a model for HpNikR activation. This model involves two mechanisms where (i) HA sites and a hydrogen bond network are required for the DNA binding and (ii) a unique X site modulates HpNikR DNA binding to low-affinity promoters, possibly by incorporating a different metal ion.

GRV16
Direct stimulus perception and transcription activation by a membrane-bound DNA binding protein

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Signal transduction across the bacterial cell envelope is most commonly achieved through two-component regulatory systems, of which one protein is a membrane-bound sensor, while the second protein generally is a transcriptional regulator. BcrR of *Enterococcus faecalis* has been identified as a novel regulatory protein required for high-level bacitracin resistance and consists of a DNA binding domain and four transmembrane helices. It was therefore proposed to act as a one-component signal transduction system, combining the detection of an extracellular stimulus (bacitracin) with an intracellular response (induction of gene expression) within a single protein. To date, few membrane proteins with a role in transcriptional regulation have been studied, and all of these require auxiliary proteins for stimulus perception. Here, we provide *in vivo* and *in vitro* evidence that BcrR alone is sufficient to both detect bacitracin and induce transcription from the promoter of the bacitracin resistance operon, *bcrABD*, in response to bacitracin. BcrR therefore constitutes a unique bona fide one-component transmembrane signal transduction system, which functions independently of auxiliary proteins.

GRV17
Regulatory mechanisms in response to thiol-reactive electrophiles in *Bacillus subtilis*

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Electrophiles include quinones and carbonyl compounds which are redox-active compounds with electron-deficient centres that target cysteine residues via thiol-(S)-alkylation. Using proteomic, metabolomic and thiol-redox proteomic approaches we showed that quinones and carbonyls deplete the cellular thiol-redox buffer cysteine and thiol-containing proteins due to the thiol-(S)-alkylation chemistry in *B. subtilis*. Moreover, quinones lead to aggregation of thiol-containing proteins *in vivo* as main mechanism of quinone toxicity.
Genome-wide expression profiling revealed the common induction of the Spx, CtsR, CymR, PerR, ArsR, CzrA, CsoR and SigmaD regulons by quinones, carbonyls and diamide in *B. subtilis*. Besides this general electrophile-stress response, *B. subtilis* expresses also unique genetic responses to quinones, diamide and carbonyl electrophiles. Transcription factor/transformations arrays identified several novel MarR-type repressors that are involved in sensing electrophiles. The specific response to quinones and diamide is mediated by the MarR-type repressors YodB, YvaP and MhqR. These control the expression of quinone or azocompound reductases and thiol-dependent dioxygenases that function in detoxification of these electrophiles. YodB and YvaP are MarR/DUF24-family repressors that are conserved among Gram-positive bacteria. The conserved N-terminal Cys residue is essential for redox regulation of YodB. YodB resembles a two-Cys-type redox sensing repressor that is inactivated by Cys6-Cys101 intersubunit disulfides by diamide and via thiol-(S)-alkylation by quinones.
In conclusion, genome-wide approaches have revealed novel thiol-based redox sensing regulators for electrophilic quinones and diamide in *B. subtilis* that control specific detoxification pathways which differ from the oxidative stress response.

GRV18

The anaerobic formation of acetoin regulated by the transcriptional regulator AlsR – part of pH homeostasis in *Bacillus subtilis*

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Bacillus subtilis is able to grow in the absence of oxygen using nitrate ammonification or fermentative processes. Hereby, lactate, acetate, acetoin and 2,3-butanediol were identified as major fermentation products. Anaerobic formation of acetoin envolves acetolactate synthase and -decarboxylase encoded by the alsSD operon. The two-component regulatory system ResDE, the redox regulator Fnr and the transcriptional modulator ArfM are the currently known parts of the regulatory system for anaerobic adaption. Surprisingly, the anaerobic expression of *alsSD* was found independent on *fnr* and *resDE*.
In addition to the anaerobic induction of the *alsSD* operon, expression was also found dependent of the presence of acetate in the growth medium, reduction of external pH and stationary growth phase. Common for all these different growth conditions is the accumulation of acetate in the cytoplasm which may result in a decrease of cytoplasmic pH. Therefore, formation of acetoin and 2,3-butanediol is induced to limit the amount of acetate.
The *alsR* gene, encoding a member of the LysR family of transcriptional regulators, is needed for *alsS-lacZ* expression under all growth conditions tested. Using promoter deletion constructs fused to the *lacZ* reporter gene we could narrow down the binding region of AlsR to 36 bp located a position -86/-50 with respect to the transcriptional start site of *alsS*. We postulate a palindromic sequence TAAT-N11-ATTA located at position -79 to -64 as AlsR recognition sequence. Further deletion of the *alsS* promoter sequences to position -72 leaded to a complete loss of ß-galactosidase activity. To verify the role of the postulated AlsR binding site we analysed the expression of mutated promoter fragments. This binding site characterization was confirmed by *in vitro* binding studies using heterologously produced and purified AlsR.

GRV19

Bacterial Cruise Control

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Many bacteria swim by means of rotating flagella that are powered by proton influx through membrane-spanning motor proteins. Much is known about how bacteria control direction of their net migration and perform chemotaxis. Here we show that Escherichia coli bacteria can adjust their swimming velocity. We unravel the molecular mechanism underlying this behaviour, by demonstrating that flagellar motor output is controlled by a signaling network that adjusts the intracellular levels of the nucleotide cyclic dimeric-GMP. This bacterial second messenger activates YcgR, a protein which curbs motor output in a brake-like fashion by interacting directly with the motor protein MotA. Brake engagement involves electrostatic interactions between stator and rotor and individual stators are limited independently from each other. Second messenger signaling mediated deceleration coincides with nutrient depletion in stationary phase and might be represent an energy saving strategy.

GRV20

Modulation of the c-di-GMP turnover by two new integral sensor proteins in the opportunistic pathogen *Pseudomonas aeruginosa*

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In order to cope with different environmental conditions bacteria have evolved a variety of different regulatory systems, which sense environmental stimuli and convert them into internal signals. A key player in such a regulatory system is the second messenger c-di-GMP, which is involved in the regulation of different cellular functions like motility or biofilm formation. Two distinct proteins are involved in the turnover of c-di-GMP: diguanylate cyclases with a characteristic GGDEF domain build up c-di-GMP from two molecules of GTP, while EAL domains act as phosphodiesterases and break down c-di-GMP into two molecules of pGpG. Like other bacteria, *Pseudomonas aeruginosa* has an abundance of proteins with a GGDEF and/or an EAL domain. These domains are often combined with signal input domains reflecting the ability to react to different environmental conditions. One novel sensor domain which is connected with GGDEF and EAL domains is the so called MHYT domain.
Here we present a combination of biochemical and genetic experiments to gain insights in the function of two novel membrane sensor proteins in *P. aeruginosa*. The gene products of PA1727 and PA3311 consist of an N-terminal membrane sensor domain MHYT and a C-terminal cytoplasmic GGDEF- and EAL-domain. Different protein variants were constructed and tested for their catalytic activity *in vitro*. PA1727 harbours diguanylate cyclase as well as phosphodiesterase activity, while PA3311 seems to be an active phosphodiesterase whose enzymatic activity is stimulated by binding of GTP to the imperfect GGDEF motif. A deletion of PA3311 results in an increased production of exopolysaccharides and a concurrent restricted swarming motility. Our data suggest that both proteins have opposing functions. Further experiments to test this hypothesis will be presented.

GRV21

Overlapping alternative sigma factor regulons in the response to singlet oxygen in *Rhodobacter sphaeroides*

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Organisms performing photosynthesis in the presence of oxygen have to cope with the formation of highly reactive singlet oxygen and need to mount an adaptive response to this photooxidative stress. Recently we demonstrated that the alternative sigma factor $RpoH_{II}$ is triggered by singlet oxygen in an RpoE dependent manner, thereby a sigma factor cascade is formed (Nuss *et al*., 2009). Here we show that the alternative sigma factors $RpoH_I$ and $RpoH_{II}$ are both involved in the singlet oxygen as well as in the heat stress response in *Rhodobacter sphaeroides*. We propose $RpoH_{II}$ to be the major player in the singlet oxygen response, whereas $RpoH_I$ is more important for the heat stress response. Mapping of the 5´ ends of $RpoH_{II}$- and also $RpoH_I$/$RpoH_{II}$-dependent transcripts revealed clear differences in the –10 region of the putative promoter sequences. By using bioinformatic tools we extend the $RpoH_{II}$ regulon, which includes genes specifically induced by singlet oxygen. These genes encode proteins which are e.g. involved in methionine sulfoxide reduction as well as in maintaining the quinone pool. Furthermore we identified small RNAs which depend on $RpoH_I$ and $RpoH_{II}$ and are likely to contribute to the defense against photooxidative stress and heat stress.

[1] Nuss, A.M., J. Glaeser and G. Klug. 2009. RpoH(II) activates oxidative-stress defense systems and is controlled by RpoE in the singlet oxygen-dependent response in *Rhodobacter sphaeroides*. J. Bacteriol. 191(1):220-30.

GRV22

Characterization of the novel group ECF41 σ factors

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Adaptation to changing environmental conditions often necessitates transmembrane signal transduction to orchestrate differential gene expression. One major principle of bacterial signal transduction are ECF (extracytoplasmic function) σ factors. ECF σ factors recognize alternative promoter sequences, are usually autoregulated and co-transcribed with a transmembrane protein functioning as the anti-σ factor. A comprehensive comparative genomics analysis based on sequence similarities and genomic context conservation identified more than 40 conserved groups of ECF σ factors, including novel groups with unique features [1]. EcfI, one of nine ECF σ factors present in the genome of *Bacillus licheniformis* [2], belongs to one of these newly identified groups, termed ECF41. This group contains proteins from five different phyla and is characterized by a large and conserved C-terminal extension of the ECF, which plays an important regulatory role. Moreover, genes encoding ECF41 σ factors show a conserved genomic context: a gene encoding a CMD (carboxymuconolactone decarboxylase) or an oxidoreductase is located directly up or downstream of the σ factor, whereas no putative anti-σ factor could be detected. A highly conserved sequence motif similar to typical ECF promoters was found upstream of both the genes encoding CMD proteins and oxidoreductases. This promoter was confirmed by 5' RACE in *B. licheniformis* (low G+C Gram-positive) and *Rhodobacter sphaeroides* (α-proteobacteria). The CMD-encoding gene *ydfG* is EcfI-dependently expressed during stationary phase in *B. licheniformis*. This dependence was verified both in a heterologous expression system in *B. subtilis* and for the homologous system in *R. sphaeroides*. Subsequent in-depth profiling of ECF41-dependent gene expression demonstrate that the widely distributed group of ECF41 proteins regulate only one single target gene.

[1] Staroń *et al.* (2009), *Mol. Microbiol.* 74:557-581
[2] Wecke *et al.* (2006), k>J. Bacteriol.</i> 188:7500-7511

GRV23
A flavonoid-dependent regulatory pathway in *Bradyrhizobium japonicum*

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An initializing step in the rhizobia-legume symbiosis is the secretion of flavonoids by plants, which leads to the expression of nodulation genes in rhizobia. A genome-wide analysis of the transcriptional response of *Bradyrhizobium japonicum* to genistein, an isoflavone secreted by soybean, indicated the induction of several genes that are likely to be involved in transport processes [1]. *bll4319*, *bll4320* and *bll4321* show high similarity to genes encoding RND (Resistance Nodulation and Cell Division)-type multidrug efflux systems. According to data base analysis, Bll4319 belongs to the intermembrane multidrug efflux proteins of the RND permease superfamily, Bll4320 is a membrane fusion protein and Bll4321 is likely to form a channel in the outer membrane. The gene *blr4322*, coding for a TetR-type regulator, is located alongside. By using a translational *bll4319-lacZ* fusion, we could demonstrate the induction of *bll4319* by genistein and daidzein. Based on the known mechanism of transcriptional regulation by TetR regulators, we propose that Blr4322 acts as a transcriptional repressor, which binds to an operator site next to the promoter of regulated genes and that this interaction is prevented by the binding of genistein to the protein. In order to define the operator site located in the intergenic region between *bll4321* and *blr4322*, we performed mobility shift assays with purified Blr4322. By nucleotide exchange, the operator site was characterised in more detail.

[1] Lang, K. Lindemann, A., Hauser, F., and Göttfert, M. 2008. The genistein stimulon of *Bradyrhizobium japonicum*. Mol. Genet. Genomics 279:203-211.

GRV24
Evolution and function of circadian clock proteins: lessons from bacteria

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Circadian rhythms are characteristic for almost all light-perceiving organisms. In case of photosynthetic cyanobacteria, the circadian timing machine is assumed to be a post-translational oscillator made of 3 proteins only. Together with ATP these 3 Kai (Japanese for cycle) proteins produce 24-hour rhythms of phosphorylation and dephosphorylation even in a test tube. KaiC-hexamers are the central protein component of the Kai-clock combining three intrinsic enzymatic activities - autokinase, autophosphatase and ATPase - which are reversely modulated by KaiA and KaiB.
Recent results suggest that an alternative pacemaker exists in cyanobacterial cells, because KaiC hyperphosphorylation, although disrupting the Kai-clock, did not abolish the gene expression rhythms observed *in vivo*. Once again a transcriptional / translational feedback loop (TTFL) is assumed, which might provide additional robustness to the oscillatory system. TTFL is a well-established model explaining the molecular core mechanism of the eukaryotic clock. Although no homologs to the cyanobacterial Kai proteins are found in the eukaryotic kingdom, comparing circadian clocks of organisms as diverse as bacteria and humans might tell us more general features of biological timing mechanisms.

HEP01
A Novel Electrochemical Method to Determine the Viability of Pathogenic Yeasts (*Candida albicans*)

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Candida albicans is an opportunistic fungal pathogen which shows a significant similarity to the non-pathogenic yeast *S. cerevisiae*. Thus, diagnostic methods, which allow the rapid and specific detection of the viable pathogen are urgently needed. In the past direct or mediated electron transfer reactions from intact microorganisms to electrodes were already applied to the detection of bacteria or to the determination of bacterial substrates, and are nowadays investigated with respect to the development of biofuel cells. 2,6-dichlorophenolindophenol (DCIP) was introduced as an electron mediator, which leads to the oxidation of NADH. This reaction was shown to be catalysed by complex I of the respiratory chain. There is no homologue to this enzyme in *S. cerevisiae* but in *C. albicans*, so that we wanted to prove whether we can use this principle to distinguish between both yeasts.
We investigated the relationship between the oxidation current of DCIP and the viable cell density, and the results suggest that the anodic current can be employed as an indicator for the cell viability. We also confirmed that this method is able to distinguish between *C.albicans* and *S.cerevisiae*, because with *S. cerevisiae* only the non-enzymatic turnover between NADH and DCIP was observed. A supplement to this mediated reaction is the direct electron transfer from yeasts to electrodes, and here we could show that this reaction is dependent on complex IV of the respiratory chain. This was proven by the application of antifungal inhibitors of the electron transport chain. Therefore, bio-electrochemical method cannot only be used as diagnostic tools but can be considered as a new analytical tool to study intracellular electron transfer mechanisms of viable *C. albicans*.

HEP02
Follow the light: Live cell imaging of endocytosis and intracellular transport of a viral A/B toxin

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K28 toxin, secreted by virus-infected killer strains of the yeast *Saccharomyces cerevisiae*, is a α/β heterodimeric protein of the A/B toxin family. After initial toxin binding to the surface of sensitive target cells, K28 is taken up by receptor-mediated endocytosis and subsequently delivered to an early endosomal compartment from where it is transported backwards through the Golgi and the endoplasmic reticulum (ER) to the cytosol. Within the cytosol, the toxin´s β-subunit is polyubiquitinated and targeted for proteasomal degradation, while α enters the nucleus and causes a G1/S cell cycle arrest and cell death.
Both, toxin uptake and intracellular transport crucially depend on the cellular HDEL receptor Erd2p which ensures that the toxin is targeted from the plasma membrane to the secretory pathway of intoxicated cells. Thus K28 represents a powerful tool and substrate for general studies of endocytosis and endosomal trafficking in eukaryotic cells. To elucidate the trafficking route of the toxin, biologically active K28/mCherry fusion proteins were designed and expressed in *Pichia pastoris* and will be used to track the toxin´s *in vivo* transit through the endocytic pathway. Purified K28/mCherry chimeras will also be used for toxin uptake and transport studies in wild-type and yeast mutants specifically blocked in cytoskeleton components or in distinct steps of vesicle transport and targeting.
Furthermore, we were able to show (for the first time in yeast) that AP2 complex components are involved in K28 endocytosis, indicating that AP2 represents a cargo-selective component of the endocytic machinery in yeast.

HEP03
Mode of action of a cell cycle arresting yeast killer toxin

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K28 is a heterodimeric A/B toxin secreted by virally infected killer strains of the yeast *Saccharomyces cerevisiae*. After binding to the cell wall of sensitive yeasts the α/β toxin enters cells via receptor-mediated endocytosis and is retrogradely transported to the cytosol where it dissociates into its subunit components. While β is polyubiquitinated and proteasomaly degraded, the α-subunit enters the nucleus and causes an irreversible cell cycle arrest at the transition from G1 to S phase. K28-treated cells typically arrest with a medium-sized bud, a single nucleus in the mother cell and show a pre-replicative DNA content (1n) - a "terminal phenotype" which differs from that of other cell cycle arresting killer toxins like zymocin from *Kluyveromyces lactis* or *Pichia acaciae* toxin PaT. Since the precise mode of action of K28α is still unknown and to gain deeper insights into the mechanism(s) of how K28α arrests the cell cycle, we studied the influence of the toxin on transcription of cell cycle and G1-specific genes. Interestingly, G1-specific *CLN1* and *CLN2* mRNA levels rapidly decrease after toxin treatment, though it is unclear if this decline is due to a direct effect. Potential toxin targets were verified *in vivo* by bimolecular fluorescence complementation analysis using a "split-mCherry" reporter system. To confirm that the nucleus represents the compartment where *in vivo* toxicity occurs we constructed protein fusions between K28α and mRFP and analysed their intracellular localisation.

HEP04
Chimeric A/B toxins: a tool to study endocytosis and *in vivo* toxicity of ricin A in yeast

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The plant protein ricin, one of the most powerful poisons of biological heritage, is a heterodimeric glycoprotein belonging to the class of microbial A/B toxins. It consists of a B-chain (RTB) which mediates toxin binding to the cell surface and subsequent toxin uptake by mammalian target cells. The cytotoxic A chain (RTA) - linked to RTB via a single disulfide bond - catalyzes the N-glycosidic cleavage of a specific adenine residue in the sarcin/ricin loop of 28S ribosomal RNA. After endocytotic uptake and retrograde transport, ricin enters the endoplasmatic reticulum from where it translocates into the cytosol most likely by passing through the Sec61p translocon. While RTB is subsequently polyubiquinated and proteasomaly degraded, RTA largely escapes degradation and rather causes cell death by rapidly inhibiting protein synthesis. Since yeast in contrast to mammalian cells is not killed by the external application of ricin, we fused biologically active RTA to the β-subunit of the yeast K28 viral toxin in order to create chimeric RTA/K28β protein fusions. To improve toxin folding, a GGGGS(2) linker was inserted in-between the two toxin subunits and all internal lysine residues in K28β had been replaced by arginine to prevent ubiquitination and proteasomal degradation. After *in vivo* expression and secretion of the toxin chimeras in *S. cerevisiae* and/or *P. pastoris*, the recombinant toxins will now be used to study the processes of receptor-mediated endocytosis and intracellular toxin transport in yeast as model organism.

HEP05
Positive and negative regulation of CWI signaling in *S. cerevisiae*

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The cell wall of *Saccharomyces cerevisiae* is essential to ensure cell shape and integrity. A set of plasma membrane spanning sensors detect cell surface stress and activate a conserved MAPK (mitogen activated protein kinase) cascade. In general, the CWI (cell wall integrity) signaling pathway ultimately governs appropriate gene transcription and cell cycle regulation.
Two small protein families constitute the sensors, comprising Wsc1, Wsc2 and Wsc3 on one hand, and Mid2 and Mtl1 on the other. Wsc1 and Mid2 are believed to be the most important members.
While Mid2 resides fairly statically and evenly distributed within the plasma membrane of *S. cerevisiae*, Wsc1 accumulates at sites of cell wall synthesis and is dynamically distributed between the cytoplasm, the plasma membrane and the vacuole. We show that Wsc2 distributes in a very similar manner as Wsc1. These dynamics depend on differing endocytosis pathways: Whereas Wsc1 takes the clathrin-dependent route *via* its NPFDD motif, Wsc2 uses a ubiquitin-dependent pathway. We present data on the half-lifes of the two sensors in different endocytosis mutants.
In an independent genetic screen we identified an as yet uncharacterized open reading frame of *S. cerevisiae* as a negative regulator of CWI signaling. Preliminary results indicate that the encoded protein may serve a function in intracellular vesicular transport. This may indicate an important function of such transport mechanisms from and to the plasma membrane in CWI signaling.

HEP06
Protein production with *Pichia pastoris*: Is metabolism limiting?

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Pichia pastoris has become one of the major microorganisms for protein production in the recent years. This development was mainly driven by the readily available genetic tools (e.g., expression plasmids with strong promoters like the methanol inducible *AOXI*) and the ease of high cell-density cultivations using methanol (or methanol/glycerol mixtures) as inductor and carbon source. To overcome the observed limitations such as high heat development, cell lysis, and explosion hazard, we here revisited the possibility to produce proteins with *P. pastoris* using glucose as sole carbon source. Metabolism of *P. pastoris* is respiro-fermentative when grown in aerobic conditions in the presence of excess glucose, with the consequence that about 10% of the carbon taken up is converted to ethanol and acetate. Interestingly, a strain producing a recombinant protein had significantly reduced by-product formation with the consequence of increased biomass yield. As the rate of growth was lower in this strain, we do not know if the rate of growth and/or glucose uptake rate and not protein production had an influence on cell physiology as reported before for *Saccharomyces cerevisiae*. Consequently, we grew *P. pastoris* under glucose limiting conditions during high cell-density fed-batch fermentations at a constant rate of growth to quantify the impact of heterolog protein synthesis on central carbon metabolism. Specifically, we used 13C-tracer metabolic flux analysis to quantify the metabolic impact of protein synthesis. Under these experimental conditions *P. pastoris* redistributed resources from biomass synthesis toward energy generation, suggesting an increased energy demand during heterolog protein synthesis. The results are discussed in the context of protein production with *P. pastoris* using glucose as sole carbon and energy source.

HEV01
Purification and topology determination of the heterologously expressed membrane subunit ND5 of *Yarrowia lipolytica* complex I

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NADH:quinone oxidoreductase, or complex I, represents the largest of four membrane-bound enzyme complexes in the mitochondrial respiratory chain and catalyses the transfer of electrons from NADH to ubiquinone. It is generally accepted that complex I translocates H^+ and thereby participates in the generation of a proton membrane potential that is subsequently used as a driving force for ATP synthesis. The complete reaction mechanism of complex I is still unknown, however it is assumed that the membrane embedded ND5 subunit is involved in cation transport. Its bacterial homolog, subunit NuoL, acts as cation transporter in the absence of other complex I subunits [1]. ND5 is homologous to Na^+ / H^+ antiporters of the *mrp* type [2]. Some complex I subunits, in particular ND5, were identified as mutational hotspots for severe neurodegenerative disorders, which points to their structural or functional importance in complex I.
We predicted a 16 transmembrane helix model of the ND5 subunit of complex I and used native membrane vesicles derived from the ER of *Saccharomyces cerevisiae* containing GFP-ND5 fusion protein to determine the topology and orientation of the protein by fluorescence protease protection assay. We report the solubilisation of the ND5 protein from the yeast *Yarrowia lipolytica* produced in *Saccharomyces cerevisiae*. This paves the way for purification of the ND5 fusion protein and its reconstitution into liposomes for functional tests.

[1] Gemperli AC, Schaffitzel C, Jakob C & Steuber J (2007) Transport of Na^+ and K^+ by an antiporter-related subunit from the *Escherichia coli* NADH dehydrogenase I produced in *Saccharomyces cerevisiae*. Arch Microbiol 188: 509-521.
[2] Mathiesen C & Hägerhäll C (2003) The ´antiporter module´ of respiratory chain complex I includes the MrpC/NuoK subunit -- a revision of the modular evolution scheme. FEBS Lett 549: 7-13.

HEV02
Investigations of cytokinesis regulators in *Saccharomyces cerevisiae* and *Kluyveromyces lactis*

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The essential gene *INN1* (*YNL152w*) encodes a regulator of cytokinesis in *Saccharomyces cerevisiae* and is required for plasma membrane ingression during actomyosin ring constriction. We identified Cyk3 as a novel interaction partner of Inn1 in addition to Hof1. Interactions are mediated by the proline-rich carboxy-terminal part of Inn1 and the SH3 domains of Cyk3 and Hof1, respectively. By live-cell fluorescence microscopy, we observed that Inn1-GFP fusions localize to the bud neck in mitotic anaphase in a specific temporal order relative to the other two regulators of cytokinesis. Genetic analyses and depletion studies suggest that Cyk3 and Hof1 act consecutively to recruit Inn1 to the contractile ring. In its N-terminal half, Inn1 also carries a C2 domain which presumably mediates the contact with the plasma membrane at the bud neck.

In a comparative investigation of the protein domain functions, we characterized the *INN1*, *HOF1* and *CYK3* homologs from the milk yeast *Kluyveromyces lactis*. The deduced amino acid sequences share 33% - 42% identity between milk- and Baker´s yeast for the three proteins. *KlINN1* cannot rescue the lethal phenotype of an *Scinn1* deletion, when heterologously expressed from a multicopy vector under the control of either its own promoter or that of the strong *PFK2* promoter. Thus, species-specific functions must be exercised by the non-conserved protein regions. In contrast, expression of *KlHOF1* and/or *KlCYK3* restores viability to the synthetically lethal *Schof1 Sccyk3* double deletion. Unlike in *S. cerevisiae*, both single deletions are lethal to haploid *K. lactis* strains.
For further investigation of the domains functions, yeast two-hybrid analyses with the key regulators of cytokinesis of both organisms as well as with hybrid-constructs will be presented.

HMP01
Virulence properties of *Legionella pneumophila* GDSL lipolytic enzymes

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Legionella pneumophila expresses a multitude of lipolytic enzymes falling into three groups that may be involved in pathogenesis. One of these groups, the GDSL hydrolases, comprises enzymes of prokaryotic and eukaryotic origin with phospholipase, acyltransferase, and hemolytic activity. Enzymatic here activity depends on a conserved nucleophilic serine embedded into the GDSL motif as well as aspartate and histidin together building up the catalytic triad. The *L. pneumophila* genome codes for three GDSL-hydrolase genes: *plaA*, *plaC* and *plaD*. The three enzymes show lysophospholipase A (LPLA) and phospholipase A activity with PlaA being the major secreted LPLA. PlaC additionally displays acyltransferase activity, post-transcriptionally regulated by *L. pneumophila* ProA, a secreted zinc metalloprotease. The sequences of PlaA and PlaC harbour N-terminal signal peptides for Lsp type II-dependent protein secretion, whereas the secretion mode of PlaD is still unclear. Since phospholipases are important virulence factors that have been shown to promote bacterial survival, spread and host cell modification or damage, we here aimed to investigate the contribution of GDSL enzymes to *L. pneumophila* virulence. Our studies of amoeba and macrophage infection showed that single and double *gdsl*-knock outs do not affect intracellular bacterial infection, pointing to overlapping functions of the three enzymes. Interestingly, the interleukin 8-release (IL-8) by lung epithelial cells infected with *gdsl* double mutants (*plaA-/plaC-* and *plaA-/plaD-*) and a *plaA* single mutant was reduced up to 60% in comparison to infection with the wild type. Secretion of IL8 can be induced via the NfκB-signaling pathway. Accordingly, we found less p65 translocation from cytoplasm to nucleus with the *gdsl* mutants compared to the wild type. These results indicate that GDSL-enzymes interfere with signalling cascades during infection and contribute to the pathogen-directed manipulation of the host cell.

HMP02
Small molecules from *Photorhabdus* and *Xenorhbdus*: More than just toxins

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Photorhabdus and *Xenorhabdus* live in symbiosis with *Heterorhabditis* and *Steinernema* nematodes, respectively. The bacteria-nematode complex is highly entomopathogenic and is used in organic farming to kill different insect pests. Moreover, the difference between symbiosis (towards the nematode) and pathogenesis (towards the insects) can be studies using these bacteria and we have started to look in detail into the role of bacterial secondary metabolites, which might play a role in both processes. In the last few years we could identify (i) new secondary metabolites, (ii) the corresponding biosynthesis gene clusters, and (iii) could also propose functions to some of these compounds within the complex life cycle. One example is the biosynthesis of isopropylstilbenes. Stilbenes are important virulence factors against insects as they inhibit the insect phenoloxidase, are required for nematode development, and act as antibiotics to kill food competitors. Other examples are cyclic and linear peptides, anthraquinones, simple amides, unusual lipids, as well as more complex secondary metabolites, which have been identified in *Photorhabdus* and/or *Xenorhabdus*. In my talk I will present the identification and biosynthesis of these and other compounds and would like to suggest functions for these compounds within the complex bacteria-nematode-insect relationship.

HMP03
Effect of aflatoxin B1 on killing capacity of bovine neutrophils against *Escherichia coli* and *Staphylococcus aureus*

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Neutrophils are key players in immune reactions and their migration into infected tissue is pivotal to confining a pathogen to a local site. They have enormous potentials to eventually kill engulfed microbes. Aflatoxin B1 (AFB1) is thought to be a potent immunotoxic. To investigate the effect of AFB1 on blood neutrophils bactericidal activity, the *in vitro* effect of the AFB1 on killing capacity of neutrophil against two worldwide superbugs in animals, *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*), was investigated. Healthy dairy cows (n=8) were used as a source of neutrophils. The isolated blood neutrophils were exposed with and without 0.5 ng/ml of AFB1 for 3 hours; their capacity to kill *E. coli* and *S. aureus* was then monitored by a bactericidal assay using sample cultivation, accordingly. Briefly, 100 µl live bacteria ($5*10^7$ / ml) were added to 500 µl viable neutrophils ($5*10^6$ / ml), or to 500 µl of normal saline containing no neutrophils, incubated for 1 hour, and finally serially diluted. After dilution and plating out, they were mixed with a sterile loop in duplicate onto Columbia sheep blood agar (Biokar Diagnostic, Beauvois, France). The plates were incubated overnight at 37°C and colony counts were performed. Results from the bactericidal assay are expressed and compared as the percentage of killed *E. coli* and/or *S. aureus* (% killing). The % killing of *E. coli* and *S. aureus* by AFB1-treated neutrophils was significantly lower than those of non-treated ones. The killing activity of neutrophils against *E. coli* in AFB1-treated and non-treated groups was 48.7 ± 3.6% and 69.5 ± 3.9 %, and against *S. aureus* was 19.3 ± 2.5 % and 33.4 ± 3.7 %, respectively.
The higher growth of *E. coli* and *S. aureus* in AFB1-treated neutrophils confirm the fact that the AFB1 is immunotoxic in dairy cows. Further studies are in progress in our laboratories to explain the mechanism of this immunotoxicity.
This work was supported in part by the Deutscher Akademischer Austauschdienst (DAAD) and the Stiftung Tierärztliche Hochschule Hannover, Germany as well as Ferdowsi University of Mashhad, Iran.

HMP04
Influence of microorganism and their products on hyaluronan metabolism and related signal transduction

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Through the secretion of interstitial extracellular matrix (ECM), fibroblasts regulate epithelial cell differentiation, are responsible for wound healing, control inflammation process and seem to play an important role in cancerogenesis. Bacteria and their products play an important role in prevention/promotion of chronic inflammatory diseases leading to cancer. In this regard, hyaluronan (HA) metabolism is linked to cellular signal transduction including Toll-like-receptor (TLR) interaction. We investigated how far microorganisms/microbial products influence the metabolism of HA and TLR activation.
For this purpose we stimulated human dermal fibroblasts with the staphylococcal toxins TSST-1 and enterotoxin B (SEB), lipopolysaccharide (LPS), peptidoglycan (PGN) as well as with culture supernatants of clinic *Staphylococcus aureus*, *S. epidermidis* and *Lactobacilus crispatus* isolates. The release of the chemokine IL-8 was detected by ELISA as well as by qualitative and real-time PCR (RT-PCR). In addition TLR activation (TLR 3-5, 8-9) was analyzed by RT-PCR, hyaluronan acid synthases (HAS 1-3) and hyaluronidases (HYAL 1-3) by qualitative PCR.
Fibroblasts respond to LPS, TSST-1, *S. epidermidis* and *L. crispatus* with the release of high amounts of IL-8 and corresponding mRNA levels. The highest mRNA expression was seen for HAS 2 and HYAL 2, moderate expression was seen for HAS 3 and HYAL 3, no signal was obtained for HAS 1 and HYAL 1. However, all microorganisms and the microbial products under study seem not to modulate HA metabolism. The most pronounced changes in TLR expression were seen for TLR 4, TLR 5 and TLR 9 by *S. epidermidis*, the toxins TSST-1 and SEB predominantly activate TLR 4. *L. crispatus* only activates TLR 3 and TLR 4.

Our data clearly show that fibroblasts interact with distinct microorganisms/microbial products to a different degree, e.g. through TLRs. However, a possible role of HA metabolism in host-microorganism interacting needs further studies.

HMP05
Interaction of *Staphylococcus aureus* with the human host. The effect of skin fatty acids on bacterial survival and virulence determinant production

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The ability of *Staphylococcus aureus* to survive as a commensal organism in the nose and on the skin requires a complex interaction with the human host.
We have previously determined that skin fatty acids are effective antistaphylococcal agents and have the ability to inhibit virulence determinant production.
Cis-6-hexadecenoic acid (C-6-H) is the most effective human skin fatty acid to prevent *S. aureus* infections. I have found *S. aureus* responds to sub-lethal
C-6-H treatment by the induction of a resistance mechanism. In order to define bacterial components important in the resistance to C-6-H and how the effect on virulence determinant expression is mediated, a transcriptomic and proteomic study of the response of *S. aureus* to C-6-H was carried out. This revealed multiple significant effects on gene expression and the production of proteins. Within 10 min C-6-H incubation the transcriptome data indicate that 319 genes are up regulated and 318 genes are down regulated more than 2-fold in the presence of C-6-H.
After 60 min C-6-H incubation, 94 genes are upregulated and 59 genes are downregulated. Within the regulated genes specific sets can be recognised which allude to the potential pathways of not only the killing effect of C-6-H, but also the regulation of virulence determinant expression. Interestingly, the genes required for capsular biosynthesis are upregulated by C-6-H and so this may be part of the mechanism to resist its deleterious effects. Several virulence determinants are downregulated by C-6-H, in particular hla, hld and spa. Six regulators are affected by C-6-H and using RT-PCR we have found that the two component regulator system SaeR/S is part of the signal transduction pathway which acts in response to C-6-H.

HMP06
Comparison of the genome sequences of two Bifidobacteria species, probiotic B. bifidum S17 and non-probiotic *B. longum* E18

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Various strains of bifidobacteria are known to exhibit health promoting effects on the host. One of the reported health benefits is the suppression of intestinal inflammation in patients suffering from inflammatory bowel diseases (IBD). Two intestinal isolates of bifidobacteria, *B. bifidum* S17 and *B. longum* biovar infantis E18 were compared in respect to their anti-inflammatory capacity *in vitro* and two models of murine colitis. *B. bifidum* S17 was shown to adhere strongly to intestinal epithelial cells (IECs) and had potent anti-inflammatory activity both *in vitro* and *in vivo*. On the other hand longum biovar infantis E18 strain adhered weakly to IECs and had negligible anti-inflammatory effects. In order to identify specific traits that contribute to the probiotic and anti-inflammatory properties of S17 strain, both genomes of S17 and E18 were sequenced. Here we present the draft genome analysis of both strains as well as a preliminary comparison of two genomes. Further analysis will be performed with particular emphasis on secreted and cell wall associated proteins, which might potentially be involved in the adhesion and/or interaction with the host.

HMP07
On the role of stress activated kinases in response to pore forming toxins

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Pore forming toxins represent a large, structurally diverse group of bacterial virulence factors. Susceptible nucleated target cells are not inevitably killed by membrane pore formation, but may survive, apparently depending on their ability to eliminate or neutralize membrane pores. Transient perforation by PFT is evidenced by reversible drop of intracellular potassium- and ATP-levels, and in the case of large pores, e.g. such formed by SLO, also by transient permeability for vital dyes. Various signaling pathways are activated by PFT, the p38-MAPK pathway being the first to emerge as a survival pathway in *C. elegans* and mammalian cells. Subsequent work suggested that critical roles of p38 in this context are to promote reconstitution of membrane integrity early after perforation, and to activate sub-routines of the unfolded protein response, also triggered by ER-stress. Here we report that PFT can activate JNK in epithelial cells, and that this SAPK assumes a vital role for perforated cells under conditions which sustain ER-stress response. The data reveal differential roles of SAPK and novel cross-talk between stress-responses.

HMP08
Role of pH-regulated antigen 1 in the interaction of *Candida albicans* with human neutrophils

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Candida albicans is an opportunistic human-pathogenic yeast and is a leading cause of severe and invasive fungal infections in immunocompromized individuals. The yeast-to-hypha morphological switch of the fungus is an important virulence factor and influences interactions with host phagocytes. The pH-regulated antigen 1 (Pra1p) of *C. albicans* is a cell wall-associated and also a secreted protein that is highly expressed in the hypha form. The aim of the present study was to analyze the role of Pra1p in the interaction of human neutrophil granulocytes with *C. albicans*.
Recombinant Pra1p bound to freshly isolated neutrophils in a dose-dependent and saturable manner, as determined by flow cytometry. Complement receptor type 3 (CD11b/CD18) was identified as the major Pra1p receptor on human neutrophils, using specific monoclonal antibodies in blocking experiments. In functional assays, neutrophil responses to wild-type and mutant *C. albicans* strains were compared. In transwell assays, a *C. albicans* mutant strain lacking Pra1p supported neutrophil migration to a lower extent than did the wild-type strain. Furthermore, the *C. albicans* Pra1p deletion mutant induced lower amounts of reactive oxygen species, determined by a fluorescence assay. Similarly, lower amounts of the antimicrobial protein lactoferrin and myeloperoxidase were released by neutrophils when stimulated with the mutant strain, measured by ELISA from supernatants of co-cultures. Neutrophils coincubated with the Pra1p-deficient strain of *C. albicans* secreted less interleukin 8.
In conclusion, Pra1p binds to neutrophils via CD11b/CD18 and *C. albicans* lacking Pra1p induces weaker neutrophil activation compared to the wild-type strain. These results suggest that Pra1p is one of the factors that mediate the stronger response of human neutrophils to *C. albicans* hyphae in contrast to the yeast cells.

HMP09

Visualizing phagolysosomal escape of *Staphylococcus aureus*

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Persistence of *Staphylococcus aureus* within human host cells has been implicated in acquisition of antibiotic resistance, establishment of chronic infections, and evasion of the host immune system. *S. aureus* is reported to escape from phagolysosomes of professional and non-professional phagocytes. Investigation of phagolysosomal escape of *S. aureus* is usually performed with electron microscopy or immunofluorescence analyses investigating absence of endosomal membranes around internalized staphylococci. Instead of such a "needle in the haystack" approach we developed an assay that selectively labels only *S. aureus* that escaped from the phagolysosome. The technique is adaptable to other bacterial species and thus might aid in assaying the contribution of phagosomal escape to a variety of pathogens and to identify virulence factors involved in relocation from the phagosome to the host cell's cytoplasm. Factors leading to *S. aureus* phagolysosomal escape are discussed.

HMP10

Interactions of wood rotting basidiomycetes and diazotrophs

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Nitrogen metabolism of wood rotting basidiomycetes is a challenging issue for biologists as well as for analysts. Although the majority of wood species are characterised by low total nitrogen content, growth of brown- and white-rotting fungi is feasible. Decades of research led to several theories of fungal nitrogen uptake, mycelial transport and association to microorganisms that fix atmospheric nitrogen (diazotrophs). To test the latter hypothesis we analyzed the interaction of own isolates as well as from already described bacteria with the wood rotters *Trametes versicolor* and *Oligoporus placenta*. Soil bacterial isolation was carried out on nitrogen limited as well as on nutrient media (Luria Bertani media 1:1 and 1:100) of buried wood samples that were previously sterilized and thereafter colonized by *T. versicolor* and *O. placenta*, respectively. Both fungi have shaped microbial community structure tremendously compared to the surrounding soil community based on cultivation dependent as well as on cultivation independent methods. In addition, we analysed the biomass of each fungi after *in vitro* incubation on different nitrogen sources. Using elementary analysis isotopic ratio mass spectroscopy (EA-IRMS) we quantified the nitrogen content and determined the isotopic ratio in the substrate as well as in the mycelium. The studies revealed that even low amounts of organic nitrogen are metabolized by both fungi. Nitrogen salts, such as ammonium and urea enhanced growth of *T. versicolor*, but not of *O. placenta*. Moreover, growth was enhanced when *T. versicolor* was co-cultured with *Azotobacter vinelandii* as well as with yet not described own isolates. Tracer experiments with isotopic labelled nitrogen ($^{15}N_2$) will elucidate if N_2 is converted to NH_4 by diazotrophic bacteria and in the following assimilated by *T. versicolor*.

HMP11

Characterization of *Escherichia coli* response to human defensins: secretion of low-weight metabolites

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Human defensins are antimicrobial peptides with important functions in innate immunity and immune regulation. They play a key role, yet not fully understood, in the pathophysiology of inflammatory bowel diseases (IBD; major types include Crohn's Disease (CD) and Ulcerative Colitis (UC)). Levels of human β-defensins, produced in the gut by epithelial cells, have been reported to be increased in UC patients and decreased in CD. This work targets the identification of metabolites secreted by the gut microflora in response to defensin activity in order to improve the understanding of host-bacteria interactions and their role in IBD pathophysiology. With this aim, E. coli cultures were treated with antimicrobial peptides (human β-defensin 2, human β-defensin 3, and human α-defensin 5), water or lysis buffer as controls. Cell growth was then monitored over the time and supernatants were collected and analyzed by LC-MS. The peaks present specifically after defensin treatment were investigated by tandem MS. Until now, five nucleoside-related compounds were detected, among them adenosine. The strongest responses are obtained after human β-defensin 2 (hBD-2) treatment. The characterized metabolites present different dynamics over time after the treatment pulse. The response to hBD-2 is dose-dependent, the dose curves also showing differences between the compounds. Inclusion of a positive control for cell lysis, and the absence of DNA degrading activity in the tested supernatants, indicate that the detection of compounds is not due to intracellular content leakage. The results obtained suggest that nucleosides may be produced by the bacteria in response to host defense. This is especially important considering that adenosine is a known immune modulator.

HMP12

Different *Staphylococcus epidermidis* biofilm types interfere with macrophage activation and phagocytosis

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Formation of adherent biofilms is the key mechanisms involved in *Staphylococcus epidermidis* virulence during device-associated infections. Aside from polysaccharide intercellular adhesin PIA, accumulation associated protein Aap and extracellular matrix binding protein Embp act as intercellular adhesins, mediating *S. epidermidis* biofilm accumulation. The aim of this study was to characterize structural features of PIA-, Aap-, and Embp-mediated *S. epidermidis* biofilms in more detail and to evaluate, by using well characterized *S. epidermidis* strain pairs, their specific contribution to biofilm-related *S. epidermidis* immune escape. PIA-dependent biofilms were characterized by densely packed bacteria, embedded in a PIA containing extracellular matrix. In sharp contrast, though being organized in high density cell aggregates, neither Aap- nor Embp-mediated biofilms featured a significant extracellular matrix. Despite this marked difference, PIA-, Aap-, and Embp-mediated biofilms protected *S. epidermidis* with similar efficiency against up-take by J774.A1 macrophages. The reduced phagocytic up-take was likewise associated with a significantly reduced NF-kappaB activation and Il-1beta production compared to macrophage responses to biofilm-negative *S. epidermidis* strains negative for the respective intercellular adhesins. Thus, interference of *S. epidermidis* biofilm formation is due to over-all reduced phagocyte activation. Moreover, contact with biofilm forming *S. epidermidis* also induced a reduction in macrophage's LPS responsiveness, suggesting biofilm-related induction of anti-inflammatory host cell events. Our results not only demonstrate that independent mechanisms of biofilm formation are similar effective in protecting *S. epidermidis* from phagocytic up-take, but point towards specific events during *S. epidermidis* – macrophage interactions which interfere with phagocyte activation. As these result in failure to take up and eradicate *S. epidermidis* they could contribute to persistence during device infections. Thus, a detailed elucidation of mechanisms involved could open new directions for development of therapeutic strategies to combat *S. epidermidis* infections.

HMP13
Epibacterial colonisation of marine macroalgae: From communities to compounds

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In the marine environment macroalgae are exposed to a strong bacterial fouling pressure. Bacterial colonisation changes the properties of algal surfaces and may have positive and negative effects on the host. To investigate the relationship between marine macroalgae and epiphytic bacteria we addressed whether the compositions of bacterial communities on algae were host-specific and persisted under varying biotic and abiotic environmental conditions throughout the year. Bacterial communities on *Fucus vesiculosus, Gracilaria vermiculophylla* and *Ulva intestinalis*, co-occurring macroalgae in the Baltic Sea, were repeatedly sampled in 2007 and 2008. In parallel we analysed the pool of potential bacterial foulers that algal surfaces were exposed to. While the bacterial composition on the surface of investigated algae was host-specific on a phylum level and seasonal changes were reproducible between different years, the composition of the biofilms varied between seasons. Strains found on the algal surfaces were never a random subsample of the colonizers pool, but rather the result of selective recruitment. For instance, Gammaprotebacteria dominated the pool of potential foulers, while they were almost completely absent on algal surfaces irrespective of sampling time. For the brown algae *F. vesiculosus* we were able to demonstrate that the surface coating by two different chemical repellent of compounds were responsible to deter the settlement of Gammaproteobacteria and Firmicutes.

HMP14
Simplified models for mechanistic studies on host-microbe interactions

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Knowledge on functions of the intestinal microbiota has considerably increased, but little is known on how the host affects intestinal bacteria. To study the impact of host factors on gut microbes, we used mice mono-associated with *Escherichia coli* MG1655 as a simplified model.
Two-dimensional difference gel electrophoresis followed by electro-spray ionization-tandem mass spectrometry was used to identify proteins differentially expressed in *E. coli* collected from cecal contents of mono-associated mice and in *E. coli* grown under anoxic conditions on a complex medium reflecting the substrate composition of the animal diet. We observed thirteen differentially expressed proteins (>3-fold, $p<0.05$). Eight proteins were up- and five were down-regulated in cecal cells. Proteins with the highest up-regulation were aspartate carbamoyltransferase (13-fold) and SAICAR synthase (7-fold). They play a role in the biosynthesis of purines and pyrimidines, whose availability in the cecum is limited, prompting *E. coli* to induce enzymes required for their synthesis. The N-acetylneuraminate lyase (NanA) was up-regulated threefold in the cecum, reflecting the utilization of host glycoproteins by *E. coli*. NanA was shown to be required for the successful establishment of *E. coli* in the gut. Our results indicate that intestinal substrate availability is a key factor for successful colonization.
We also established a model of a simplified human intestinal microbiota in rats. Eight bacterial species known to be dominant in the human gut mimic at large the metabolic activity of the human gut microbiota: all selected bacteria were able to establish in the gut of previously germfree rats. The consortium is transferred to the offspring and can be easily stored as a cryo-culture. The defined microbiota responded to dietary changes by changes in the relative proportions of the community members when inulin, pectin, resistant starch or a Western diet were fed.

HMP15
Caveolin recruitment prevents premature host cell entry of type IV pili producing *Neisseria gonorrhoeae*

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Type IV pili (Tfp) confer the initial attachment of many bacterial pathogens to human mucosal surfaces as well as the endothelial lining of the vascular system. Here we demonstrate that attachment of piliated Neisseria gonorrhoeae (P+GC) to human cells induces an immediate tyrosine phosphorylation–dependent recruitment of caveolin-1 (Cav1). Cav1 recruitment, in conjunction with F-actin effectively prevents bacterial host cell entry. Down-regulation of Cav1 resolves blockage of cell entry. An unbiased analysis of potential interaction partners for tyrosine-phosphorylated Cav1 hints towards an indirect involvement of the small GTPase RhoA in the uptake process. Thus, we describe a novel Tfp dependent mechanism that stabilizes the extracellular stage of typically extracellular bacterial pathogens preceding secondary infection processes, such as host cell entry via Opa proteins.

HMP16
Role of the β-hemolysin for survival of *Streptococcus agalactiae* in monocytic cells

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Streptococcus agalactiae is an important human pathogen and the β-hemolysin is a major virulence factor of this species. Controversial reports exist in regard to the role of the beta-hemolysin for survival in professional phagocytes. We investigated the role of the β-hemolysin for intracellular survival in the monocytic cell line THP-1 using a serotype Ia *S. agalactiae* wild type strain and an isogenic nonhemolytic deletion mutant of this strain. Both strains were fluorescently labeled with an EGFP expressing plasmid. Following infection of the THP-1 cells with a multiplicity of infection of 5, 10 and 25 for different time points ranging from 45 min to 3 hours, the intracellular *S. agalactiae* cells were evaluated by FACS analysis and plating of intracellular bacteria. Interestingly the nonhemolytic mutant displayed a significantly better ability to survive the intracellular environment than the hemolytic strain. To rule out, that the effect was due to an increased lysis of THP-1 cells by the hemolysin and did not represent a true difference between the two strains, LDH determinations were carried out and confirmed the better ability of the nonhemolytic strain to withstand the intracellular environment. Since the β-hemolysin has been described to induce IL-8, we tested the IL-8 release following infections of THP-1 cells with the two strains. However, we could not observe any significant difference in the ability of hemolytic or nonhemolytic strains to induce IL-8. To further investigate mediators of IL-8 release in THP-1 cell infected with *S. agalactiae*, IL-8 determinations were carried out after challenge of the eukaryotic cells by cell wall preparations from both strains. In these assays it could be confirmed that gram-positive cell wall preparations are potent inflammatory inducers. In conclusion the β-hemolysin of *S. agalactiae* seems to impair the ability of *S. agalactiae* to survive in THP-1 cells.

HMP17
Abuscular mycorrhizal fungi in semiarid soil (Baja California): effect of land use

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Arbuscular mycorrhizal fungi (AMF) colonize the roots of the majority of land plants and can improve plant growth. They are obligate biotrophs that can only be cultured in the presence of their host plant. Different AMF species usually occur in the same roots, but the limited variation in hyphal morphology in either plant or soil makes identification extremely difficult. The fundamental problems of identification and classification in AMF limit the study of natural communities. In this study semiarid soil samples, from cultivated and uncultivated areas (alfalfa and shrubland), were taken in the year 2008 from two different sites (site 4 and site 7). Total community DNA was extracted and AMF-specific 18S rRNA gene fragments were amplified with the primers AM1 and NS31 as described by Vallino *et al*. (2006) with some modifications. Amplicons of 550 bp size were cloned and approx. 100 clones per site and treatment were analyzed by RFLP analysis in order to explore changes in the relative abundance of AMF in response to the land use. The RFLP analysis and the sequencing revealed new RFLP types belonging to the genus Glomus and Diversiscosporacae. The four soils differed from each other in the AMF species composition and in the relative abundance of the dominant RFLP types. Specific groups of AMF were detected in each soil. The comparison between soil planted with alfalfa and shrubland soil, at both sites, showed that agriculture affected the AMF species composition. How the changes in the relative abundance of different AMF observed in the soils with different land use translate into changes of soil functions needs be shown.

[1] Vallino M., Massa N., Lumini E., Bianciotto V., Berta G. and Bonfante P. 2006. Assessment of arbuscular mycorrhizal fungal diversity in roots of *Solidago gigantea* growing in a polluted soil in Northern Italy. Environ Microbiol 8: 971–983.

HMP18
The *Legionella pneumophila* phagosome proteome

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Legionella pneumophila, the agent of Legionnaires´ disease replicates intracellularly within specialized phagosomes of human macrophages and amoebae. *Legionella* alter the maturation of their phagosomes and reside within a compartment that resists acidification and fusion with lysosomes. To define the molecular composition of this compartment, we developed a novel method for obtaining highly purified phagosomes from infected *D. discoideum* and analyzed the phagosomes by mass spectrometry-based proteomics. We analyzed 157 host identified proteins on *Legionella phagosomes* for physical and functional interaction using the STRING database. Network analyses of phagosomal proteins allowed the identification of protein clusters relevant for molecular functions associated with pathogenic phagosomes. Comparative proteomics of phagosomes containing highly virulent *L. pneumophila* Corby versus less virulent *L. hackeliae* revealed distinctive protein expression patterns, e.g., an abundance of RhoGDI and FttB in *L. hackeliae* degrading phagosomes versus little RhoGDI and FttB in *L. pneumophila* Corby replicative phagosomes. We present a kinetic dissection of phagosome maturation including the complex alterations of the phagosome protein composition. A reference flow chart suggests thus far unrecognized consequences of infection for host cell physiology, actin degradation on phagosomes and a putative interference of cysteine proteinase inhibitor with lysosomal enzyme sorting and activation processes.

HMP19
Denitrifiers Differ in the Alimentary Canals of the Earthworms *Octochaetus multiporus* (Megascolecidae) and *Lumbricus rubellus* (Lumbricidae)

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Complete denitrification to N_2 occurs in the anoxic alimentary canal of New Zealand´s native earthworm *O. multiporus* (Megascolecidae) and the introduced species *L. rubellus* (Lumbricidae). *L. rubellus* emits denitrification derived nitrous oxide (N_2O) whereas *O. multiporus* does not. The denitrifier communities in the earthworm alimentary canals of both earthworm species and their surrounding soil (pasture soil for *L. rubellus* and forest soil for *O. multiporus*) were assessed with the structural gene marker *nosZ* encoding nitrous oxide reductase. 255 *nosZ* amino acid sequences formed 16 distinct OTUs at a species-level dissimilarity threshold of 14 %. *nosZ* diversity was high in *O. multiporus* (11 OTUs) and surrounding forest soil (11 OTUs) and low in *L. rubellus* (5 OTUs) and surrounding pasture soil (2 OTUs). Alimentary canal derived *nosZ* sequences of *O. multiporus* were highly similar to those found in surrounding forest soil and approximately 50 % of the sequences occurred in OTU 2 in which the closest relatives were *Flavobacterium denitrificans*, *Rhodopseudomonas palustris*, and *Bradyrhizobium japonicum*. 95 % of the sequences from pasture soil also belonged to OTU 2. In *L. rubellus*, this OTU represented only 15 %, and the majority (80 %) was represented by another OTU which was exclusively detected in this sample and showed high similarity to *Pseudomonas fluorescens*. These findings demonstrate that denitrifiers in the alimentary canal of *O. multiporus* and *L. rubellus* are dissimilar and suggest that this dissimilarity might in part explain the contrasting denitrification activities of these two earthworms.

HMP20
Pertussis toxin-induced permeabilization of cerebral endothelial barriers potentially enhances the risk for bacterial meningitis

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The respiratory tract infection`whooping cough´caused by *Bordetella pertussis* is occasionally accompanied by severe complications such as encephalopathies leading to neurological disorders. These sequelae have been associated with the decisive virulence determinant pertussis toxin (PT), which of the cerebral barriers exclusively disrupts the integrity of the blood-brain-barrier (BBB). This might facilitate the occurence of secondary complications as well as the onset of additional bacterial infections.

To analyze whether the PT-induced transient disruption of barrier integrity of endothelial cells enhances the translocation of bacteria, we have used human brain microvascular endothelial cells (HBMEC) in an two-compartment tissue culture model.

Following treatment with PT we observed an increased paracellular traversal of *Escherichia coli* K1 (O1:K1), the main cause of neonatal meningitis, as well as of *Neisseria meningitidis* and *Streptococcus pneumoniae* strains across the HBMEC barrier. This is corroborated by transmission electron microscopy where *E. coli* K1 is solely found in-between endothelial cells.

Furthermore, we observed an enhanced migration of differentiated HL60 macrophage-like cells and of U937 monocytes through PT-treated HBMEC barriers. *E. coli* K1 is able to survive in HL60-like macrophages, in the mouse macrophage cell line J774, and in U937 monocytes. The survival of these bacteria in HL60 and U937 cells during the migration across the HBMEC barrier suggests an additional contribution of the „Trojan horse mechanism“ as a possible alternative route also for translocation *in vivo*.

These findings suggest that the PT-induced alterations of the BBB may present an additional risk factor and might further increase the burden for the central nervous system (CNS) by allowing paracellular penetration of microbes and macrophages into the brain. These processes might facilitate secondary infections of cerebral endothelial barriers such as bacterial meningitis.

HMP21
Recombinant *Clostridium acetobutylicum* expressing *Clostridium perfringens* enterotoxin (CPE) for treatment of pancreatic cancer

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Pancreatic cancer belongs to the most malignant solid tumours. This disease has a relatively poor prognosis, and the life expectancy of patients is very low. After diagnosis for most patients only a palliative treatment is possible. Genetically manipulated clostridia open a new possibility of anti-tumour treatment with enormous potential. Clostridial spores specifically colonise and germinate in the hypoxic regions of solid tumours and can deliver therapeutic proteins directly to their targets. CPE is produced by sporulating *C. perfringens* and was shown to interact with claudin receptors, which are 1000fold overexpressed in pancreatic carcinoma cell lines. The binding of CPE to this receptor results in the formation of pores that ultimately cause cell death. A modified *C. acetobutylicum* strain was able to produce and secrete the toxin into the surrounding medium. However, production proved to be too low for therapy in an *in vivo* mouse model. Thus, an improved expression system is required. For this purpose, a number of different clostridial signal peptides and promoter sequences were tested. The Tet-system was chosen as a potential expression system. In *E. coli*, the signal peptide of a putative pectate lyase of *C. acetobutylicum* allowed high-level expression.

HMP22
Adhesion and internalization of *Corynebacterium diphtheriae* by host cells

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Corynebacterium diphtheriae is the causative agent of diphtheria, a toxaemic localized infection of the respiratory tract. By vaccination diphtheria is well-controlled in e. g. Western Europe; however, this disease is still a cause of morbidity and mortality in less developed countries. While the production of diphtheria toxin has been well-established as a major virulence factor, little is known about *C. diphtheriae* factors crucial for colonization of the host and corresponding host receptors recognized by these factors, although colonization is an essential step of pathogenicity. Adhesion of different *C. diphtheriae* strains to epithelial cells and internalization of bacteria by these cells was characterized using a combination of gentamicin protection assays and immuno-fluorescence microscopy. Adhesion and invasion are not strictly coupled processes although in general the studied toxin-producer showed lower adhesion and invasion rates compared to the non-toxigenic strains investigated. Based on ultrastructure analyses by atomic force microscopy and RNA hybridization experiments, pili length and abundance is crucial for adhesion. Furthermore, strain-specific pili expression patterns were observed. None of the studied *C. diphtheriae* strains had a dramatic detrimental effect on host cells as indicated by measurements of transepithelial resistance of Detroit 562 cell monolayers and fluorescence microscopy. Using green fluorescent protein-labeled bacteria we could show that *C. diphtheriae* is able to persist within mouse macrophages. After 12 hours of phagocytosis even formation of microcolonies was detectable, while 16 hours after infection, the number of bacteria decreased.

HMP23
The role of the Cpx two-component system in the invasion of *Salmonela enterica serovar typhimurium* – In search of the missing link between CpxA and HilA

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The Cpx two-component system plays a crucial role in the envelope stress response, cell division, biofilm formation and pathogenesis of the food borne gram-negative pathogen *Salmonella enterica serovar typhimurium*. During invasion the precise sensing of activating external stimuli like pH shift or increasing osmolarity is essential for the pathogen to assign its location, to time the production of virulence factors, and to defend itself against the host cell response. These stimuli are sensed by the Cpx-system which consists of the membrane-anchored sensor kinase CpxA, the response regulator CpxR and the periplasmic inhibiting protein CpxP. Under invasion-inducing (SPI-1) or mildly acidic conditions the expression of the master regulator of invasive genes HilA is controlled by CpxA independently of its cognate transcriptional regulator CpxR (Nakayama et al., 2003). This implies an unknown regulatory interaction partner of CpxA or a cross-talk between the Cpx system and another two-component system regulating hilA expression.
By using "Strepprotein-interaction-experiment" (SPINE) we will elucidate the "interactome" of CpxA under SPI-1 growth conditions by *in vivo* cross-linking with formaldehyde. The method (Membrane-SPINE) has now been established and proven for the interaction between the membrane protein CpxA and its cognate cytosolic partner CpxR under Cpx-activating conditions in our lab. By creating a snapshot of the interactom of proteins under specific growth conditions it is feasible to show the physical interaction by this biochemical approach *in vivo*.
The aim of this study is to identify possible new regulatory interaction partners of CpxA which are responsible for the activation of hilA expression by CpxA in the absence of CpxR. After the identification we will attempt to demonstrate the functional interaction between reconstituted CpxA in proteoliposoms and the new interaction partner by using a phosphor kinase assay (Fleischer *et al.*, 2007) *in vitro*.

HMP24
Bacteria in leaf nodules of plants

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Bacteria are the only cells which synthesize vitamin B_{12}. Plants and other eukaryotic cells are apparently unable to produce this corrinoid, which is a cofactor of different enzymic reactions.
Some tropical plants comprise symbiotic bacteria within leaf nodules. The nodules of two of these plants, *Psychotria punctata* and *Ardisia crenata*, have been analyzed for their bacterial populations. The symbiotic bacteria were classified by their 16S rRNA gene sequences as *Burkholderia* species. Their genomes have been further investigated for genes encoding vitamin B_{12} biosyntheses and putative vitamin B_{12} transporters.

HMP25
The double life of *Candida albicans*

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Candida albicans is a commensal fungal organism in the microflora of the oral cavity, gastrointestinal tract and vagina. But *C. albicans* also is a pathogen leading to superficial or systemic infections. In the last years systemic candidiasis has become the fourth most frequent cause of nosocomial bloodstreams infections in the United States with mortality rates of up to 50%.
Important risc factors for a candida infection are intravascular catheters or a compromised immune system, as it is present in neutropenic patients and patients with an HIV infection or those under chemotherapeutic or broad spectrum antibiotic therapy. The molecular mechanisms, which allow the host to control the commensal state of the yeast or which lead to the switch from the commensal organism to the pathogen are still only partly understood.
We establish an infection model comprising a human epithelial cell line (A431) in direct contact to *Candida albicans* and neutrophils or macrophages (THP-1) as representatives of the immune system. The combination of these three cell-types allows the simulation of the commensal state, whereas removal of the immune cells induces the pathogenic state. Key components, which are already known to protect the epithelial cells from damage by *C. albicans*, are cytokines such as the tumor necrosis factor TNFα, which are produced by the immune cells and induce an enhanced expression of the toll like receptor 4 (TLR4) in the epithelial cells. This in turn stimulates the epithelial cells to produce protecting compounds themselves or induce chemical protection by the macrophages. Among those chemicals probably are antimicrobial peptides. Thus we comprehensively analyse the supernatant of the cell system to obtain dynamic and quantitative information about secreted components. To dissect the contributions of the single cell-types to the overall systems behaviour these data will be complemented by the responses of each of the cell-types.

HMP26
Pathogenic and symbiotic plant-microbe interaction require bacterial phosphatidylcholine

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Several distinctly related bacteria, including members of the *Rhizobiaceae* produce the typical eukaryotic membrane lipid phosphatidylcholine (PC). Many of these prokaryotes interact with eukaryotic hosts. The phytopathogenic *Agrobacterium tumefaciens* and the soybean symbiont *Bradyrhizobium japonicum* both posses PC as a major membrane lipid.
Bacterial PC biosynthesis occurs via the methylation pathway and the phosphatidylcholine synthase (Pcs) pathway. An agrobacterial mutant lacking both pathways does not produce any PC [1]. It furthermore shows a dramatic virulence defect which is due to a lack of the type IV secretion complex. A bradyrhizobial mutant in the methylation pathway shows a severely reduced PC content. This phenotype was accompanied by a severely reduced nodule occupancy and nitrogen fixation activity. Recent studies showed that the methylation pathway of *B. japonicum* requires two phospholipid *N*-methyltransferases (Pmt) with distinct substrate specificities [2].
Purified *A. tumefaciens* PmtA is a monomeric enzyme capable of catalyzing all three methylations of PE to form PC. *In vitro* activity of PmtA is inhibited by its endproducts PC and SAH. On the other hand, PmtA activity is stimulated by phosphatidylglycerol, one of the main phospholipids found in *A. tumefaciens* membranes. PmtA binds SAM only in the presence of one of the substrate lipids or the endproduct PC [3]. SAM-binding occurs at the N-terminal part of PmtA, as shown via site-directed mutagenesis and radioactive SAM-binding studies. These data provide first insights into mechanism and structure-function relationship of a bacterial phospholipid *N*-methyltransferase.

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HMP27
Proteomic and Functional Analysis of *Legionella pneumophila* Outer Membrane Vesicles

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Legionella pneumophila, the causative agent of an atypical pneumonia, mainly replicates within alveolar macrophages. In addition extracellular bacteria can spread within the lung tissue and into other organs like the spleen due to destruction of the lung epithelial barrier. Secreted effector molecules are critical for the extracellular pathogenicity of *Legionella pneumophila*. Specific secretion machineries, which are responsible for the subfraction of secreted proteins (soluble supernatant proteins, SSPs), and the production of bacterial outer membrane vesicles (OMVs) both contribute to the protein composition of the extracellular milieu. We performed a comprehensive proteome comparison of proteins secreted by different secretion systems (SSPs) and the OMV fraction of proteins of *L. pneumophila*. Protein identification revealed a total of 181 supernatant proteins, 33 of which were specific to OMVs and 107 of which were specific to the remaining SSP. A large proportion of the identified OMV proteins are involved in the pathogenesis of Legionnaires' disease. Additionally, the binding of immunofluorescently stained OMVs to alveolar epithelial cells suggested that there is delivery of a large and complex group of proteins and lipids into the infected tissue. Enzyme assays performed in our study revealed that the SSP and OMV fractions possess proteolytic and lipolytic enzyme activities. The observed proteolytic effects could be due to several identified proteins as shown by extracellular matrix degradation assays. Hence, SSP and OMV may promote the dissemination of *L. pneumophila* within the lung tissue by degrading extracellular targets of the lung epithelium barrier.

HMP28
Mutants of *Xanthomonas campestris* pv. *vesicatoria* devoid of aconitase B exhibit reduced pathogenicity on pepper leaves

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Aconitases are [4Fe-4S] proteins that have two different functions depending on the cellular iron level. If iron is sufficient the enzyme possesses a labile [4Fe-4S] cluster and is functional in the TCA-cycle. If iron becomes limiting aconitase loses its [4Fe-4S] cluster and adopts a post-transcriptional regulatory function as an iron regulatory protein (IRP). Aconitases are also important factors in controlling the superoxide stress response and consequently can have a role in pathogenesis. *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*) is an obligate aerobic γ-proteobacterium that causes bacterial spot disease on pepper and tomato plants. The genome of *Xcv* encodes three aconitases. Two of the aconitases belong to the AcnA family while the third is a member of the AcnB family. The *acnB* gene forms an operon with two small genes *Xcv1925* (*orfX*) and *Xcv1926* (*orfY*) whose products have no known function, although they share amino acid sequence similarity with nucleic acid-binding proteins. In an attempt to determine whether the AcnB enzyme of *Xcv* might be required for infection of host plants and to identify the role of *orfX* and *orfY* we created defined deletion mutants and characterized them with respect to growth and pathogenicity. Both an *acnB* mutant, as well as an *orfX-orfY-acnB* triple deletion mutant exhibited reduced growth *in planta*, a reduced ability to infect susceptible pepper plants and showed a decreased hypersensitive response (HR) in resistant pepper lines. This is possibly due to altered iron or oxidative stress levels to which the bacterium is exposed during infection of the host plants. Complementation of these mutations restored growth *in planta* and pathogenicity. Current studies are focussed on analysing the effects of iron depletion and oxidative stress on the interaction of the bacterium with pepper plants and the role that aconitase B might play in this process.

HMP29
Impact of hemin and iron on Sae activity in *Staphylococcus aureus*

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Staphylococcus aureus (SA) is a major human pathogen and a common cause of nosocomial infections. SA produces a large arsenal of virulence factors including the hemolysins. They allow the bacteria to lyse erythrocytes and thereby release large amounts of hemoglobin (approx. 5 mM per lysed erythrocyte). The released heme is thought to be the main iron source of this organism during the course of infection, and is considered to be crucial for bacterial proliferation *in vivo*. High concentrations of heme and its degradation products, on the other hand, are known to be toxic for the mircoorganism. Thus, the control of heme release from red blood cells, and its uptake by SA needs to be tightly regulated. Since expression of hemolysins is controlled mainly by the two-component regulatory system Sae, a major regulator of exoprotein production, we wondered whether and how presence of heme and iron affect the activity of this two-component system.
Here we present data showing that transcription of sae is increased under iron restricted conditions in SA strain LS1. The expression of *hla*, encoding for α-hemolysin, the major hemolysin of SA, was found to be increased under these conditions as well, supporting previous observations suggesting that *hla* and *sae* transcription is positively affected by Sae activity. Concentrations as low as 1 – 4 µM of hemin (the oxidized form of heme), on the other hand, were sufficient to decrease *sae* and *hla* transcription in a dose-dependent manner, irrespective of the amount of iron being present in the media. Expression of *eap* (encoding extracellular adherence protein), another gene that is under positive control of Sae, was also found to be induced under iron limiting conditions and repressed in presence of hemin. Our data suggest that SA uses the *sae* regulatory functions to deal autonomously with different iron concentrations and to fine-tune the hemoglobin release of erythrocytes.

HMP30

Substrate specificity switching during type III secretion in the plant pathogen *Xanthomonas campestris* pv. *vesicatoria*

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The gram-negative bacterial plant pathogen *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*) employs a type III secretion (T3S) system to translocate a large set of bacterial effector proteins directly into eukaryotic cells. Effector protein translocation depends on an extracellular pilus, which serves as a protein transport channel to the host-pathogen interface, and a predicted translocon in the plant plasma membrane. Essential for the efficient assembly of the T3S system is the cytoplasmic HpaC protein, which inhibits secretion of the pilus assembly protein HrpB2 and promotes secretion of translocon proteins, presumably after pilus formation. HpaC thus acts similarly to known T3S substrate specificity switch (T3S4) proteins from animal pathogenic bacteria that alter the substrate specificity of the T3S system from early to late substrates. The substrate specificity switch often depends on a conserved C-terminal domain of T3S4 proteins, termed T3S4 domain, which is also predicted in HpaC from *Xcv*. To identify functional domains in HpaC, we introduced serial deletions of 10 amino acids and analyzed the corresponding HpaC deletion variants by functional assays and protein-protein interaction studies. Our results reveal that the C-terminal T3S4 domain of HpaC is crucial for protein function. Furthermore, the T3S4 domain mediates the interaction of HpaC with the C-terminal cytoplasmic domain of HrcU, which is a conserved component of the T3S system at the inner membrane. HrcU is crucial for the HpaC-mediated substrate specificity switch and also provides a binding site for the pilus assembly protein HrpB2. Notably, no interaction was observed between HrcU and other T3S substrates including pilus, translocon and effector proteins. This is in contrast to HrcU homologs from animal pathogenic bacteria and suggests important differences in substrate recognition by T3S systems from plant and animal pathogenic bacteria.

HMP31

Systemic administration of high doses of *Escherichia coli* Nissle 1917 causes colonization of gall bladders in mice

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The use of live attenuated bacteria as vaccines constitutes a successful health measure in human and veterinary medical practice. In addition, bacteria-based medicines are currently developed for the treatment of tumors in which bacteria are systemically administered to the patient. In this situation non-pathogenic bacteria should be used that only target the tumor but otherwise are readily eliminated from all body cavities. However, during experiments in which we injected inducible bioluminescent *E. coli* Nissle 1917 (EcN) to tumor bearing Balb/c mice for therapeutic intervention we observed incidentally characteristic light emission from the abdominal region in some animals. Further experiments revealed that these prominent signals originate from the gall bladder of mice. Moreover, bioluminescence imaging and plating of tissue homogeneates indicated that bacteria are shedded from the colonized gall bladder into the gut lumen and finally are excreted via feces. By administration of escalating doses to animals we observed that high doses are required for gall bladder colonization. Despite high bacterial numbers inside the gall bladder mice did not show any signs of discomfort and colonization did not become chronic.

HMP32

Characterization of RND-type multidrug efflux pumps in the plant pathogen *Pseudomonas syringae*

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Plant-pathogenic bacteria are continually exposed to deleterious chemicals naturally occurring in their environment such as antimicrobial plant metabolites and toxins produced by other epiphytic microorganisms. Bacteria have developed various ways to resist the toxic effects of antimicrobial compounds. Extrusion of toxic agents from cells by multidrug efflux (MDE) is one of these mechanisms. This project aims to identify and characterize RND-type pumps of the plant pathogen *Pseudomonas syringae* and to gain knowledge about their natural functions.

Pseudomonas syringae pv. *tomato* DC3000 represents a model for studying the molecular basis of pathogenesis, as it infects different plant species, including tomato, several crucifers, and the model plant *Arabidopsis thaliana*. The availability of the complete sequence of *P. s.* pv. *tomato* DC3000 has allowed functional genomics approaches to identify candidate genes encoding for MDE transporters. At least 11 possible operons encoding RND-type transporter were identified in the *P. s.* pv. *tomato* DC3000 genome. Knockout mutants were generated to study the role of RND-type transporters in multidrug resistance and virulence of *P. s.* pv. *tomato* DC3000. To learn more about the natural functions of these transporters and their role during pathogenesis, we will analyze the expression of these efflux pumps during infection of the host plant.

HMP33

Yop translocation into host cells requires beta 1 integrins and RhoGTPases

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Yersinia enterocolitica (Ye) evades the immune system of the host by injection of *Yersinia* outer proteins (Yops) via a type three secretion system into host cells. In this study a reporter system comprising a YopE-β-lactamase hybrid protein and a fluorescent staining sensitive to β-lactamase cleavage was used to track Yop injection in cell culture. Cell culture experiments using siRNA and inhibitors demonstrated that β1 integrin mediated signalling and the RhoGTPases RhoA and Rac1 are crucial for Yop injection demonstrating that Yop translocation is a process which needs the activation and modulation of host cells. Further studies are in process to get better insights in the mechanism on the host cell which regulate Yop injection.

HMP34

A proteogenomic comparison of conspecific bacterial endosymbionts from two deep-sea tubeworms

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The closely related tubeworms *Riftia pachyptila* and *Tevnia jerichonana* live in proximity to hydrothermal vents, the oases of the deep sea, which are only accessible with submersibles. Interestingly, both worm genera show a temporally differing colonization of new vent sites after deep-sea eruptions. It is therefore all the more surprising that the tubeworms completely depend on the same species of endosymbiotic γ-Proteobacteria - the first described occurrence of this kind among sulfur-oxidizing microorganisms.
To examine the role of the endobacteria within their hosts, this study has focussed on a comprehensive proteogenomic comparison of the symbionts. Based on a significantly improved bacterial genome database (FLX/Titanium pyrosequence), the number of protein identifications could be more than doubled. The genomic results confirmed the high similarity between both symbionts, since intraspecific variations of key enzymes and indications for potential subspecies were not detectable.
However, the protein profiles, compared by superposing both bacterial proteome reference maps, revealed substantial regulatory differences between the microorganisms concerning the energy metabolism, carbon fixation and stress response. This allowed us to exemplify a direct link between the different nutrient availability outside the hosts and the metabolic conditions of the endosymbionts.

HMP35

Microbial TLR-signaling at the intestinal epithelial surface stimulates the polarized secretion of CXCL8 and autocrine stimulation of epithelial repair

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The commensal microbiota of the intestinal tract is largely symbiotic in nature and over eons of co-evolution multiple adaptations appear to have developed to help maintain this peaceful co-existence. Certain probiotic strains have given significant and promising results in human clinical trials and experimental animal models of colitis. Nevertheless the precise mechanisms by which probiotic bacteria can protect against colitis are largely unknown. Here we present a protective homeostatic mechanism involving a polarised host response to indigenous microbial TLR ligands. Stimulation of Caco2 monolayers with specific ligands for TLR1/2, TLR2/6, TLR5 and NOD-2 or cytokines TNFα and IL-1β induced NF-κB activation and polarized secretion of CXCL8 (also known as Interleukin-8), according to the direction of stimulation. Basolaterally secreted CXCL8 is a known inflammatory initiator of leukocyte migration and activator of neutrophils but the consequences of apical secretion in an intact polarized epithelium are unknown. CXCR1, the major receptor for CXCL8, was shown to be constitutively expressed and located in the apical membrane suggesting that it might participate in an autocrine pathway. In a model of the wounded epithelium addition of CXCL8 stimulated epithelial cell migration. These findings demonstrate that the polarity of intestinal epithelial cells has a major effect on the host response to microbial TLR ligands and the directionality of IL-8 secretion. Apical secretion of IL-8 would promote epithelial repair through autocrine stimulation of the CXCR1 receptor whereas basolateral stimulation would augment inflammatory immune response through the recruitment of leukocytes. This homeostatic mechanism implicates a role for microbial ligands in the process of epithelial restitution and repair.

HMP36

Simkania negevensis vacuoles are embraced by host endomembranes

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Simkania negevensis is a chlamydia-like emerging pathogen implicated in pulmonary diseases. We investigated the intracellular accommodation of S. negevensis strain Z in human epithelial cells. Growth of *S. negevensis* was slow with vacuole formation apparent earliest at day 2 post infection. Pathogen containing vacuoles failed to fuse with lysosomes. Interestingly, vacuole expansion was accompanied by massive spatial reorganization of the host mitochondria and endoplasmic reticulum. The *S. negevensis* vacuole was pervaded and intimately embraced by these endomembrane systems. This was not the case for the Golgi apparatus which appeared to be intact and positioned between nucleus and *S. negevensis* vacuole. Ultrastructural analysis and 3D reconstruction showed that S. negevensis forms one large vacuole located within the endoplasmic reticulum lumen. Our data provide the initial characterization of the S. negevensis vacuole, its fate during bacterial growth and the unexpected interaction with host mitochondria and endoplasmic reticulum.

HMP37

Chlamydia trachomatis protein CT166 induced actin reorganization – possible involvement of Rac1 glucosylation

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Recently, we provided first evidence for the ability of CT166 from *Chlamydia trachomatis* to induce actin reorganization of the host cell cytoskeleton. Now we broadened our findings focusing on infection experiments. CT166 shares homology to clostridial glucosylating toxins (CGT) such as Toxin B (TcdB) from *Clostridium difficile* particularly due to a CGT specific aspartate – any amino acid – aspartate (DxD) motif. Infection with high MOI of *C. trachomatis* serovar D containing the CT166 ORF affects the host cell cytoskeleton resulting in cell rounding and a decreased cell diameter. We could show that the putative chlamydial effector protein CT166 ectopically expressed in HeLa cells caused an identical phenotype. In contrast, CT166 with a mutated DxD-motif (CT166-mut) exhibited almost unchanged actin dynamics and delayed TcdB mediated actin reorganization. Intriguingly, HeLa cells expressing CT166-mut were also protected against *C. trachomatis* D mediated cell rounding. These data suggest that CT166 and TcdB share a common substrate and that overexpressed CT166-mutant competes with endogenous CT166 during infection. *E. coli* necrotizing factor 1 (CNF1) toxin activates Rho-GTPases and transiently protects them against glucosylation. Thus, CNF1 delayed cytoskeletal changes induced by TcdB. More important, CNF1 reverted the rounded phenotype of CT166 expressing cells - further supporting the hypothesis that Rho-GTPases are targets of CT166. Rac1 is participating in chlamydial uptake. TcdB inactivates Rho GTPases such as Rac1 and RhoA. Overexpression of a constitutive active non-glucosylable Rac1-mutant reverted the CT166 induced morphological changes whereas overexpression of a corresponding RhoA-mutant did not. Finally, expression of CT166 in HeLa cells decreased infection rates. Our data might explain how *C. trachomatis* D ensures its balanced uptake into host-cells by interfering with excessive Rac-dependent cytoskeletal changes.

HMP38

Mercury methylation by microorganisms from the gut of soil invertebrates

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Mercury and its derivates are widely distributed in the biosphere. There are different anthropogenic and natural sources known. Mercury is mainly released by anthropogenic activities such as stationary combustion like coal fired power plants, waste disposal and cement production. Furthermore, it is used for batteries, energy saving bulbs or in medicine for dental amalgams. It accumulates in the food chain and in consequence in human beings. Generally, mercury and its derivatives are harmful and polluting. Especially monomethylmercury is 100 times more poisonous than inorganic mercury-(II). It is known as a potent neurotoxin due to its ability to cross the blood-brain barrier.
The methylation of mercury can be a biotic reaction mainly performed by microorganisms. Methylmercury can also be produced abiotic e.g. by photochemical reactions. Methylation of mercury by aquatic microorganisms is well studied. In contrast, there is less knowledge about such reactions in soil and the features of the involved microorganisms.
In this study was shown that the earthworm Eisenia foetida accumulated methylmercury in tissue when it was fed with soil enriched with mercurychloride. We could isolate mercury methylating mixed bacterial cultures from the gut. The detection of mercury and methylmercury in cultural media was performed with gas chromatography atomfluorescence spectrometry (GC-AFS). The isolated microorganisms were identified by 16S rDNA-analysis. The different 16S rDNA amplificates were separated by denaturating gradient gel electrophoresis (DGGE) and by constant denaturating gel electrophoresis (CDGE). Our experiments demonstrated that Enterobacteriaceae and Pseudomonadaceae are responsible for mercury methylation in the gut of invertebrates such as earthworms.

HMP39

Integrin-linked kinase is involved in uptake of *N. meningitidis* by human host cells

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N. meningitidis, the causative agent of meningitis and septicaemia, is able to attach to and invade a variety of host cell types. Entry of *N. meningitidis* into human host cells is mediated by fibronectin bound to the outer membran protein Opc forming a bridge to α5β1 integrins. This interaction leads to cytoskeletal rearrangement and uptake of the bacteria. We postulated that the integrin-linked kinase (ILK), which directly associates with integrins, plays a crucial role in this process.

In this study we showed, that ILK expression and activity are upregulated in response to Opc expressing *N. meningitidis*, but not in response to an isogenic opc-deficient mutant strain. Inhibition of ILK expression by siRNA silencing reduced Opc-mediated invasion into human brain microvascular endothelial (HBMEC) and human kidney (293T) cells up to 90%. Moreover, ILK-deficient fibroblasts were impaired in their ability to internalize *N. meningitidis* and showed reduced phosphorylation of the cytoskeleton and decreased development of stress fibres.
Having recently shown the requirement of Src family PTKs in meningococcal uptake by host cells, this study enhances our knowlegde about the intracellular signals involved in integrin-initiated uptake of *N. meningitidis* and suggests that *N. meningitidis* evolved different molecules and stratagies to exploit the host integrin signalling pathway for endocytosis.

HMP40
Differential proteome analysis (*in vitro* vs. *in vivo*) of *Mycobacterium avium* ssp. *paratuberculosis*

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Mycobacterium avium ssp. *paratuberculosis* (MAP) is the causative agent of Johne's disease (JD) in ruminants. Clinical similarities between JD and Crohn´s disease (CD) in humans and the detection of MAP in affected tissue in men is the cause for an ongoing discussion on the possible relevance of MAP in CD. Research in diagnostics and pathology of JD is hampered by the lack of knowledge on MAP metabolism and protein expression in the host. In an attempt to improve the understanding in this area MAP was isolated from the intestinal mucosa of three cows with clinical JD, and proteins present in membrane-enriched MAP fractions were compared to the respective preparations of cultured isolates. The yield of MAP isolated from mucosa was calculated in relation to total mucosa wet weight and varied from 1.4% to 4.4%. Membrane-enriched fractions prepared by ultracentrifugation of culture- and mucosa-derived MAP-lysates were subjected to a tube-gel trypsin digestion and subsequently investigated by nUPLC-MS/MS. In addition, a two dimensional differential gel electrophoresis (2D-DIGE) was performed to determine relative differences in expression between mucosa- and culture-derived MAP. A total of 172 proteins were detected in mucosa-derived MAP including 59 proteins of bovine origin. 199 proteins were detected in culture-derived MAP. 55% of culture- and 65% of mucosa-derived MAP were predicted as being membrane-associated due to the presence of membrane spanning regions and a signal peptide. 84 of these proteins were detected in both, mucosa- and culture-derived MAP. 29 proteins were found to be expressed in mucosa-derived MAP only. Among them, one protein (MAP2611c) was detected in the preparations from all three cows and four proteins (MAP1775, MAP2643, MAP3968, MAP4336) were detected in preparations from two cows. 21 of the 29 *in vivo* expressed proteins were membrane-associated; ten of the proteins were of unknown function, and for 19 proteins a putative function was predicted. One of these was a putative Radical SAM superfamily FO synthase (FbiC). Another interesting candidate for further investigations is MAP3968, a homologue of the heparin-binding hemagglutinin adhesin-like protein (HBHA) which has been reported as being expressed *in vivo* in *Mycobacterium tuberculosis* and *Mycobacterium leprae*.

HMP41
Analyzing host-bacterium interaction involved in asymptomatic colonization of the urinary bladder

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Escherichia coli asymptomatic bacteriuria (ABU) isolate 83972 was first discovered to have colonized a young Swedish school girl for at least three years. It has the ability to persist in the human bladder for extended periods without producing overt symptoms of infection. In addition, ABU is able to outcompete uropathogenic *E. coli* (UPEC) strains for growth in urine. These findings show that *E. coli* 83972 possesses a sort of probiotic effect with its ability to rid the urinary bladder of other bacteria through its colonization without generating symptoms on the part of the host. These characteristics prompted ABU's use for prophylactic purposes in patients with recurrent urinary tract infection (UTI).
ABU *E. coli* isolates were geno- and phenotypically analyzed to characterize bacterial traits that allow asymptomatic colonization of the urinary bladder. Results show that ABU frequently lost the ability to express functional virulence-associated factors such as adhesins, toxins and LPS O-side chain expression due to genomic deletions, point mutations and DNA rearrangements. The absence of these virulence-associated factors explains why these strains do not cause mucosal inflammatory responses in the bladder by the innate immune system. In very rare cases, however, some patients who are stably colonized with *E. coli* 83972 complained of developing episodes of UTI symptoms. It is the aim of this project, therefore, to compare re-isolates from symptomatic episodes with their parent strain 83972 initially used for colonization and to investigate whether these re-isolates differ in their ability to interact with bladder epithelial cells.

HMP42
Proteomics of the bacterial endosymbiont from the deep sea tube worm *Riftia pachyptila*

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The hydrothermal vent tube worm *Riftia pachyptila* lives in a highly specific symbiosis with a single species of sulfide-oxidising γ-proteobacteria. The lack of a digestive system makes the worm entirely dependent on its endosymbionts for nutrition. The symbionts are contained in a specialised host organ: the trophosome. They rely on the energy derived from sulfide oxidation to assimilate CO_2 and to produce organic carbon compounds that feed their host. The extraordinary efficiency of this symbiosis makes *R. pachyptila* one of the fastest growing marine invertebrates.
In this study the genome of the *R. pachyptila* endosymbionts was resequenced by 454 pyrosequencing, which led to a considerable reduction of the number of individual contigs. The thus improved genome sequence facilitated an in-depth proteome analysis of the yet uncultured symbionts. Both the intracellular and the membrane protein fraction of the bacterial symbionts were analysed by polyacrylamide gelelectrophoresis and mass spectrometry. Additionally, first attempts were made to specifically address membrane proteins associated to periplasmic sulfur globules. The thus established proteome reference maps present a comprehensive picture of the symbionts' fundamental metabolic pathways. These include the energy-generating sulfide oxidation pathway and the two alternative CO_2 fixation mechanisms Calvin cycle and reverse TCA cycle. Furthermore, the effect of sulfide availability and sulfide depletion in the hydrothermal vent environment on the endosymbionts was analysed using a comparative proteomic approach. Our results indicate that both sulfide oxidation and CO_2 fixation reactions are regulated according to the prevailing energy situation.

HMP43

Active fermenters in the alimentary canal of the earthworm *Lumbricus terrestris*

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The *in situ* conditions of the earthworm gut favor heterotrophs capable of anaerobiosis. The earthworm alimentary canal contains high amounts of mucus- and plant-derived saccharides such as glucose. High concentrations of hydrogen and organic acids in the anoxic alimentary canal indicate that fermentative processes are ongoing. Thus, the central objective of this study was to link fermentations in the earthworm alimentary canal to active fermenters by the use of 16S rRNA stable isotope probing with [^{13}C]glucose as a model substrate. Ten millimolar supplemental glucose was consumed within 48 hours of incubation in anoxic microcosms with midgut and hindgut contents. The formation of acetate, butyrate, formate, lactate, propionate, succinate, ethanol, CO_2, and H_2 was indicative of diverse fermentations in the alimentary canal. Organisms of the families *Clostridiaceae* and *Enterobacteriaceae* were dominant utilizers of glucose-derived carbon and might have used glucose for butyrate and mixed acid fermentation, respectively. Additional active phyla identified by 16S rRNA analysis of fresh midgut and hindgut contents included *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Chloroflexi*, *Cyanobacteria*, *Gemmatimonadetes*, *Nitrospirae*, *Planctomycetes*, *Proteobacteria*, *Tenericutes*, and *Verrucomicrobia*. These findings suggest that (a) both obligate anaerobes and facultative aerobes contribute to the degradation of mucus- and plant-derived saccharides during gut passage and (b) diverse microorganisms are active in the alimentary canal of the earthworm.

HMP44

Staphylococcus aureus has versatile strategies to infect and persist within endothelial cells

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S. aureus is an important human pathogen of endovascular diseases, such as endocarditis, vascular graft infections or sepsis. Clinically, they can take an acute and destructive course and/or can develop in a chronic infection with a high relapsing rate despite of antimicrobial treatment. The different courses of infections might be induced by diverse infection strategies of *S. aureus* in endovascular tissue. So far, persistent and antibiotic-refractory infections have been largely associated with a subpopulation of *S. aureus*, the so-called "small-colony variants" (SCVs), which express less virulence factors.
In this work we used endothelial cells to investigate the effect of the highly virulent wild-type isolate (6850), the corresponding stable SCV mutants with a gene defect in the hemin-biosynthesis and the complemented mutant. All strains were invasive in endothelial cells, but largely differed in their ability to induce a host cell response. Microarray analysis showed that wild-type 6850 and the complemented mutant up-regulate a huge number of endothelial genes, including many genes involved in innate immunity, whereas the SCV mutants did not induce these dramatic changes in endothelial gene expression. The inflammatory endothelial response was strongest directly after infection and largely declined within the following days. However, despite this strong and acute inflammatory response we found that not only the SCV mutants but also the wild-type 6850 can persist intracellularly and was detected at low numbers within the host cells even after 5 days.
These results suggest that upon entering endothelial cells *S. aureus* can induce hugh inflammation, but can also persist within endothelial cells at low numbers without provoking the host defending system. Intracellular *S. aureus* persistence was not only restricted to SCVs with a stable mutation (e.g. hemin biosynthesis), but was also found with the highly virulent wild-type isolate 6850. This most likely represents an important strategy of *S. aureus* to hide within in the host cells, which could be a reservoir for chronic infections.

HMP45

The developmental cycle of *Protochlamydia amoebophila* in *Acanthamoeba* sp.

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All known members of the obligate intracellular *Chlamydiae* alternate between infectious elementary bodies (EBs) and dividing reticulate bodies (RBs). While the developmental cycle of several chlamydial pathogens has been studied intensively, detailed insights into the life cycle of *Protochlamydia amoebophila* are missing though this amoeba symbiont constitutes the only environmental member of the phylum *Chlamydiae* with a sequenced genome. In this study, the developmental cycle of *P. amoebophila* was characterized in *Acanthamoeba* sp. as a host. By monitoring the course of infection using fluorescence *in situ* hybridization, immunofluorescence and quantitative real-time PCR, key events of the *P. amoebophila* developmental cycle could be phenotypically and quantitatively determined: intracellular bacteria were detected as early as one hour post infection (hpi); transition of EBs to RBs occurred within 24 hpi, initiating a phase of RB replication lasting through 72 hpi; RB-to-EB transition and release of bacteria took place between 72 and 96 hpi, after which the cycle became asychronous, indicating completion of the productive growth cycle of *P. amoebophila*. Moreover, lysis of the *Acanthamoeba* hosts was not observed, suggesting a primarily non-destructive exit mechanism and a stable, rather than a parasitic host-endosymbiont relationship. Iron depletion induced a variation of this growth cycle characterized by aberrantly enlarged chlamydial cells – an effect known as persistence for known chlamydial pathogens. This observation suggests that persistent infection of eukaryotic cells may be an ancient feature that was present in the last common ancestor of all chlamydiae.

HMP46

Matrix metalloproteinase (MMP)-8 and apoptosis-independent cell detachment during *N. meningitidis* infection – association with blood-brain barrier damage

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Disruption of the blood-brain barrier (BBB) is a hallmark event in the pathophysiology of bacterial meningitis. Several inflammatory mediators, such as tumor necrosis factor alpha (TNF-α), nitric oxide and matrix metalloproteinases (MMPs) contribute to this disruption.
Based on the finding of elevated concentrations of MMP-8 in cerebrospinal fluid of patients suffering from meningococcal meningitis, we analysed the role of MMP-8 during infection with *N. meningitidis* using an *in vitro* blood-brain barrier model system. Monolayers of human brain microvascular endothelial cells (HBMEC) were infected with several *N. meningitidis* isolates belonging to different clonal lineages and the release of active MMP-8 as well as the integrity of the cell monolayers during infection were measured. Interestingly, increase of MMP-8 activity in the supernatant was accompanied with enhanced cell detachment. Injury of the HBMEC monolayer required direct cell contact because no detachment was observed when bacteria were placed above a transwell membrane. Inhibition of MMP-8 activity could be demonstrated to protect the monolayer from cell loss. Detached cells in the supernatant did not display apoptotic signs such as defragmented DNA or extracellular translocated annexin-V. The influence of several virulence factors of *N. meningitidis* on cell detachment and MMP release was estimated and the lipopolysaccharide (LPS) was found to contribute to both active MMP-8 release and cell detachment. Taken together our data indicate that the release of MMP-8 is involved in an apoptosis-independent cell detachment contributing to the pathogenesis of meningococcal meningitis.

HMP47
Massive proliferation of IS elements and contiguous transcription of IS elements and their downstream genes in the genome of the amoeba symbiont '*Candidatus* Amoebophilus asiaticus'

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Mobile genetic elements, such as IS elements, can have profound effects on genome structure and evolution including inactivation of genes, recombination, deletions, inversions, or the acquisition of novel genes (e.g. antibiotic resistance). By these mechanisms, mobile genetic elements play an important role during adaptation to changing environmental conditions. In this study we analyzed the mobile genetic elements present in the genome of '*Candidatus* Amoebophilus asiaticus', an obligate intracellular amoeba symbiont belonging to the *Bacteroidetes*. While the density of IS elements is generally below 3% in bacterial genomes, the genome of *A. asiaticus* shows massive proliferation of mobile genetic elements (mainly IS elements), corresponding to 24% of all predicted open reading frames. The IS elements were classified into 18 different groups, belonging to 9 different IS element families, with copy numbers ranging from 1 to 20 copies. Many of the IS elements are highly conserved within the *A. asiaticus* genome, indicating that they might be transpositionally active. Southern blot analyses, however, revealed no indications for recent transpositional activity. On the other hand, using reverse-transcriptase PCR we could show transcription of selected IS elements. In 10 cases we observed contiguous transcription of IS elements with their (non-pseudogene) downstream genes. This suggests that some *A. asiaticus* IS elements are influencing the expression of neighboring genes, a feature which is often found among IS elements. Interestingly, some of the genes co-transcribed with IS elements are predicted to be important for host cell interaction including proteins with eukaryotic domains. This suggests that proliferation of IS elements and interference of IS elements with gene regulation was an important process during the adaptation of *A. asciatus* to an obligate intracellular life style.

HMP48
Apoptosis induction in insect cells after infection with environmental chlamydiae

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Apoptosis, a form of programmed cell death, not only exerts a key function in development and tissue homeostasis in multicellular eukaryotes, but is also an effective defense mechanism against pathogens. The significance of its protective role is highlighted by the multitude of anti-apoptotic strategies evolved by various microbes, including the obligate intracellular bacteria of the family *Chlamydiaceae*. Although *Chlamydiaceae*, such as *Chlamydia trachomatis* and *Chlamydophila pneumoniae*, are well-known due to their medical importance for humans and animals, the pathogenic potential of their closest relatives, the environmental chlamydiae, is still debated. As inhibition of apoptosis is assumed to be essential for efficient infection of a multicellular host by chlamydiae, we aimed to analyze the anti-apoptotic capacity of the *Parachlamydiaceae*, naturally occurring as symbionts of free-living amoebae. We therefore applied insect cell lines as an infection model and monitored host cell death in infected cultures. Within few hours typical apoptotic features were observed, such as formation of apoptotic bodies, DNA condensation and nucleosomal DNA fragmentation. The apoptotic nature of cell death was confirmed by the detection of caspase 3-like activity in infected cultures. In addition, apoptosis induction was dependent on infectious bacteria, as heat- or UV-inactivated bacteria did not cause detectable cell death. The significance of early host cell death on the infection efficiency is not clarified yet, but is currently subject of further investigations that aim to analyze infection progress and host cell death on a single-cell level. Moreover, we plan to extend our studies to a mammalian host cell system in the near future. These studies will help to decipher the significance and evolution of host cell death modulation by chlamydiae. They will also contribute to our understanding of the role of environmental chlamydiae as possible emerging pathogens.

HMP49
First cultivation-independent analysis of the bacterial community in a cockroach hindgut

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Termite guts harbor a remarkably diverse and metabolically active community of bacteria and protists, which enable the host to feed on wood or other lignocellulosic diets. Surprisingly little is known about the gut microbiota of cockroaches, the closest phylogenetic neighbors of termites. Most previous studies, typically employing highly biased culture-based techniques, have focused on the cockroach as a pest species and its gut as a reservoir for pathogens. Here, we present the first cultivation-independent characterization of the gut microbiota of a cockroach. We constructed a clone library of the 16S rRNA genes in the hindgut of *Shelfordella lateralis*, an omnivorous cockroach belonging to the family *Blattidae*, a sister group to termites. The dominant clones were identified as members of the *Bacteroidetes*, *Firmicutes* (mainly *Clostridia*) and δ-*Proteobacteria*. *Fibrobacteres* and *Spirochaetes*, the major cellulolytic groups in higher termites, were absent. Nevertheless, many of the clones clustered with sequences previously obtained from the termite gut, some of them belonging to *Bacteroidetes* and *Elusimicrobia* (formerly Termite group 1) lineages that comprise endosymbionts of termite gut flagellates. Since *Shelfordella lateralis* does not harbor such protists, it is likely that these sequences belong to free-living forms. Community fingerprinting (T-RFLP analysis) of individuals maintained on different diets indicated that the gut microbiota comprises both stable and variable elements. Our results show that the cockroach gut contains a highly diverse and complex bacterial community that partly depends on their particular diet and partly reflects the close phylogenetic relationship between cockroaches and termites.

HMP50
Losing the partner - now what? Effects of host loss on transcription in arbuscular mycorrhizal fungi

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Arbuscular mycorrhizal fungi are an ancient fungal phylum (Glomeromycota) that coevolved with plants for the last 400 million years, assisting the colonization of land masses by higher plants. Today, these fungi associate with 70-90% of all plant roots and form intimate and, in most cases, mutualistic symbioses. Collectively referred to as arbuscular mycorrhiza (AM), this association is regarded as the most widespread terrestrial symbiosis. The interaction is mainly characterized by fungal arbuscules, i.e. tree-shaped subcellular structures within plant cells that are the main site of nutrient exchange between the fungal and plant symbiotic partners. The fungal partner provides water, phosphate and other nutrients which are taken up via its extensive hyphal network from the soil. In return AM fungi obtain carbohydrates from their plant partner. Up to 20% of the photosynthesis products of terrestrial plants (roughly 5 billion tonnes of carbon per year) are estimated to be consumed by AM fungi. Therefore, the AM symbiosis contributes significantly to global phosphate and carbon cycling and influences primary productivity in terrestrial ecosystems.
While the symbiosis is well characterized in regard to nutrient and signal exchange, little is known regarding the senescence of AM fungi. The life time of extraradical hyphae (i.e. hyphae outside the root) has been shown to be relatively short, on average 5-6 days, and intraradical hyphae (hyphae inside the root) as well as arbuscules have been described to be turned over equally rapidly. The aim of our experiments is to characterize such senescence processes on a molecular level. Currently we induce fungal senescence by separating AM fungi from their plant partner, which ultimately results in hyphal ageing and death of fungal hyphae, if the fungal partner is prevented to re-establish the symbiosis. This experimental system will be used to monitor changes in transcription and to detect key genes involved in fungal senescence.

HMP51
Identification of volatile metal(loid) compounds formed by intestinal microorganisms by use of simultaneous EI-MS and ICP-MS detection after gas chromatographic separation

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For investigation of biotransformation processes of heteroelements in the environment, both molecular and element-sensitive detection systems are used in hyphenation to chromatographic separation. In this work, we studied the potential of our recently developed gas chromatographic system with parallel electron impact mass spectrometry and inductively coupled plasma mass spectrometry (GC/EI-MS/ICP-MS) for non-target screening and subsequent identification of volatile arsenic compounds formed by intestinal microorganisms. Therefore, either fresh fecal slurries or continuous culture sampled from the Simulator of the Human Intestinal Ecosystem (SHIME), an *in vitro* gastrointestinal model, was amended with inorganic metal(oid) salts (Ge, As, Sn, Sb, Te, Hg, Pb and Bi) as well as the nonmetal selenium.
While for Sb, Te and Bi only permethylated species were detected, a broad range of complex volatile As and Se species was observed. By combined use of molecular and elemental detection after gas chromatographic separation (GC-EI-MS/ICP-MS) as well as synthesis experiments, these compounds were identified as methylthio species (dimethyl-methylthio-arsine, (CH3)2AsSCH3, methyl-di(methylthio)-arsine, CH3As(SCH3)2, methyl-methylthio-selenide, CH3SeSCH3, di(methylthio)-selenide (CH3S)2Se), methyldithio species, (dimethyl-methyldithio-arsine, (CH3)2AsSSCH3, methyl-methyldithio-selenide CH3SeSSCH3) as well as methyl-methylthio-ethylthio-arsine, CH3As(SCH3)(SC2H5), dimethylthioarsinous acid, (CH3)2AsSH and thio-bis(dimethylarsine), ((CH3)2As)2S. Furthermore, one mixed arsenic/selenium compound, dimethyl-methylseleno-arsine, (CH3)2AsSeCH3 was identified.
Five of these species, namely methyl-di(methylthio)-arsine, dimethyl-methyldithio-arsine, thio-bis(dimethylarsine), methyl-methylthio-ethylthio-arsine and dimethyl-methylseleno-arsine have not been described in environmental or human matrices before. Finally, the advantages of elemental and molecular detection after gaschromatographic separation are discussed.

HMP52
Role of *Salmonella* Pathogenicity Island 2 effector proteins in formation of *Salmonella*-induced filaments and maintenance of the SCV membrane

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Salmonella enterica is a pathogen that escapes host defence systems by living in a membrane-bound compartment called *Salmonella*-containing vacuole (SCV). The survival and replication of the bacteria in the SCV requires the expression of virulence factors encoded by *Salmonella* pathogenicity island 2 (SPI2). The SPI2 encoded effector proteins are translocated into host cell cytoplasm and interfere with host endosomal trafficking and the microtubule cytoskeleton. A subset of effector proteins affects the membrane integrity and intracellular location of the SCV, and leads to induction of tubular structures originating from SCV, called *Salmonella*-induced filaments (SIF). We have shown that Sifs are highly dynamic structures which extend, contract and continuously interact with the host cell endosomal system. In the present study, we screened the SPI2 effector proteins for their contribution to Sif formation and maintenance of SCV integrity using a live cell approach. Similar to previous reports, the results revealed an important role for SifA in the formation of Sifs and maintenance of intact SCV membrane. The deletion of pipB2 and sseF revealed novel SIF phenotypes. While the absence of PipB2 leads to bulky Sifs, the lack of SseF resulted in very thin SCV and SIF membrane. Further, based on the bioinformatic structural modelling of SifA, we set out to determine amino acid residues that are crucial for the function of SifA. Following site-directed mutagenesis of the predicted amino acids we found that the specific amino acids were crucial for the functioning of SifA protein and there by the formation of Sifs.

HMP53
Evaluation of FRET in *Salmonella enterica* for assessing protein-protein interaction of virulence factors

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Salmonella enterica is a common facultative intracellular pathogen that causes diseases ranging from self-limiting gastrointestinal infections to life-threatening typhoid fever. Pathogenicity of *Salmonella* serovars relies on the coordinated function of different virulence factors. Investigation of interactions between these factors is fundamental for understanding the molecular bases of Salmonellosis.
Fluorescence Resonance Energy Transfer (FRET) is a well known method for monitoring dynamic protein-protein interactions in real time *in vivo*. FRET relies on the distance- dependent energy transfer from an excited donor- to an acceptor fluorophore.
For establishing FRET in *Salmonella* a positive control is of main interest. Previous studies could demonstrate dynamic interaction of CheY and CheZ, two components of the *Escherichia coli* chemotaxis machinery, using fusions of CheY to yellow fluorescent protein (YFP) as acceptor and CheZ to cyan fluorescent protein (CFP) as donor. CFP-YFP is the most popular functional donor-acceptor pair for FRET.
Based on this work, *Salmonella* homologs of CheY and CheZ were fused to the improved monomeric fluorescent proteins SYFP2 and SCFP3a, respectively. The fusion genes are expressed under control of the arabinose- (pBAD) or tetA-promoter. Both of which allow tight regulation of expression. To determine functionality of the positive control, FRET measurements were carried out in *Salmonella enterica* serovar Typhimurium with both fusion proteins co-expressed.
Further evaluation of FRET in *Salmonella* should help to understand the kinetics of dynamic interactions between virulence associated proteins *in vivo*.

HMP54
The major *Staphylococcus aureus* autolysin Atl mediates host cell invasion by interacting with human heat shock cognate 71 kDa protein

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The ability of *S. aureus* to invade human cells is considered to be the main reason why *S. aureus* infections are extremely difficult to eradicate. Host cell invasion is mainly dependent on the interaction between staphylococcal fibronectin binding proteins (FnBPs) and host $\alpha_5\beta_1$ integrins. However, strains that lack FnBPs are still internalized to a certain amount.
The major *S. aureus* autolysin Atl consists of three repeat regions, each of which contains two glycine-tryptophane (GW) dipeptides. GW repeats are present in a number of surface proteins of Gram-positive bacteria, such as in the *Listeria monocytogenes* invasion protein InlB, where the GW repeats mediate host cell adhesion and invasion.
We found that the GW-protein Atl is involved in adhesion to and internalization by EA.hy 926 cells. Recombinant Atl (rAtl) binds to EA.hy 926 cells and interacts with human heat shock cognate 71 kDa protein (Hsc70). Surface-associated Hsc70 has been shown to be involved in virus cell entry, as well as *Brucella abortus* internalization. The ability of Atl-deficient *S. aureus* SA113 (SA113*atl*) to be internalizated by EA.hy 926 cells is reduced by 49,24% ± 8,23%, compared to the wild type. Furthermore, internalization of *S. aureus* SA113 can be decreased by rAtl (-48,57% ± 2,40%) and an antibody raised against Hsc70 (-32,96% ± 8,32%). Impaired invasiveness of SA113*atl* can be nearly restored by expressing plasmid-encoded *S. epidermidis atlE* in the mutant strain.
We additionally tested whether infection of EA.hy 926 cells with different *S. aureus* strains stimulates an increased expression of surface-associated Hsc70, since it is known that heat shock protein 70 is upregulated upon stress response during *S. aureus* infection. Indeed, we detected an increased level of surface-localized Hsc70 in *S. aureus* infected cells.
Our results show that *S. aureus* Atl interacts with Hsc70 on host cells and that this interaction represents an additional *S. aureus* internalization mechanism by non-professional phagocytes.

HMP55
The role of amino acid and peptide metabolism on intracellular growth in *Salmonella typhimurium*

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Salmonellosis is a worldwide health problem for both humans and animals. Hosts are generally infected through contaminated food or water. During infection, *Salmonella* first crosses the host intestinal epithelia layer and can be carried by marcophages to other organs and lymphoid tissues. Within infected host cells, *Salmonella* replicates within a membrane-bound vacuole known as the Salmonella-containing vacuole (SCV). While much is known about the process of infection and the effects of *Salmonella* on host cell responses and modulation of intracellular trafficking, little is known about how *Salmonella* acquires nutrients to support growth within the SCV.
Salmonella spp. are heterotrophic bacteria, capable of utilizing multiple carbon sources for growth. However, amino acid auxotrophs remain completely dependent upon the host as a source of these metabolites regardless of the type of carbon sources available. To understand the metabolism of intracellular *Salmonella*, we have constructed directed gene deletion mutants in amino acid biosynthetic pathways, peptide uptake and regulatory genes and compared the intracellular replication of mutants with the wildtype strain in standardised infection/invasion assays *in vitro*. Of approximately 200 different mutants harboring single or combined mutations in amino acid metabolism, no significant differences were observed relative to the wildtype strain, with the exception of mutations affecting glycine metabolism, which affect central C1 carbon metabolism. The results of these studies indicate that intracellular *Salmonella* have direct access to host amino acid pools, either as free amino acids or in the form of peptides.
These results will be discussed with regard to future studies aimed at identifying the intracellular sources of amino acids accessed by *Salmonella*, as well as possible application of the knowledge gained for development of antibacterials targeting intracellular pathogens.

HMP56
In vivo time-course monitoring of bacterial infections

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In an era of emerging and spreading antibiotic resistance, new antiinfective compounds have to be identified and developed. In this context, not only the search for new drugs should be enforced, but also the testing of new promising drugs should be improved and accelerated.
Here, we evaluated MRI (magnetic resonance imaging) as a new imaging method to localize, quantify and monitor infections. Therefore, we used proton-based and contrast-agent enhanced MRI methods to visualize *Staphylococcus aureus* infections in a thigh abscess model in the mouse.
T_2-maps proved to be a suitable native MRI-method to visualize abscess formation and growth. It delivers even at very early stages of disease development contrast to the surrounding muscle tissue.
We used USPIO and Perfluorocarbon contrast agents to visualize abscess formation and inflammation in the infected muscle. Both contrast agents accumulated in/around the abscess area and formed a hollow sphere.
These MRI-methods proved to be very powerful to visualize and monitor *staphylococcal* infection, at least in this mouse model. The abscess area was visualized with high spatial resolution *in vivo* and non-invasively. But this is only the first step of our investigation. Next will be the visualization of renal abscess formation and the evaluation of several promising antiinfective compounds.

HMP57
Influence of *Helicobacter pylori* on the expression and activity of deubiquitinating enzymes of human gastric epithelial cells

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Immunoevasive and immunomodulatory mechanisms of human or animal associated virus and bacteria were shown to frequently target the host ubiquitin-proteasome system (UPS). Ubiquitylation and deubiquitylation of proteins regulate essential cellular processes. Helicobacter pylori is able to persist within its host and therefore needs efficient strategies to guide the immune responses. In susceptible hosts these modulations may promote cancerogenesis. So far, little is known about the potential of *H. pylori* to interfere with the host UPS. The *H. pylori* cag pathogenicity island (cagPAI) is associated with disease severity and carcinogenesis, suggesting that cag functions interfere with the immune responses. This project investigates effects of *H. pylori* on ubiquitin-related pathways of human gastric epithelial cells.
Firstly, influences of *H. pylori* wild type and different isogenic mutants on host deubiquitinating enzymes (DUBs) were analysed using activity probes in coincubation experiments with lysates of various human gastric epithelial cells. We were able to identify active DUBs in several human gastric cell lines. cag-dependent and cag-independent influences of the *H. pylori* infection on the expression and activity of defined cellular DUBs were clearly determined, affecting TRAF6 and p53 expression.
Secondly, we concentrated on putative *H. pylori* encoded effectors responsible for the observed influences on the host UPS. Using BLAST searches, specific pattern searches, and hidden Markov models to identify short catalytic residues from different DUB families in a specific DUB database (Sompallae *et al.*, 2008) we obtained several interesting hits in *H. pylori*. For further functional assays, we purified the most interesting protein candidates and tested their interaction with different ubiquitin and ubiquitin-like substrates (activity probes, ubiquitin-GFP, fluorogenic substrates). Results of the biochemical analysis of candidate proteins will be presented and discussed.

HMP58
A novel *Salmonella* gene involved in regulation of expression of the SPI4-encoded adhesin, SiiE

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Salmonella spp. are facultative intracellular pathogens which actively invade many cell types including non-phagocytic epithelial cells. Invasion of epithelial cells is mediated by the expression of genes located in *Salmonella* pathogenicity island 1 (SPI1), the invasion gene locus. In contrast to SPI1, the functions of the other SPIs in *Salmonella* virulence are less well understood. *Salmonella* pathogenicity island 4 (SPI4) has recently been shown to encode a non-fimbrial adhesin, SiiE, involved in attachment to epithelial cell surfaces. The expression of SPI4 genes coincides with the activation of invasion genes in *Salmonella*, and recent studies have shown that the expression of SPI1 and SPI4 are co-regulated through the same regulatory networks.
Previous studies in our laboratory showed that the expression of the *Salmonella yciGFE* operon was highly activated under SPI1-activating conditions. The *yciGFE* operon is conserved in numerous enteric bacteria, but its biological function is still unknown. Here we show that the *yciGFE* operon is involved in the co-regulation of SPI1 and SPI4 gene expression. Deletion mutants in the *yciGFE* operon were constructed and the effects on *Salmonella* invasion were determined. We observed decreased expression of genes encoded within both SPI1 and SPI4 in Δ*yciGFE* and Δ*yciF* mutant strains, but higher expression levels of the SPI4-encoded *siiE* gene in the Δ*yciG* deletion background despite reductions in expression of SPI1-encoded regulatory genes. A Δ*yciG* mutant strain showed increased adherence to human intestinal epithelial cells relative to the wildtype, and elevated levels of SiiE expression at the bacterial cell surface. Our data indicate that the *yciG* gene product is somehow involved in the coupling or co-regulation of these two pathogenicity islands, assigning a first possible biological function for the *yciGFE* genes.

HMP59
Identification and characterization of a novel extraintestinal pathogenic *Escherichia coli* fimbrial adhesin

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The extraintestinal pathogen, avian pathogenic *E. coli* (APEC), known to cause systemic infections in chickens, is responsible for large economic losses worldwide. To identify genes involved in adhesion and colonization, Signature-tagged mutagenesis (STM) was applied to a previously established lung colonization model of infection by generating and screening 1,800 mutants of an APEC strain IMT5155 (O2:K1:H5; Sequence type complex 95).
The study led to the identification of new genes of interest, including an adhesin, coding for a novel APEC fimbrial adhesin (Yqi) not described for its role in APEC pathogenesis to date. Its gene product has been temporarily designated ExPEC Adhesin I (EA/I) until the adhesin-specific receptor is identified. Deletion of the EA/I gene resulted in reduced colonization by APEC strain IMT5155 *in vitro* and *in vivo*. Furthermore, complementation of the adhesin gene restored its ability to colonize epithelial cells *in vitro*. The ExPEC adhesin I protein was successfully expressed *in vitro*. Electron microscopy of an afimbriate strain *E. coli* AAEC189 over-expressed with the putative EA/I gene cluster revealed short fimbrial like appendages protruding out of the bacterial outer membrane.
The adhesin coding gene *yqi* was prevalent among extraintestinal pathogenic *E. coli* (ExPEC) isolates, including APEC (54.4%), Uropathogenic *E. coli* (UPEC) (65.9%) and Newborn meningitic *E. coli* (NMEC) (60.0%), and absent in 153 intestinal pathogenic *E. coli* strains tested, thereby validating the designation of the adhesin as ExPEC Adhesin I. In addition, prevalence of EA/I was most frequently associated with the B2 phylogenetic group and ST95 complex of the multi locus sequence typing (MLST) scheme, with evidence of a positive selection within this highly pathogenic complex.
This is the first report of the newly identified and functionally characterized ExPEC adhesin I and its crucial role during APEC infection in chickens.

HMP60
Biological role of pseudomonic acid derivatives produced by a sponge-symbiotic *Pseudomonas* strain

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Marine sponges provide a unique habitat for a great diversity of bacteria. The sponge Halichondria panicea was shown to harbour members of different genera, e.g. *Streptomyces*, *Micromonospora*, *Bacillus*, *Vibrio*, *Shewanella*, and *Pseudomonas* respectively. In a study, which focussed on sponge-associated bacteria exhibiting antimicrobial activity one *Pseudomonas* sp. isolate was of special interest, because it showed a strong inhibition of several *Staphylococcus* sp. strains including methicillin-resistant *Staphylococcus aureus* (MRSA). Not only clinically relevant strains were inhibited by the *Pseudomonas* sp. isolate, but also further H. panicea associated isolates, e.g. representatives of the genera *Bacillus*, *Streptomyces* or other members of the genus *Pseudomonas.* Chemical analysis and the detection of PKS-I gene fragment revealed the presence of pseudomonic acid derivatives. Besides this compound is known for 3 decades and used as drug against infection diseases caused by MRSA, the biological role of this substance has to be considered. We proposed, that the producer might asserts itself against antagonistic bacteria, contributes to the structuring of the relationship between the sponge and the associated organisms and protects its host against pathogenic microorganisms.

HMP61
The production of autoinducer signals of *D. shibae* DFL-12^T during cocultivation with its algal host

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Dinoroseobacter shibae DFL-12^T is a member of the globally important marine *Roseobacter* clade. *D. shibae* lives as epibiont on the benthic dinoflagellate *Prorocentrum lima* which causes toxic algae blooms, so-called red tides. The bacteria provide the grow-limiting vitamins B_1 and B_{12} to the dinoflagellate host. One of the most important signal transduction systems in bacteria, quorum sensing, is involved in many regulatory circuits in *Alphaproteobacteria*, for example in the control of communication between rhizobia with their eukaryotic host. In *D. shibae* acylated homoserinelactones (AHL) with chain lengths up to 18 carbon atoms were identified but traits regulated by quorum sensing are not known so far. As part of an investigation to understand the role of bacteria in dinoflagellate physiology, we started to cultivate *D. shibae* with bacteria free cultures of *Isochrysis galbana* CCMP1323, a haptophyt and the dinoflagellate *Prorocentrum minimum* CCMP1329. Bacteria and host grow in completely defined mineral media lacking the required vitamins and carbon sources for both. Studying the cell numbers of bacteria and algae during cocultivation clarifies real interaction and the reciprocal exchange of metabolites. To unravel the role of the *D. shibae* quorum sensing regulon in algae symbiosis a GFP expressing strain was constructed which harbours the C12-AHL specific sensor plasmid pKR-C12 whose GFP is only expressed in the presence of long-chain AHLs. At present, it is not known if and how the exchange of metabolites between bacteria and algae works but we could show that vitamins in the medium can be replaced by a small inoculum of *D. shibae*. Furthermore the production of long-chain AHLs during cocultivation experiments was shown. Thus, AHL signalling could play a role for the symbiosis.

HMP62
"Extracellular adherence protein (EAP)" of *S. aureus* irreversibly bound to PSS-PAH multilayer films maintains anti-proliferative activity

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EAP belongs to a new group of highly cationic, secreted *S. aureus* adhesins (SERAMs) and was detected in practically all *S. aureus* strains examined so far. In contrast to MSCRAMMs, it has an extremely broad binding spectrum, including various extracellular matrix proteins as well as cellular receptors of the immunoglobulin superfamily. *In vivo* it was found to exert potent immunomodulating and anti-angiogenic effects. On the molecular level these are principally due to one of two mechanisms, involving either ICAM-1 (blockage of leukocyte transmigration and pro-inflammatory signals) or inhibition of Ras-activation (anti-angiogenic and anti-proliferative effects).
As EAP was also shown to propagate *S. aureus* cellular invasion, there has been some dispute to whether Ras-blockage would require cellular uptake of the protein. However, for a future clinical application of EAP, local immobilisation would be desirable, since some authors reported an EAP induced glomerulonephritis in mice.
Polyelectrolyte multilayer (PEM) films made from poly(allylamine hydrochloride) (PAH) and poly-4-styrene sulfonate sodium salt (PSS) were used as solid carrier. Deposited PEM architecture was $(PAH\text{-}PSS)_3$ and $(PAH\text{-}PSS)_3\text{-}PAH$ as determined by QCM-D. EAP was adsorbed very quickly onto PSS ending films (negative surface charge), but not to PAH ending films (positive surface charge). Binding between EAP and PSS was an endothermic process and adsorption of irreversible nature. The adsorption isotherm reached a pseudo plateau at protein concentrations above 0.01 mg/mL. Investigation of adsorbed protein by AFM revealed aggregates with a diameter of 200-400 nm. While the diameter of these deposits was unaffected by protein concentration, their surface density increased with the concentration of dissolved protein.

When HUVEC or fibroblasts were seeded on these EAP coated PEM films, but not when seeded on naked PEM, ERK-dependent growth was inhibited to a similar degree as with dissolved protein added.

HMP63

Complex interaction of biocontrol strains, *Rhizoctonia solani* and indigenous microbial community in the rhizosphere of lettuce unraveled by molecular methods

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The control of the soil-borne plant pathogen *Rhizoctonia solani* is difficult because of its wide host range and its ability to survive as sclerotia under adverse environmental conditions. In the present study we aimed to explore the potentials to improve biocontrol efficiency by combining antagonists that supposedly colonize different niches in the rhizosphere and target *R. solani* via different mechanisms. *Trichoderma viride* strain GB7 (Grosch *et al.*, 2005) and *Serratia plymuthica* strain 3Re4-18 (Berg *et al.*, 2005) were selected for growth chamber experiments. Different monitoring strategies were used to study the colonization patterns of the inoculants and to compare the effects of the inoculation of single and of the combined antagonists in the presence of *R. solani* AG1-IB on the composition of bacterial and fungal communities in the rhizosphere of lettuce. In addition, biocontrol efficiency was measured. At two time points during plant development lettuce plants were destructively sampled and 16S rRNA gene or ITS fragments were amplified from total community DNA and analyzed by denaturing gradient gel electrophoresis. The study showed that the applied 16S or ITS-based fingerprinting methods provided insights into the complex interaction in response to the pathogen presence and to biocontrol strain inoculation. A significantly improved biocontrol effect was observed for the combined application of *S. plymuthica* 3Re4- 18 and *T. viride* strain GB7 but also a more pronounced effect on the bacterial and fungal community composition compared to the application of single inoculants.

HMV01

Characterization of two members of a novel porin family discovered in the amoeba symbiont *Protochlamydia amoebophila*

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Proteins in the outer membrane of chlamydiae are of great importance as they are involved in attachment to and entry into host cells, uptake of nutrients, disposal of waste products and recognition by the immune system of the host. Due to their potential as vaccine candidates, these proteins have been studied extensively for members of the Chlamydiaceae. However, the genome sequence of *Protochlamydia amoebophila* revealed that a homologue to the most abundant outer membrane protein of the Chlamydiaceae, the major outer membrane protein (MOMP), is missing in this amoeba endosymbiont, highlighting a significant difference between the Chlamydiaceae and their environmental counterparts. We recently identified a novel family of putative porins in the genome of *P. amoebophila* by in silico analysis. Two of these porins, pc1489 and pc1077, are highly abundant in outer membrane preparations of this organism. In this study, we purified the putative porin pc1489 directly from elementary bodies of *P. amoebophila* for antibody production and functional characterization. The location of pc1489 in the outer membrane was confirmed by immunofluorescence analysis and immuno-transmission electron microscopy. We could further show that the gene is transcribed and the corresponding protein is present in the outer membrane throughout the whole developmental cycle, thereby suggesting an essential role for *P. amoebophila.* Lipid bilayer measurements using purified pc1489 demonstrated that pc1489 is a functional porin. The second porin, pc1077, was heterologously expressed in *E. coli* and purified for the production of antibodies. We could also show localization to the outer membrane of *P. amoebophila* for this protein. Taken together, our results suggest that pc1489, possibly in collaboration with pc1077 and other members of this porin family, is the functional equivalent of the MOMP in *P. amoebophila.* This work contributes to our understanding of the adaptations of symbiotic and pathogenic chlamydiae to their different eukaryotic hosts.

HMV02

Commensalism, parasitism and global environmental change: Insights into the lifestyle switch of pandemic *Vibrio parahaemolyticus* in association with the bivalve host

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The bacterium *Vibrio parahaemolyticus* is ubiquitous in the marine environment and can lead to acute seafood- and water-borne infections in humans and aquatic wildlife. The recent worldwide increase in incidence of *V. parahaemolyticus* epidemics is associated with the emergence and the pandemic spread of the serovar O3:K6 and its variants. *V. parahaemolyticus* is found at particularly high densities in association with marine bivalves, which implicates life in bivalves as the preferred environmental niche of pathogenic and non-pathogenic *V. parahaemolyticus*. While epidemiological data on the occurrence and distribution of epidemic genotypes have been accumulating, knowledge on the adaptive mechanisms and the selective forces favouring the evolution of pathogenic clones remains scarce. Our aim is to understand the environmental and genetic determinants favouring colonization and accumulation of *V. parahaemolyticus* in the bivalve host. Specifically, our research tests the hypothesis that adaptations to the bivalve host drive the evolution of virulence traits and pathogenic potential. We studied interactions of *V. parahaemolyticus* and the blue mussel *Mytilus edulis in vitro* and *in vivo*. Transcriptional profiling combined with the evaluation of specific knockout mutants provided evidence for (i) the involvement of a type III secretion system in the colonization and asymptomatic carriage of *V. parahaemolyticus* in bivalve hosts, (ii) the induction of O-antigen modifications and biofilm formation upon exposure to mussel hemolymph, (iii) a lifestyle switch from commensalism to pathogenicity induced by elevated seawater temperature, and (iv) its reciprocal regulation by cell-density dependent quorum sensing. Taken together, our findings provide first insights into the molecular machinery employed by *V. parahaemolyticus* to grow inside an environmental host organism and have implications for our understanding of transmission and infection processes of this emerging pathogen.

HMV03

Dual symbiosis of the vent shrimp *Rimicaris exoculata* with filamentous gamma- and epsilonproteobacteria at four Mid-Atlantic Ridge hydrothermal vent fields

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The shrimp *Rimicaris exoculata* from hydrothermal vents on the Mid-Atlantic Ridge (MAR) harbors bacterial epibionts on specialized appendages and the inner surfaces of its gill chamber. Using comparative 16S rRNA sequence analysis and fluorescence *in situ* hybridization (FISH), we examined the *R. exoculata* epibiosis from four vents sites along the known distribution range of the shrimp on the MAR. Our results show that *R. exoculata* lives in symbiosis with two types of filamentous epibionts. One belongs to the Epsilonproteobacteria, and was previously identified as the dominant symbiont of *R. exoculata*. The second is a novel gammaproteobacterial symbiont that belongs to a clade consisting exclusively of sequences from epibiotic bacteria of hydrothermal vent animals, with the filamentous sulfur oxidizer *Leucothrix mucor* as the closest free-living relative. Both the epsilon- and the gammaproteobacterial symbionts dominated the *R. exoculata* epibiosis at all four MAR vent sites despite striking differences between vent fluid chemistry and distances between sites of up to 8500 km, indicating that the symbiosis is highly stable and specific. Phylogenetic analyses of two mitochondrial host genes showed little to no differences between hosts from the four vents sites.

In contrast, there was significant spatial structuring of both the gamma- and the epsilonproteobacterial symbiont populations based on their 16S rRNA gene sequences that was correlated with geographic distance along the MAR. We hypothesize that biogeography and host - symbiont selectivity play a role in structuring the epibiosis of *R. exoculata*.

HMV04
Host-specific nitrogenase gene expression in the gut of dry-wood termites (Kalotermitidae)

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The nitrogen-poor diet of wood-feeding termites is compensated by the capacity of the gut microbiota to fix atmospheric nitrogen. Nevertheless, the bacteria responsible for this activity are largely unknown. In the present study, we studied expression of nitrogenase genes (*nifH* homologs) in dry-wood termites (Kalotermitidae) – a group of termites known to possess the highest rates of nitrogenase activity in the acetylene reduction assay. Terminal-restriction-fragment length polymorphism (T-RFLP), cloning, sequencing, and phylogenetic analyses of *nifH* homologs and their mRNA transcripts demonstrated that despite a high diversity of nitrogenase genes, only a core set of four homologs is expressed. The patterns of gene expression were specific for the termite species studied and were correlated with the hydrogen partial pressure in the hindgut, measured by microsensors, but were not affected when termites were incubated under external hydrogen. In *Kalotermes flavicollis* and *Incisitermes marginipennis*, the most highly expressed genes belonged to the *Treponema*, *Bacteroidales*, and the Proteobacteria-Cyanobacteria group. In *Neotermes castaneus* and *Cryptotermes longicollis*, however, the most abundant transcripts fell into the previously detected termite-gut specific *anfH* group. Recovery of the *anfH* genes from capillary-picked flagellate suspensions of *Devescovina* flagellates provides strong evidence that they belong to "*Candidatus* Armantifilum devescovinae", the ectosymbiont covering the surface of these flagellates, which have coevolved with their host flagellates in dry-wood termites.

HMV05
In vivo model of a simplified and defined human intestinal microbiota

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Owing to the complexity of the microbial community in the gastrointestinal tract, its interactions with the host are difficult to study. Therefore we established an *in vivo* model of a simplified and defined human intestinal microbiota in Sprague Dawley (SD) rats. We selected bacteria that represent dominant species in the human gut, whose genome sequence is available and that mimic at large the metabolic activity of the human gut microbiota: *Bacteroides thetaiotaomicron*, *Bifidobacterium longum*, *Anaerostipes caccae*, *Blautia producta*, *Clostridium butyricum*, *Clostridium ramosum*, *Lactobacillus plantarum* and *Escherichia coli*. Our analyses show that all these species were able to establish in the gastrointestinal tract of previously germfree rats. We observed the same colonisation pattern in the caecum, the colon and in the faeces. The microbiota responded to dietary modifications by changes in the relative proportions of the community members. Such changes were observed in reponse to inulin, pectin, resistant starch or a Western style diet.
It has been proposed that the fermentation product butyrate may be an important factor in the prevention of colorectal cancer. However, it is still an open question whether these preventive effects are due to the presence of the butyrate formed from fiber, the fiber itself, or the bacteria involved in the fiber conversion. To answer this question we compared rats that differ in their microbiological status and in their diet. Conventional rats and rats associated with the human simplified microbiota were injected with azoxymethane (15mg/kg BW) to induce colon cancer. Both groups were fed a fiber-rich or a control diet. The animals were killed in week 24 and the total number of preneoplastic lesions (aberrant crypt foci, ACF) and tumors were scored.
Independent of the microbial status both types of rats produced more butyrate when fed a fiber-rich diet (conventional: 9.0 mmol/g dry matter (DM), simplified microbiota: 3.6 mmol/g DM), as compared to the control diet (conventional: 4.2 mmol/g DM, simplified microbiota: 0.8 mmol/g DM). The simplified microbiota-associated rats had significantly ($p < 0.01$) more ACF (333) in their colon than the conventional animals (270).

HMV06
Host driven micro-evolution of asymptomatic bacteuria *Escherichia coli* strain 83972 in the human urinary tract

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Escherichia coli, the major cause of uncomplicated symptomatic urinary tract infections (UTI), may also cause asymptomatic bacteriuria (ABU), i.e. a carrier state without symptoms. In contrast to acute pyelonephritis isolates, many ABU strains fail to express functional virulence factors, suggesting that the difference in virulence might explain the severity of infection. The molecular mechanisms that underpin ABU are not well understood. Bacteria associated with ABU are often left untreated and may even be beneficial in preventing symptomatic infection by more virulent organisms (bacterial interference). In this study the strain 83972, that was originally isolated from a young female with long term ABU, was used for deliberate colonization of patients with recurrent UTI. To study adaptational changes upon long term growth in the bladder, consecutive re-isolates were analyzed with regard to changes in their transcriptome and proteome as well as genome rearrangements. The results obtained were compared with those from re-isolates from a control experiment, in which strain 83972 was propagated over two months in pooled human urine under *in vitro* conditions. Whole genome sequences analysis of the prototype asymptomatic bacteriuria strain *E. coli* 83972 and its re-isolates obtained from several patients demonstrated that host-specific adaptation drives genome, transcriptome and proteome divergence. This is the first genome-wide example of bacterial evolution in human hosts and provides the surprising insight that individual hosts essentially will imprint their microflora through alterations in metabolic and virulence-related genes and in pleiotropic regulators of bacterial gene expression.

HMV07
Complement factor H and CFHR1 facilitate adhesion to *Candida albicans* and enhance antimicrobial activity of human neutrophils

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The host complement system plays an important role in protection against infections. Several pathogenic microbes acquire complement inhibitory molecules, such as factor H, in order to evade complement. Human factor H was shown to bind to the opportunistic human-pathogenic yeast *Candida albicans* and also to host cells. In this study we analyzed whether factor H and other factor H family proteins play a role in host-pathogen interaction.
Both *C. albicans* and blood-derived human neutrophils bound factor H, factor H-like protein 1 (CFHL1) and factor H-related protein 1 (CFHR1), from human plasma and also as recombinant proteins, determined by flow cytometry, Western blot and ELISA. The main binding sites responsible for interaction with neutrophils were localized to the seventh and the two C-terminal domains of factor H, using recombinant factor H fragments in flow cytometry experiments. Complement receptor type 3 (the integrin CD11b/CD18) was identified as the major cellular receptor for factor H, CFHL1 and CFHR1 on human neutrophils, using receptor specific antibodies in blocking experiments. Factor H and CFHR1 supported neutrophil migration in a transwell assay. Adhesion to and phagocytosis of *C. albicans* by neutrophils was increased by factor H, CFHL1 and CFHR1. Furthermore, yeast-bound factor H and CFHR1 enhanced generation of reactive oxygen species, determined by a fluorescence assay, and the release of the antimicrobial protein lactoferrin by neutrophils, measured by ELISA from supernatants of co-cultures. The presence of factor H and CFHR1 on *C. albicans* resulted in an increased killing by neutrophils.
In conclusion, we show that factor H, CFHL1 and CFHR1 enhance neutrophil adhesion via CD11b/CD18. Furthermore, the results indicate that factor H and CFHR1, when bound on the surface of *C. albicans*, enhance antimicrobial activity of human neutrophils and lead to a more efficient killing of the pathogen.

HMV08
Time resolved quantitative proteome profiling of host-pathogen interactions: The *S. aureus* RN1HG response to human S9 cells after internalization

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Staphylococcus aureus is both a successful colonizer and an important pathogen in humans and can cause a wide spectrum of infectious diseases. Although this microorganism has traditionally not been considered as an intracellular pathogen, there are a number of reports of its ability to invade and persist in a variety of laboratory cell culture models [Garzoni & Kelley 2009]. However, due to the low numbers of bacteria available from such internalization experiments, the investigation of bacterial adaptation reactions to internalization at the genome-wide level is still a challenge. In this study, we developed a new workflow for the analysis of internalized *S. aureus* cells by a combined pulse-chase and gel-free proteomics approach.
Before infection, *S. aureus* RN1HG cells carrying plasmid pMV158GFP [Nieto & Espinosa 2003] with a *mobM-gfp* reporter gene fusion were fully labeled with heavy amino acids using SILAC [Ong *et al.* 2002] in an adapted cell culture medium (pMEM). After complete labeling has been achieved, *S. aureus* RN1HG cells were co-cultivated with S9 human bronchial epithelial cells [Zeitlin *et al.* 1991] in non-labeled MEM medium allowing internalization by the S9 cells. Subsequent to the killing of non-internalized *S. aureus* cells samples were examined over a period of 6 hours. Since *S. aureus* harbors a plasmid-encoded *mobM-gfp* reporter gene fusion, bacteria could be enriched by FACS after lysis of eukaryotic cells. Bacteria were collected using a vacuum filtration device and subjected to tryptic digestion on the filtration membrane. The peptides generated were measured by ESI-LC-MS/MS with an LTQ-Orbitrap mass spectrometer and data were analyzed with the Rosetta Elucidator software suite. Using a sorting time of 45 minutes and roughly 2-5x10^6 *S. aureus* cells, we were able to identify and quantify between 300 and 700 *S. aureus* proteins over a period of six hours post-internalization. The proteomic view of this time resolved analysis of the adaptation of *S. aureus* RN1HG to internalization by human epithelial cells will be presented.

HMV09
Quantitative Phosphokinome Analysis of the Met Pathway activated by the Invasin InlB from *Listeria monocytogenes*

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Stimulated by its physiological ligand HGF, the transmembrane receptor tyrosine kinase Met controls mitogenic, motogenic and morphogenic responses. Remarkably, the food-borne human pathogen *L. monocytogenes* also promotes autophosphorylation of Met through its virulence factor InlB and subsequently exploits Met signalling to induce phagocytosis into a broad range of host cells. Although the interaction between InlB and Met has been studied in detail, the signalling specificity of components involved in InlB-triggered cellular responses remains poorly characterized. The analysis of regulated phosphorylation events on protein kinases is therefore of particular relevance, although this could not as yet be characterized systematically by proteomics. Here, we have implemented a new pyridopyrimidine-based strategy that enabled the efficient capture of a considerable subset of the human kinome in a robust one-step affinity chromatographic procedure. To gain functional insights into the InlB/Met-induced invasion process, a quantitative survey of the phosphorylation pattern of these protein kinases was accomplished. With this improved chemical proteomics strategy, we have relatively quantified 143 phosphorylation sites detected on 94 human protein kinases. Interestingly, InlB mediated signalling shows striking similarities compared to the natural ligand HGF. In addition, this systematic approach reveals a new subset of protein kinases including Nek9, which are phosphorylated after 4 min treatment of cells with the Met-activating $InlB_{321}$. Thus this quantitative phosphokinome study suggests a general, hypothesis-free concept for the detection of dynamically regulated protein kinases as novel signalling components involved in host-pathogen interactions.

HMV10
Virulence and communication of *Legionella pneumophila*

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Legionella pneumophila is an amoebae-resistant opportunistic pathogen, which employs a conserved mechanism to replicate within "*Legionella*-containing vacuoles" (LCVs) in amoebae and macrophages. Formation of LCVs involves the bacterial Icm/Dot type IV secretion system and more than 150 translocated "effector" proteins that subvert host cell signaling and vesicle trafficking pathways. Some Icm/Dot substrates bind distinct host phosphoinositides (PIs), and thus exploit these signaling lipids to anchor to the LCV membrane and interfere with vesicle trafficking pathways [1].
LCVs avoid the fusion with lysosomes, intercept the early secretory pathway and interact with the ER. Intact LCVs can be purified from infected *Dictyostelium* amoeba by immuno-magnetic separation using an antibody against an Icm/Dot-translocated effector protein localizing exclusively to LCVs [2]. The proteome of purified LCVs revealed more than 560 host proteins, including novel small GTPases implicated in secretory or endosomal vesicle trafficking pathways.
L. pneumophila employs a biphasic life cycle to replicate in host cells and spread to new niches. To regulate gene expression the bacteria use the signaling molecule 3-hydroxy-pentadecan-4-one (LAI-1, *Legionella* autoinducer-1) [3]. The *lqs* (*Legionella* quorum sensing) gene cluster encodes the PLP-dependent autoinducer synthase LqsA, the putative cognate sensor kinase LqsS and the response regulator LqsR. Functional studies and transcriptome analysis revealed that *lqsA*, *lqsS* and *lqsR* regulate phagocyte interactions, extracellular filaments and a genomic "fitness" island.

[1] Weber, S.S., *et al*., Pathogen trafficking pathways and host phosphoinositide metabolism. Mol Microbiol, 2009. 71: 1341-52.
[2] Urwyler, S., *et al*., Proteome analysis of *Legionella* vacuoles purified by magnetic immunoseparation reveals secretory and endosomal GTPases. Traffic, 2009. 10: 76-87.
[3] Tiaden, A., *et al*., Bacterial gene regulation by α-hydroxyketone signaling. Trends Microbiol. Submitted.

HMV11
Impact of TNF on *Listeria monocytogenes*-containing phagosomes

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TNF is an essential effector molecule for the eradication of intracellular pathogens. Lack of TNF or TNF-receptor 1 (TNFR1) is associated with a high susceptibility to infections with intracellular bacteria such as *Listeria monocytogenes* (LM). We have shown that internalized TNFR1 complexes are instrumental for mediating apoptosis and activation of the lysosomal compartment. Therefore, we hypothesised that these TNFR1 receptosomes deliver activated lysosomal components to the LM-containing phagosomes to mediate their listericidal effects. By confocal microscopy we were able to demonstrate the colocalisation of TNFR1 receptosomes with LM-containing phagosomes. We have adopted a protocol to magnetize LM and to purify phagosomes from infected cells to analyse the recruitment of signature proteins. We found that the recruitment of rab proteins and lysosomal proteins such as cathepsin D to the Listeria-containing phagosome is altered by TNF treatment. We conclude from our findings that TNF causes specific changes in the phagosomal maturation process. Our observations identified a TNF-specific effect on Listeria-containing phagosomes that could lead the way to elucidate the specific contribution of TNF for the eradication of intracellular pathogens.

HMV12
Neisseria gonorrhoeae delays apoptosis in polymorphonuclear leukocytes

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After maturation polymorphonuclear leukocytes (PMNs) circulate in the bloodstream and are attracted by infection sites to parry pathogens and induce an inflammatory response. Without the recognition of an infection, PMNs undergo apoptosis after a lifespan of about 36h and are phagocytosed by macrophages. It is well known that *Neisseria gonorrhoeae* ends up in PMNs after invading the tissue where this pathogen survives and even replicates. Spontaneous apoptosis in PMNs is repressed for approximately 72h, when the cells are infected by *Neisseria*. Delay in apoptosis was estimated during a time course experiment after addition of different *Neisseria* strains, isolated membrane vesicles or lipooligosaccharides from *N. gonorrhoeae*. Activities of key apoptotic enzymes were analysed by Western Blot, ELISA and flow cytometry. The possible signal pathways responsible for delay of apoptosis in PMNs by *N. gonorrhoeae* will be discussed.

HMV13
Commensal and pathogenic staphylococci activate different signalling pathways to modulate the innate immune response in human primary keratinocytes

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Little is known about the impact of different microbial signals on skin barrier organ function and the interdependency between resident microflora and pathogenic microorganisms. Our study shows that commensal and pathogenic staphylococci differ in their ability to induce expression of antimicrobial peptides/proteins (AMPs) and activate different signaling pathways in human primary keratinocytes. We examined the expression of antimicrobial peptides and the signalling pathways activated in human primary keratinocytes in response to stimulation with commensal and pathogenic bacteria. Pathogenic staphylococci induce significantly higher expression levels of the antimicrobial peptides HBD-3 and RNase7 compared with commensal staphylococci, whereas HBD-2 expression differs not significantly between both staphylococci. We could show that induction of expression of HBD-3 and RNase7 are mediated by secreted staphylococcal products, whereas staphylococcal envelope products are responsible for induction of HBD-2 expression. S.aureus conditioned medium induced expression of the AMPs HBD-3 and RNase7 in primary human keratinocytes not via TLR-2, EGFR- and NFκB-activation in contrast to S.epidermidis. Furthermore pathogenic staphylococci activate the MAPK- and PI3K/AKT signaling pathways and suppress NFκB activation. Interestingly, commensal bacteria are able to amplify the innate immune response of human keratinocytes to pathogens by increased induction of AMP expression and abrogation of NFκB suppression suggesting that the two activation pathways can act in a synergistic way. These data indicate that commensal and pathogenic microorganisms evolved specific mechanisms to modulate innate immunity of the skin.

HMV14
Detailed analysis of cyclic di-GMP signaling in virulence phenotypes of *Salmonella enterica* serovar Typhimurium

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Cyclic di-GMP (c-di-GMP) is a secondary signaling molecule present in many Bacteria. Recently, we have investigated the effect of c-di-GMP signaling on biofilm formation in *Salmonella enterica* serovar Typhimurium. Biofilm formation in *S.* typhimurium is characterized by the expression of proteinaceous and polysaccharide extracellular matrix components, which are regulated by the major biofilm regulator CsgD. C-di-GMP signalling stimulates the expression of CsgD and the expression of extracellular matrix components. However, *S.* Typhimurium is mainly known as a pathogen causing self-limiting gastroenteritidis in humans. Using overexpression of a potent di-guanylate cyclase, we could recently show that two basic phenotypes that are major determinants of *Salmonella* virulence, invasion of epithelial cells and induction of proinflammatory cytokines are negatively regulated by c-di-GMP signaling (Lamprokostopoulou et al., 2009). To analyze the role of c-di-GMP signaling in more detail, we tested individual mutants in c-di-GMP metabolizing proteins for their role in invasion and induction of the proinflammatory cytokine IL-8. Task distribution of individual c-di-GMP metabolizing proteins could be shown as distinct groups of di-guanylate cyclases and phosphodiesterases affect the two phenotypes. Therefore, c-di-GMP does not only mediate the transition between biofilm formation and motility, but also the transition between colonization and virulence on the mucosal surface.

[1] Lamprokostopoulou, A., Monteiro, C., Rhen, M., Römling, U. (2009) C-di-GMP controls virulence properties of Salmonella enterica serovar Typhimurium at the mucosal lining, Environ Microbiol, published online.

HMV15
H. pylori selectively disrupts SHP-2 dependent EGFR signaling to evade the antimicrobial impact of the human beta defensin hBD3

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Endogenous antimicrobial peptides have been identified as key elements of innate host defence against infection. Within this peptide family, defensins exhibit a variety of microbicidal activities against Gram-positive and –negative bacteria, mycobacteria, fungi and certain viruses. Recently, the human beta defensin 3 (hBD3) was shown to have antimicrobial potential: it efficiently killed the human gastric pathogen *Helicobacter pylori* after short contact *in vitro*. *H. pylori* type I strains persistently colonize the human stomach, thereby causing severe diseases like ulcer and gastric cancer. Whereas most bacteria are free swimming organisms in the gastric mucosa, a small percentage adheres to epithelial cells. Here, pathogenic type I strains translocate the effector protein CagA into the host cell, which dramatically interferes with cellular signaling pathways like apoptosis and cytoskeletal rearrangements. The mechanisms how *H. pylori* establishes a protective biological niche remain poorly understood. Since *H. pylori*-mediated pathogenesis is a long-term process, this particular question is an important scientific issue. Therefore, we wanted to investigate if and how *H. pylori* escapes from the antimicrobial impact of hBD3 to enable persistent colonization. Intriguingly, we found that initially induced hBD3 expression vanishes over time in order to provide extracellular survival of *H. pylori* during longer infection. Moreover, we could reveal the underlying molecular mechanism that directly leads to *H. pylori* mediated blockage of hBD3. Within this scenario both, the bacterial effector protein CagA as well as the cellular tyrosine phosphatase SHP-2 play essential roles in complex manipulations of EGFR signaling, thereby allowing long term survival of *H. pylori*. Our data provide new mechanistic insights of how *H. pylori* controls complex host cell pathways to conduct them into beneficial directions. Furthermore, they lead to the hypothesis that attachment of free swimming bacteria cause a positive impact on the bacterial population by setting up a protective biological environment.

HMV16
Chlamydia trachomatis regulates host cell metabolism and nuclear NADH signaling

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Chlamydia trachomatis is an obligate intracellular bacterium which actively modifies the metabolism of the host cell for its own growth and replication. There is a dearth of information regarding the factors that control metabolism and apoptosis in Chlamydia-infected cells. The metabolic cofactor, reduced nicotinamide adenine dinucleotide (NADH) is formed during host cell glycolysis. NADH is a freely diffusible molecule and can enter the nucleus, thus changes in cytoplasmic NADH levels due to altered metabolism are reflected in nuclear NADH levels. The transcriptional corepressor, carboxyl-terminal-binding protein (CTBP) can sense nuclear NADH levels and regulate transcription of its target genes and connect metabolism with gene transcription. One of its target genes, the protein-deacetylase, sirtuin-1 (SIRT1) is an important regulator of apoptosis and lifespan. Our studies show that *Chlamydia trachomatis* infection alters the protein binding of NADH in the host cell nucleus as monitored by fluorescence lifetime measurements by two-photon microscopy. We elucidated the role of SIRT1 in Chlamydia development by using the SIRT1 inhibitor, nicotinamide. Nicotinamide inhibited the development of Chlamydia inclusions as measured by the inclusion size and the amount of infectious Chlamydia progeny.

In addition SIRT1 was regulated in transcriptional and protein levels in Chlamydia infected cells. The changes of nuclear NADH fluorescence lifetime suggest that NADH is a possible regulator of host cell alterations during the Chlamydia development cycle controlling not only metabolic but also gene transcription changes. The findings provide new insights into the molecular mechanism of the dynamic interaction between Chlamydia and its host cells.

HMV17

Rab6A and Rab11A regulate *Chlamydia trachomatis* infection and golgin-84 dependent Golgi fragmentation

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Trägerin des Förderpreises

Chlamydia trachomatis acquires essential nutrients including sphingolipids from the host cell. The precise mechanisms underlying these transport processes are not completely understood. We have shown previously that *C. trachomatis* infection caused fragmentation of the Golgi apparatus. Golgi fragmentation is triggered by *Chlamydia*-dependent truncation of the Golgi matrix protein, golgin-84. Inhibition of golgin-84 processing by specific protease inhibitors blocked Golgi fragmentation, inhibited bacterial growth and sphingolipid acquisition. Furthermore, we could show that fragmentation of the Golgi by knock-down of golgin-84 or giantin before infection boosted *Chlamydia* progeny. These data indicated that the structure of the GA regulates *Chlamydia* replication. Rab proteins are important small GTPases that control Golgi structure and *Chlamydia* recruits specific Rab proteins to their inclusions. Therefore, we hypothesized that Rab proteins might regulate *Chlamydia*-induced Golgi fragmentation. Using a RNA interference approach we screened for Rab proteins that inhibit *Chlamydia*-induced Golgi fragmentation. Interestingly, knock-down of Rab6A and Rab11A blocked *Chlamydia*-induced Golgi fragmentation, reduced transport of sphingolipids to the bacteria and decreased bacterial replication. Interestingly, knockdown of p115 induced Golgi fragmentation independently of Rab6A and Rab11A and could rescue *Chlamydia* replication in Rab6A and Rab11A knockdown cells. This study indicates that these Rab proteins are important regulators of chlamydial propagation and Golgi structure.

HMV18

Legionella pneumophila induced IκBζ-dependent expression of interleukin-6

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Legionella pneumophila causes severe community- and hospital-acquired pneumonia. Lung airway and alveolar epithelial cells comprise an important sentinel system in airborne infections. Although interleukin-6 (IL-6) is known as a central regulator of the immune response in pneumonia, its regulation in the lung is widely unknown.
Herein we demonstrate that different *L. pneumophila* strains induce delayed expression of IL-6 in comparison to IL-8. IL-6 expression depended at early time points on flagellin recognition by TLR5, and at later time points on the type IV secretion system. In the same manner, but more rapidly, the recently described transcription factor IκBζ was induced by *L. pneumophila* infection and – binding to the NF-κB subunit p50 - recruited to the il6 promoter. Similarly, histone modifications and NF-κB subunit p65/RelA appeared at the iκbζ and subsequently at the il6 gene promoter, thereby initiating gene expression. Gene silencing of IκBζ reduced *L. pneumophila*-related IL-6 expression by 41 %.
Overall, these data indicate a sequence of flagellin/TLR5- and type IV-dependent IκBζ expression, recruitment of IκBζ/p50 to the il6 promoter, chromatin remodeling and subsequent IL-6 transcription in *L. pneumophila*-infected lung epithelial cells.

HMV19

S. *lugdunensis* SLUSH Peptides-more than haemolysins

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Coagulase-negative staphylococci (CoNS) are becoming more and more important in nosocomial and community-acquired infections such as bacteremia, nosocomial neonatal sepsis, endocarditis and meningitis and often these bacteria are resistant to several antimicrobial agents.
Staphylococcus aureus and *Staphylococcus epidermidis* have been shown to secrete phenol-soluble modulin peptides, which can be sensed by dedicated receptors of the innate immune system and lead to neutrophil responses such as chemotaxis, calium ion flux, and IL-8 release. To further elucidate the ability of neutrophils to sense further CoNS we analyzed the response of human primary neutrophils and monocytic cell lines to culture supernatants from different CoNS species. We found most CoNS species to elicit calcium ion fluxes in leukocytes.
One of these CoNS namely *S. lugdunensis* is outstanding since it behaves more like *S. aureus* than other CoNS regarding its virulence and clinical manifestation in infections. In fact, this pathogen has often been implicated in severe inflammatory infections in recent years. These may proceed aggressively and with severity similar to that of *S. aureus*.
S. lugdunensis secretes three small peptides, SLUSH-A, SLUSH-B, and SLUSH-C, which exhibit synergistic haemolytic activity with *S. aureus*. In order to characterize whether the elevated virulence of *S. lugdunensis* is linked to the presence of these peptides we are currently examining the response of human neutrophils, monocytes and monocytic cell lines to synthetic SLUSH peptides.
Due to its enhanced virulence *S. lugdunensis* is not a typical CoNS species and deserves more attention regarding its interaction with the adaptive immune system.

HMV20

Immunomodulation by YopP from *Yersinia enterocolitica* suppresses RIP1-related signaling of programmed necrosis

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Many pathogenic microorganisms has evolved strategies to modulate host cell death or survival pathways for colonizing host organisms. Enteropathogenic *Yersinia enterocolitica* triggers apoptosis in macrophages by engaging YopP which is injected by type III secretion into host cells. YopP inhibits TLR-induced NF-kappaB activation and impedes the initiation of a coordinated immune response by the infected cell. We show that immunomodulation by YopP in macrophages comprises the receptor-interacting protein (RIP1) kinase pathway which regulates inflammation and cell death. RIP1 was rapidly phosphorylated and activated in macrophages after infection with YopP-negative yersiniae or stimulation with LPS. The induction of the RIP1 kinase activity triggered caspase-independent cell death when apoptosis execution and NF-kappaB activation were prevented. Wild type Yersinia on the contrary counteracted the phosphorylation of RIP1 and repressed its phosphotransferase activity by the delivery of YopP. YopP thereby inhibited the autophosphorylation of RIP1 as well as external RIP1 phosphorylation events. The impairment of RIP1 by YopP obviated the shift of death from apoptosis to programmed necrosis. These results indicate that RIP1 plays a critical role in the regulation of cell death pathways in bacteria-infected macrophages. The action of YopP on the RIP1 pathway restricted controlled necrosis which could be beneficial for the pathogen in the course of bacterial infection.

HMV21
Systematic analysis of protein interactions between *Yersinia enterocolitica* pYV pathogenicity and human host proteins

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Yersinia enterocolitica is a frequent cause of acute gastrointestinal disease. It evades the host's immune response by injecting, via a virulence-plasmid encoded Type 3 secretion system, anti-host effector proteins into the host cell cytoplasm. To gain a better understanding of Yersinia pathogenesis, we aimed at characterizing pathogen-host protein interactions on a proteome-wide scale. We cloned all 74 open reading frames (ORFs) of the prototypical virulence plasmid pYVa127/90 plus functional domains of 27 of these ORFs into yeast-2-hybrid bait and prey vectors (194 plasmid constructs in total). This clone collection was subsequently tested for protein interactions between Yersinia proteins, and screened for pathogen-host interactions using four human cDNA libraries. We identified 264 interactions between Yersinia proteins, and 760 distinct pathogen-host protein interactions, 157 of which were high-confidence interactions. A subset of interactions was validated by LUMIER pull-down assays and approximately 50% could be confirmed. As anticipated, proteins belonging to the type III secretion apparatus predominantly interacted with themselves, whereas the secreted effectors Yop E, H, O, M, P and T showed a strong enrichment for interactions with host proteins. By connecting the Yersinia proteins into a human interactome network via the identified pathogen-host interactions, we were able to show that Yersinia proteins preferentially target highly connected human proteins similar to viruses. A functional analysis using gene ontology annotations of cellular interaction partners reveals the preferential targeting of proteins involved in cytoskeletal reorganisation, transcription, posttranslational modification and signal transduction, suggesting that these cellular processes reflect core pathogenic events during infection. Experimental assays using Yersinia mutants are currently performed to address the biological and functional significance of the identified interactions

HMV22
Control of type III secretion in the plant pathogen *Xanthomonas campestris* pv. *vesicatoria*

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The Gram-negative bacterial plant pathogen *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*) employs a type III secretion (T3S) system to translocate bacterial effector proteins into eukaryotic cells. The T3S system is a highly complex protein transport apparatus that spans both bacterial membranes and is associated with an extracellular pilus and a predicted channel-like translocon in the plant plasma membrane. Pilus formation and insertion of the translocon presumably precede the translocation of effector proteins, suggesting that T3S is a hierarchical process. In *Xcv*, secretion of different T3S substrates is controlled by the cytoplasmic proteins HpaB and HpaC, and the secreted regulator HpaA. HpaC promotes secretion of translocon proteins after pilus formation and is thus involved in the T3S substrate specificity switch. The HpaC-mediated control of T3S substrate specificity depends on the cytoplasmic domain of the conserved inner membrane protein HrcU, which is a component of the T3S system and might contribute to substrate recognition. Both HpaC and HrcU interact with each other and also bind to HpaB, which is a global T3S chaperone for effector proteins. HpaB promotes secretion and translocation of at least eleven different effector proteins. Activity of HpaB is presumably regulated by the secreted HpaA protein, which is essential for T3S. Complementation studies revealed that the function of HpaA depends on the N-terminal T3S signal and the C-terminal HpaB-binding site of HpaA. According to a current model, binding of HpaA to the T3S chaperone HpaB allows assembly of the extracellular components of the T3S system whereas secretion of HpaA liberates HpaB and is thus key for effector protein translocation.

HMV23
Breaking the code of DNA-binding specificity of TAL-type III effectors

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Pathogenicity of many bacteria depends on the injection of effector proteins via type III secretion into eukaryotic cells in order to manipulate cellular processes. Members of the large family of TAL (transcription activator-like) effectors from Gram-negative plant pathogenic Xanthomonas spp. bacteria are important virulence factors. They employ eukaryotic features to act as transcriptional activators in the plant cell nucleus. Nuclear import of TAL effectors is mediated via nuclear localization signals and host gene induction is supported by a transcriptional activation domain. TAL effectors directly bind to promoters of target host genes via a central domain of tandem repeats. Recently, we solved a 20-year enigma how specificity of TAL effectors is encoded and clarify the functionality of a novel DNA binding domain. Recognition sequences of TAL effectors (Hax2, Hax3, Hax4) with so far unknown specificity were predicted and experimentally confirmed in a transient reporter system using Agrobacterium-mediated expression in planta. The TAL repeat domain architecture enabled the construction of artificial effectors (ARTs) with novel repeat combinations. These ARTs exhibited predicted specificities, indicating that DNA-targeting domains with novel preferences can be generated. Our work characterizes a unique type of DNA-binding domain with high potential for biotechnology. TAL effectors can now be used to design DNA-binding domains with easily predictable specificities for almost any DNA target sequence.

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HMV24
Donuts and tubules: mechanism of type VI protein secretion

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The recently identified type VI secretion systems (T6SS) are present in a multiplicity of pathogenic proteobacteria and are encoded by a conserved gene cluster comprising approx. 15 open reading frames. Since the identification of T6SS large progress has been made at all levels, including regulation of T6SS, their impact on bacterial virulence, the function of effector proteins and the mechanism of T6S. T6SS contribute to virulence development of various pathogens and are often induced or activated upon contact with host cells. Recent structural and mechanistic studies revealed unique features of T6SS that separate T6SS from other export systems. Structural similarities between the T6SS-specific exoproteins Hcp and VgrG and components of the cell puncturing device of tailed bacteriophages, suggest that T6SS mimic the mechanism of bacteriophages by puncturing host cell membranes to translocate effector proteins, representing a novel mechanism of effector delivery. Secretion systems are frequently energized by hexameric ATPase components that directly act on secretory proteins. T6SS harbor a conserved and essential ring-forming AAA+ protein, ClpV, which, however, does not process the exoproteins Hcp and VgrG. Instead, ClpV disassembles VipA/VipB complexes that form large cogwheel-like tubules. While the role of this severing activity is currently unknown, this finding illuminates an unexpected role of an ATPase component in protein secretion. Novel data addressing the physiological role of ClpV-mediated disassembly of VipA/VipB tubules and the interaction of both partners will be presented.

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HYP01
No correlation between alcoholic hand rub consumption and compliance in 73 German ICU's

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Background: There are two systems available to determine hand hygiene (HH) compliance: direct measurement by observing of HH opportunities (HHO) in clinical practice and measurement of alcoholic hand rub consumption (AHC) as a surrogate parameter. Hospitals participating in the German national HH campaign are measuring AHC on a mandatory basis, while observation is voluntary.
Objectives: We correlated the AHC with the HH compliance of all ICU's providing both data in 2008.
Methods: The following data are provided annually per unit: consumption of AHC, the number of annual patient days (PD) per unit, type of unit. The AHC in ml per PD for individual units is fed back to the hospitals together with reference data stratified by type of unit. All participants used defined observation tools. Spearman correlation coefficient was used in order to define the relationship between the two parameters. Significance was tested using Wilcoxon rank sum test.
Results: Both datasets were available for 73 ICU's. There was no correlation neither for all units (ρ=0.019, p=8,809) nor for special types of units (surgery ρ=-0.068, p=0,819; internal medicine ρ=0.061, p=0,239; interdisciplinary ρ=0.234, p=0,755; neonatology ρ=-0.131, p=0,868).
Conclusion: Based on our data, there is no correlation between AHC and compliance rates. Observations to determine compliance rates are very challenging. Satisfactory inter rater reliability is hard to achieve. Evaluation of observation results revealed an inter rater reliability between 30% and 60%. Observation is an excellent tool to determine quality, e. g. whether HHO's are appropriately detected in clinical practice. However, without extensive training and evaluation, quantitative interpretations of compliance rates should be done carefully. Measurement of AHC provides a feasible and perhaps more reliable system to detect quantitative changes in HH behaviour, provided that there is a stable and unit based order system of AHC.

HYP02
Why do polyphasic nosocomial outbreaks happen? - A systematic review

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Sometimes the epidemic curves of nosocomial outbreaks show additional peaks of infection despite the introduction / enforcement of appropriate infection control measures. A systematic review was performed using the Worldwide Outbreak Database (www.outbreak-database.com), PubMed, and a hand search of reference lists. Search terms applied were "NOSOCOMIAL", "OUTBREAK", "EPIDEMIC", and "POLYPHASIC". Data got collected on setting (place, time, duration, ward, kind of unit), type of infection (number of cases, pathogen, type of infection), epidemiological features (source, mode of transmission, typing), and infection control measures. Findings from polyphasic outbreaks were then compared to monophasic nosocomial outbreaks as filed in the Outbreak Database. 124 polyphasic nosocomial outbreaks got included. Surgical departments were more often affected by polyphasic outbreaks (p<.01), and hepatitis B virus was determined as the predominanat causative agent (p<.01). The following types of infection were more frequently observed in polyphasic outbreaks: BSI (p<.05), respiratory tract infection (p<.01), wound infection (p<.01), UTI (p<.01), skin and soft tissue infection (p<.05), and meningitis (p<.05). All kinds of infection control measures (except for change of equipment, protective clothing, and vaccination) were significantly more often applied in polyphasic outbreaks. There were 245 sources identified in the 124 polyphasic outbreaks compared to 2.169 sources in the 2.089 monophasic events. The greater number of infections and infection control measures is most probably a consequence of the prolonged outbreak. Most likely the existence of an undiscovered additional source or the formation of such a secondary source during the first phase of the outbreak (rather than not identifying its primary source) was responsible for further outbreaks phases. Infection control staff should be aware of the possibility of a secondary source whenever investigating a nosocomial outbreak.

HYP03
C. difficile strain dependency of sporocidal activity of disinfectants

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Background: *C. difficile* infections (CDI) are recognized as the major causes of nosocomial gastroenteritis with increasing morbidity and mortality. A considerable part of CDI is acquired by transmission through direct or indirect contacts. Thus sporocidal activity of disinfectants is crucial for prevention of transmission.
Objective: To compare sporocidal activity of different disinfectants using spores of different genotypical and epidemiological characterized *C. difficile* strains.
Methods: B. subtilis ATCC 6633, one *C. difficile* strain isolated form only one patient, one endemic strain, ribotyp 027, 126 and 078 (1.5 to 5x106 CFU per ml) were used to test glutaraldehyde (2%), peracetic acid (PAA) (0.05%), sodium hypochlorite (500 ppm), perform® (2%) and gigasept PAA concentrate® (1 and 2%) for sporocidal activity according to DIN EN 13704. A reduction factor (Rf) of ≥ 3 was regarded as effective.
Results: Rf for glutaraldehyde (30 min) were 0.89 for *B. subtilis* and > 4 for all *C. difficile* strains. Rf for PAA (15 min) were 0.17 for *B. subtilis* and between 0.1 and 3.94 for *C. difficile* strains. Sodium hypochlorite was effective after 15 min for *B. subtilis* and 5 min for all *C. difficile* strains. Perform® was effective >120 min for *B. subtilits* and 60 to 180 min for *C. difficile*. Gigasept PAA concentrate® 2% (1%) was effective after 5 (5) min for *B. subtilis* and between 5 (15) and 15 (30) min for *C. difficile* strains. The unique *C. difficile* strain and ribotyp 027 and 078 were less susceptible to most disinfectants compared to other *C. difficile* strains and *B. subtilis* ATCC 6633 used as surrogate for sporocidal activity.
Conclusion: In comparison to *B. subtilis, C. difficile* strains were less susceptible to PAA and Gigasept PAA concentrate® and more against sodium hypochlorite. Susceptibilities to disinfectants seem to be strain dependent. Our results question the usefulness of surrogate testing for the determination of sporocidal activity of disinfectants.

HYP04
Resuscitation of *Pseudomonas aeruginosa* from VBNC-state in biofilms after disinfection in close-to-practice drinking water test units

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Biofilms in drinking water installations are a potential source for hygienically relevant bacteria. Elevated water temperatures, mainly stagnating water and a high diversity of materials may promote the colonization of biofilms by pathogens and their dissemination into the water. In the present study the long time (7 months) potential risk of tap water contamination by hygienically relevant bacteria after a single contamination incident is investigated. Experiments are performed in close-to-practice test units running a representative water consumption profile. Contamination potentials were studied for different water qualities, temperatures and installation materials. *P. aeruginosa* was added to established biofilms on (i) elastomeric material (EPDM) not recommended for application in drinking water, (ii) EPDM, (iii) plastomeric material (PEXc) and (iiii) copper the latter three recommended for application in drinking water as pipes or gasket. Depending on water quality, material and temperature, *P. aeruginosa* was detectable by cultivation methods in the stagnating water until 2 months after contamination but not in the corresponding biofilm. During the following 5 months *P. aeruginosa* was no longer detectable neither in biofilm nor in the stagnating water by cultivation methods but by the culture independent FISH method in biofilm indicating that *P. aeruginosa* had entered a VBNC-state. After disinfection 7 months after contamination with various ClO_2 concentrations (0.2 to 20 mg/L ClO_2) applied several times until rest concentrations remained, *P. aeruginosa* was again detectable in the stagnating water by cultivation methods on EPDM only. *P. aeruginosa* in VBNC-state survived even high disinfection doses in thick biofilms, disseminated culturable cells into the stagnating water and hence was resuscitated from its VBNC-state.
Acknowledgements
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HYP05

The effect of hospital volume on surgical site infection rates following orthopeadic procedures: What seems to be the most appropriate threshold?

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Background: Surgical site infections (SSI) belong to the most common nosocomial infections. The association between the operation volume and the risk of SSI is an importend issue. We wanted to investigate if high volume hospitals have lower surgical site infection (SSI) rates following orthopaedic operations.
Objectives: We used 3 orthopaedic procedures to analyse the association between the annual volume of operations and the incidence of SSI.
Method: >The German national nosocomial infection surveillance system (KISS) is focussing on surveillance of 29 selected indicator procedures. KISS is using the definitions of the Centers for Disease Control and Prevention and the method of the National Health Safety Network for recording SSI. We analysed the data for 3 orthopaedic procedures types performed between January 2001 and June 2006. 35.579 hip prosthesis procedures, 29.237 knee prosthesis procedures and 16.642arthroscopic operations were included. Two limits were considered: > 50 and > 100 procedures per year.
Results: Higher SSI rates in low volume hospitals were found for all 3 three procedure types considering the limit of an annual number of 50 operations per year with significant differences for hip and knee prosthesis. (Table).
Using the limit of > 100 operations per year the SSI rates were even lower in the group of high volume hospitals (Knee prosthesis SSI rate 0.91%, Hip prosthesis SSI rate 0.94%).

Type of operation	SSI rate in hospitals with an annual number of procedures <= 50 OPs	SSI rate in hospitals with an annual number of procedures > 50 OPs	Odds ratio	p value
Hip prosthesis	1.73 %	1.06 %	1.65	0.017
Knee prosthesis	1.91 %	1.00 %	1.93	0.016
Arthroscopic operations	0.33 %	0.27%	1.24	0.561

Conclusion: The annual volume of operations has a significant impact on SSI rates following orthopaedic procedures. The hospitals should perform at least 50 operations annually, but more than 100 operations per year is even better.

HYP06

Comparative investigation of disinfectant testing methods

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Effective disinfectants are essential for realizing hygiene in hospitals as well as in food production and animal husbandry. This cope requires the determination of effectivity of disinfectants. Commonly, methods for disinfectant testing consist of several steps and consequently are very time and material consuming. The present investigation compares quantitative methods for evaluation bactericidal and yeasticidal activity of chemical disinfectants. Aim of the study was to determine whether less extensive methods led to results comparable with those obtained by applying the method given in the guidelines of the DVG.
According to the DVG guidelines a quantitative suspension test was performed. After a contact with a disinfectant the number of surviving test organisms (viable counts) was determined by two spread plate techniques: (i) DVG method (dilution in a total volume of 10 ml) and (ii) microtiter plate method (dilution in a total volume of 250µl performed in microtiter plates) and (iii) the most probable number method (MPN) (performed in microtiter plates). Subsequently, the minimal bactericidal and yeasticidal concentrations as well as the decimal log (lg) reductions were calculated.
Three different disinfectant substances (sodium hydroxide, glutaraldehyde and peracetic acid) combined with six test organisms (according to the DVG guidelines) were included.
All three methods identified the same minimal bactericidal and yeasticidal concentrations.
Furthermore, a comparison of the viable counts and the lg reductions obtained by the spread plate techniques (DVG and microtiter plate method) revealed a complete agreement. Thus, the microtiter plate method is a suitable alternative and could help to reduce labor costs.
In contrast, 25% of the results obtained by the MPN method significantly differ from the two others methods. Hence, the MPN method cannot be recommended for effectivity testing.

HYP07

Detection of Human Adenovirus in inlet and outlet samples from a sewage treatment plant

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Wastewater contains a variety of microorganisms and in particular enteric viruses and coliphages. To designate the reduction of viruses, phages, and bacteria and to identify an indicator for viruses in sewage water, samples were collected every one or two weeks from the inlet of the activated sludge basin and the outlet of a treatment plant. The samples were filtrated using the VIRADEL method with a high-salt alkaline phosphate buffer. For human Adenovirus (HAdV) qReal-Time PCR was carried out. Somatic coliphages were quantified using the double layer plaque assay according to DIN EN ISO 10705-2. The quantification of *E. coli* and intetestinal Enterococci were done according to the standard reference method (DIN EN ISO 9308-3 and 7899-1). HAdV were detected in 100% (46/46) of the inlet and outlet samples. 100% (23/23) of the inlet samples but only 91% (21/23) of the outlet samples were positive for coliphages. All samples (100%-42/42) were positive for *E. coli* and Enterococci. The quantitative analysis has revealed an average reduction of $1.5x10^3$ for adenovirus, $2.7x10^2$ for somatic coliphages, and $5.0x10^2$ for *E. coli* and Enterococci. No correlation between the different parameters could be detected. This study shows a high load of HAdV and somatic coliphage in treated sewage water. At this time no indicator parameter could be recommended. More samples and a variety of enteric viruses have to be analyzed.

HYV01

Increase of alcoholic handrub consumption in 1261 German non-ICU units within one year

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Objective: Hand hygiene is the essential method to prevent hospital acquired infections. HAND-KISS, a module of the German Krankenhaus-Infektions-Surveillance-System (KISS), established a new module for the systematic surveillance of alcohol-based hand rub consumption (AHC) in 2007. We compared the consumption data for the years 2007 and 2008 of those hospitals, participating in HAND-KISS and the national hand hygiene campaign since 2008.
Methods: The following data are provided annually per unit: AHC, the number of annual patient days (PD) per unit and the unit characteristics. HAND-KISS calculates the consumption in ml per PD for individual units and provides reference data stratified according to the specialty of the unit. We examined the data of those units providing consecutive data for the years 2007 and 2008. Differences of consumption were tested by a Wilcoxon rank sum test for significance.
Results: 181 hospitals provided consumption data of 2041 non-ICUs in 2007, and 330 hospitals of 3328 non-ICUs in 2008. 1261 non-ICUs out of 134 hospitals provided data for both years. The median overall consumption rate for 2007 is 14.6 ml/PD (interquartile range (IQR) 10.6 - 20.8 ml/PD) and 16.9 ml/PD (IQR 12.2 – 23.8 ml/PD) in 2008.
There was a significant increase between 2007 and 2008 in all types of units. The average increase of consumption is 13%. The maximum increase is found in medical and medical-surgical units representing an increase of 14%.
Conclusion: The consumption of AHC in participating units is increasing. Since it is a mandatory measurement, all hospitals participating in the national hand hygiene campaign are also providing data to HAND-KISS. The placement of local consumption data in comparison to national reference consumption data provides a benchmarking system. This is the basis for ongoing discussions about hand hygiene and sustained sensibility for this topic in health care workers.

HYV02
Baseline hand hygiene compliance rates in 126 hospitals participating in the national German hand hygiene campaign

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Background: The national German hand hygiene (HH) campaign "AKTION Saubere Hände" started at January 1st 2008. The campaign is based on the WHO "Clean Care is Safer Care" campaign and is funded for three years be the German ministry of health. By November 1st 2009, 624 health institutions are actively participating. Among other measures, HH compliance observations are an essential but voluntary part of the campaign.
Objectives: We represent baseline compliance data from 126 hospitals stratified by indication and type of unit.
Methods: All participants used defined observation tools and where trained by the campaign team members. The definition of HH opportunities is based on the WHO Model "My 5 moments of hand hygiene".
Results: The overall median baseline compliance in 126 hospitals was 62.5% (IQR 50.49 - 73.1%). The compliance per indication is shown in table 1. There was no difference between ICU's (overall median 62.2%) and non-ICU's (overall median 62.9%). The highest compliance rate was found in neonatology units (median 72.2%, IQR 66.7 - 78.6%), the lowest in interdisciplinary units (median 60.2%, IQR 51.9 - 72.2%). Overall 92585 HH opportunities from 126 hospitals were observed. The following rates stratified by indication were observed (before patient contact 54.4% (IQR 39.1-70.7%), before aseptic task 60% (IQR 40.8-76.5%), after contact with infectious material 77.5% (IQR 61.5-92.4%), after patient contact 71.9% (IQR 61-81.7%), after patient surroundings contact 51.1% (IQR 36.8-66.4%).
Conclusions: So far only few observation data were available for German hospitals, so this is the first large scale dataset. Compared to other international campaigns, Germany starts at a fairly high level of compliance. Considering the relatively low compliance before patient contact and before aseptic tasks and the importance of these indications for patient outcome, the campaign is focussing within the next year on intervening these two situations.

HYV03
MRSA contamination in ambulance cars

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The increase of MRSA affects the logistics of ambulance services, mostly due to extensive disinfection procedures that take ambulance vehicles out of service for more than 90 min. The contamination of surfaces in ambulance vehicles after transport of an MRSA colonized patient was assessed by swabbing defined surfaces in close proximity to the patient and at distant sites of the car. Transportation time never exceeded 20 min, thus representing the majority of inner-city transports. After swabbing, MRSA was enriched in BHI medium resulting in highly sensitive detection. 90 transports were evaluated by Nov 24, 2009. MRSA could be detected in nine transports. It was exclusively found at the head rest and at the holds of the stretcher. No contamination of distant sites was detected, indicating that under the current local regulations at least 90 cars would need to be extensively disinfected and taken out of service, before one with a contaminated distant surface might be identified. The dominant spa-type was t003, most prevalent at the University Clinic of Würzburg. Limitations of the study: the area accessed by swabbing was limited; furthermore, there was no information available on individual patients and therefore more than one transport per subject might have been included. Conclusions: The data suggest that for transportation times < 20 min, disinfection procedures should focus on the head piece and other sites in close proximity to the patient. Due to the expected prevalence of unknown MRSA carriers in Germany, disinfection of patients´ contact surfaces such as head pieces - irrespective of MRSA status - appears to be more effective than exaggerated and time-consuming measures after transport of the minor fraction of identified MRSA carriers.

HYV04
Five-Years –Surveillance of invasive Aspergillosis in a German tertiary Care Hospital

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Introduction: Invasive aspergillus infections (IA) are a severe complication in immuncompromised, especially transplantated, patients. Unfortunately diagnosis can be difficult in multimorbide patients and one must assume that several patients die failing diagnosis and special treatment. The aim of this study was to detect high risk patients to introduce special preventive measures and to get information about quick diagnosis of infection.
Patients and methods: After implementation of a surveillance protocol in 2003, we surveyed all hospitalized patients by positive microbiological cultures, pathologic diagnoses and/ or pharmacologic informations about antifungal treatment in 2003-2007: Cases were reviewed and were classified according to international consensus criteria (EORTC). Possible nosocomial acquisition was determined.
Results: Among the 704 alerts, 214 IA cases were observed: 56 proven cases (26%), 25 (12%) probable cases and 133 (62%) possible cases. The incidence was 1.6 %. Among the proven and probable IA cases, we observed 56% nosocomial cases (45/81) and 21% cases of undetermined origin (17/81). Many patients (37/81) had organ transplantation, followed by bone marrow transplantated patients (8/81), but we also found a high number of other patient groups. We detected 10 malignancy tumor patients and 26 other patients with chronical organ diseases. Among the proven and probable cases we detected 53 patients undergoing immunsuppressive therapy. 45% of the proven patients died during their hospital stay. 11 of the proven patients did not get any antifungal treatment, seven of those patients died. Overall 32 patients (57%) were diagnosed by pathologic results, 16 patients were diagnosed by autopsy and seven patients (13%) would not have been diagnosed without autopsy.
Conclusion: High risk patients are not only patients undergoing transplantation, but all patients undergoing immunsuppressive therapy. Diagnosis is difficult and 13% of all cases are diagnosed by autopsy only. Thus, this study shows the importance of IA surveillance in all high-risk units to improve infection prevention measures.

HYV05
Impacts of the pandemic Influenza A/H1N1/09 on a Tertiary Care Hospital

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Methods: The epidemiology of pandemic influenza A/H1N1/09 among the patients of a tertiary care hospital in south-west Germany is recorded prospectively.
Results: Including November 2009 the number of hospitalised patients was low, whereas the burden of disease in the ambulatory health care setting was high (data from RKI-AG Influenza).
Considering the incubation period of 3 days, only single cases of possibly nosocomially acquired influenza cases were observed. Until the end of November, the vaccination rate of health care workers was: 1,539 out of 3,900 (39.5%) against the saisonal strains and : 1,392 out of 3,900 (35.7%) against the pandemic A/H1N1/09 strains.
Discussion: By the end of November 2009, the infection control measures were efficient to avoid nosocomial transmission and infection of high risk patients. The vaccination of healthcare workers is thought to be an important factor, as well the consequent infection control measures (isolation, masks, hand hygiene).

HYV06
Outbreak of *Burkholderia cepacia complex* in a German university hospital caused by contaminated alcohol-free mouthwash

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Objective: We investigated an outbreak with *B. cepacia complex* (Bcc) in 3 different intensive care units (ICUs) of a German university hospital in 2009.
Methods: Cases were defined as detection of Bcc in any material sent to the laboratory. Patients´ charts were reviewed. Retrospective database analysis was performed. Products for oral hygiene toothpaste, one-way toothbrush, moisturizing lip-cream, alcohol-free mouthwash, shaving cream and sterile water filters were investigated. To prove clonal identity PFGE (SpeI digest) was performed.
Results: 10 cases were diagnosed with Bcc on 3 ICUs. 9 in respiratory specimens, 1 in maxillary wound swab. All patients were intubated and at least 4 patients had a ventilator-associated pneumonia due to Bcc. 1 patient died related to his underlying diseases. Environmental research was looking for equipments for oral hygiene. Bcc was found in opened and factory-closed flasks of alcohol-free mouthwash. Contamination was up to 6.3x105 CFU per ml. After recognition of the source health care authorities were informed. This mouthwash was exclusively distributed by chemist`s all lots were recalled by "Rote Hand". PFGE proved indistinguishable clone in clinical specimens and mouthwash. After elimination of the mouthwash no more cases occurred.
Conclusion: Intrinsically contaminated alcohol-free mouthwash was identified as cause of this Bcc outbreak. This source of Bcc in nosocomial outbreaks is already described. The infection control team was informed and identified the source through detailed questioning of ICU staff. Thus further cases were prevented and the outbreak was stopped rapidly. Detection of Bcc in clinical specimens from ICU patients should always be alarming for an external source and investigation should be started immediately. Health care authorities should be informed to prevent further cases in other hospitals. The safety of non-pharmaceutical products used in the care of ICU-patients should be better regulated in future.

ICP01
Rational development of a Helicobacter pylori-vaccine to prevent gastric cancer

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Objectives: Chronic infection with Helicobacter pylori (HP) has been identified as main risk factor for developing gastric malignancies. Vaccination against HP could potentially prevent many cases of gastric adenocarcinoma. So far no regimen immunizing humans efficiently against the bacterium exists. However, it is possible to immunize mice by oral administration of HP sonicate with Cholera toxin (CT) as adjuvant, reducing bacterial counts after challenge by about 2 log units. Our aim is to identify the cell populations targeted by this vaccine and the mechanisms involved in bacterial clearance, in order to explore alternative adjuvants suitable for human use.
Methods: We used various knock-out mice and depleted immune system components in wild type C57BL/6 mice during immunization and/or challenge phase of the vaccination protocol to assess the outcome on vaccination efficiency. Furthermore, we replaced CT by several other adjuvant candidates and varied antigen dose and administration routes.
Results: CD4 $^{+}$ effector T cells are essential for vaccine-induced protection against HP and caspase 1, TNF-alpha and IFN-gamma strongly promote efficient vaccination. Dendritic cells are required for presentation of HP antigen, whereas absence of Tregs, CD8+ T cells, B cells or macrophages does not influence vaccination outcome. Subcutaneous administration of sonicate with a liposome-based adjuvant formulation resulted in a significant reduction of HP.
Conclusion: A strong Th1-biased adaptive immune response triggered by innate signals is the basis for vaccine-induced protection against HP in C57BL/6 mice. However, the final effector mechanisms leading to eradication of HP remain unclear. Once further optimized, alternative adjuvants could allow for an efficient human vaccine formulation. Our finding that CD4 $^{+}$-T-cell derived IFN-gamma also mediates the formation of preneoplastic lesions upon HP infection underlines the necessity to achieve sterilizing immunity.

ICP02
Quantitative proteomics targets the kinome in renal cell carcinoma

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Renal cell carcinoma (RCC) accounts for 2-3% of all cancers, making it the tenth common form with an annual increase in incidence of 2%. Clear cell renal cell carcinoma, the most common type of RCC, is linked to an inactivation of the VHL tumor suppressor gene in more than 60% of the patients. Loss of VHL function leads to the upregulation of several growth factors like VEGF, PDGF and TGF-α. Therefore, multitarget kinase inhibitors such as Sunitinib and Sorafenib, focusing on the inhibition of the involved pathways, constitute the current gold standard in therapy. However, non-responders, drug resistance and site effects suggest that our knowledge about the affected signal networks in renal cancer is still incomplete.
The aim of this project is to analyze human kinases (kinome) as major and druggable signalling components in renal cancer patients. Kidney cancer samples and "healthy" counterparts dissected from nephrectomies are used as starting material to affinity purify more then 150 kinases by chemical proteomics. Kinase enriched fractions are then comparatively analyzed by quantitative proteomics and phosphoproteomics. iTRAQ peptide labelling in combination with a novel statistical validation method allows the characterization of human kinases both at the level of expression and site-specific phosphorylation signifying their activity status. Beside of known cancer-related proteins this approach suggests novel kinases that have to be considered for diagnosis and as potential drug targets.

ICP03
Antitumoral & immunostimulatory effects of a mixed bacterial vaccine -Coley`s Toxin-

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Background & Aims: Here, we assessed the potential of Coleys Toxin (CT), a mixture of heat-inactivated gram-positive *S. pyogenes* and gram-negative *S. marcescens* , as an active immunotherapeutic compound for treatment of pancreatic cancer (PaCa) *in vitro* and *in vivo*.
Material & Methods: Established human PaCa cell lines were treated with increasing concentrations of CT (0.5 x 10^6 – 12.5 x 10^6cfu/ml) for 24 and 48 hours. Tumor cell proliferation and viability were assessed by BrdU incorporation assay & Calcein AM staining. Apoptotic tumor cells were detected by Sub-G1-peak analysis. Additionally, antitumoral effects of CT (2.5 x 10^6cfu/ml) in the presence of peripheral blood leukocytes (PBL) from healthy volunteers were explored using co-culture experiments. *In vivo*, Panc02-tumor carrying C57Bl/6 mice were treated with CT (4 injections/twice a week, n=7). Control mice received saline (n=5). Tumor growth was monitored for 28 days, lymphocyte subpopulations were examined from blood samples.
Results: Treatment with CT mediated a dose and time-dependent influence on tumor growth in 4/5 cell lines (responder: AsPC1, BxPC-3, MiaPaCa-2, T3M4, non-responder: Capan-1). These direct antitumoral effects were accompanied by reduced cell viability and increases in apoptotic sub-G1-peak. Antitumoral and immunostimulatory effects of the toxin were boosted after co-culture with PBLs. In these experiments numbers of viable cells were significantly reduced (24h: BxPC-3: 60 % vs. control, T3M4: 20 % vs. control). *In vivo*, CT induced a delay of Panc02 tumor growth (day 28 (x-fold increase from therapy start): 6.8±1.3 (CT) vs. 20.6±4.2 (control)). The antitumoral effect was accompanied by raised levels of circulating CD11b^{+}/CD62L^{+} granulocytes/monocytes, NK1.1^{+} NK-cells and γ/δ TCR^{+} T cells.
Conclusions: Data presented herein prove the antitumoral potential of CT that comprises direct growth inhibitory as well as immunostimulatory potential and thus warrants further investigations on this historical, but still current immunotherapeutical approach.

ICP04
Combined bacterial antibody therapy for colorectal carcinoma

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We analyzed the potential of heat inactivated *Staphylococcus aureus* loaded with therapeutic monoclonal antibodies (mAb), for treatment of colorectal carcinomas *in vitro* and *in vivo*. The aim was to induce an inflammatory reaction against bacteria, finally activating tumor specific immune responses.
Colorectal carcinoma cell lines, established in our lab, were treated with *S. aureus*, antibodies (Panitumumab (anti-EGFR), or Trastuzumab (anti-Her2neu)) or a combination of both for 24 and 48 hours. Cell proliferation and viability were assessed by BrdU-incorporation assay and Calcein AM staining. Cell cycle analysis was performed by flow cytometry. Subsequently, antitumoral effects were examined in co-culture experiments using peripheral blood mononuclear cells (PBMCs) from healthy donors. *In vivo*, Balb/c mice with Her2neu transfected CT26 tumors received local injections of the therapeutics or saline (10 injections/twice a week, n=6). Tumor-infiltrating leukocytes were analyzed by immunohistochemistry.
Treatment with *S. aureus* and the combination mediated an S-Phase arrest in all tested cell lines, while the mAbs had minor effects on cell cycle. Proliferation and survival of tumor cells was also slightly affected by the therapeutical agents. However, antitumoral effects were boosted after co-culture with PBMCs in the presence of bacteria and mAbs. Flow cytometric analysis of PBMCs revealed an increase of $CD4^+/25^+$ and $CD8^+/25^+$ cells (24 h), indicative for activation of T cells by *S. aureus. In vivo, S. aureus* treatment induced a delay of tumor growth. These effects were accompanied by increased numbers of tumor-infiltrating $CD11b^+$ granulocytes/macrophages, $CD4^+$ and $CD8^+$ T cells. Similar results were obtained after combination therapy.
Our results demonstrate that *S. aureus* is capable to elicit direct antitumoral and immunostimulatory effects without the need of a targeted therapy with mAbs. Current investigations focus on testing specific antitumoral T cell responses subsequent to stimulation with *S. aureus*.

ICP05
Inflammation (infection) induced cancer development

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Hepatitis B and C viruses (HBV, HCV) cause chronic hepatitis and hepatocellular carcinoma (HCC) by poorly understood mechanisms. We show that cytokines lymphotoxin (LT) a, b and their receptor (LTbR) are upregulated in HBV- or HCV-induced hepatitis and HCC. Liver-specific LTab expression in mice induces liver inflammation and HCC causally linking hepatic LT overexpression to hepatitis and HCC. Development of HCC, composed in part of A6+ oval cells, depends on lymphocytes and IKappa B kinase b expressed by hepatocytes but is independent of TNFR1. *In vivo* LTbR stimulation implicates hepatocytes as the major LT-responsive liver cells and LTbR inhibition in LTab-transgenic mice with hepatitis suppresses HCC formation. Thus, sustained LT signaling represents a pathway involved in hepatitis-induced HCC.

IIP01
Natural immunization against malaria: pre-exposure prophylaxis with antibiotics

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Malaria remains the most important vector-borne infectious disease. Current antimalarial drug intervention strategies cure malaria or prevent infections but lack a sustained public health impact, for instance by expediting the acquisition of protective immunity. We show that antibiotic cover during parasite transmission results in swift acquisition of long-lived, life-cycle specific protection against re-infection with live sporozoites in mice. Antibiotic treatment specifically inhibits the biogenesis and inheritance of the apicoplast in Plasmodium liver stages resulting in continued liver stage maturation but subsequent failure to establish blood stage infection. Exponential expansion of attenuated liver stage merozoites from a single sporozoite induces potent protection. If confirmed in malaria-endemic areas periodic pre-exposure prophylaxis with safe and affordable antibiotics may offer a powerful shortcut towards a needle-free surrogate malaria immunization strategy.

IIP02
Cell-Penetration and Down-Regulation of Pro-Inflammatory Cytokines by a Gram-negative Bacterial Effector Protein

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Pathogenic Gram-negative bacteria frequently employ type III secretion systems (T3SS) to translocate effector proteins directly into host cells, where they compromise the innate immune response of the host. Here, we identified and characterized the ability of YopM of pathogenic *Yersinia enterocolitica* to penetrate host cells and to localize to the cytosol without the requirement for additional factors. This T3SS-independent translocation of YopM into the host cell cytoplasm was confirmed in various cell types, demonstrating that host cell penetration is an intrinsic ability of YopM. Molecular analysis using truncated versions of YopM revealed that the N-terminal α-helices of YopM mediate translocation into the host cell. Moreover, this domain is also capable of delivering heterologous cargo as demonstrated for the green fluorescent protein (GFP). Most interestingly, once inside the cells YopM down-regulates the transcription of several pro-inflammatory cytokines (TNFα, IL-12, IL-15, IL-18 etc.), while the mRNA level of anti-inflammatory IL-10 is not significantly altered. Beyond the newly identified function and effect of YopM on host cells, our findings imply a potential new tool for protein delivery, open the possibility to study molecular interactions of YopM without interference by additional bacterial components and, moreover, immediately suggest a potential therapeutic application. YopM can be considered as a novel locally applicable, self-delivering immunotherapeutic molecule, especially for the treatment of autoimmune diseases characterized by elevated levels of pro-inflammatory cytokines such as TNFα.

IIP03

Age-Dependent Susceptibility to *Streptococcus pyogenes* Infection in Mice: Underlying Immune Dysfunction and Strategies to Enhance Immunity

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Epidemiological studies have shown that the elderly are at higher risk of severe *Streptococcus pyogenes* infections. However, the immunological basis of this age-associated susceptibility to infection has not yet been investigated. In this study, we have used a mouse model that display the age-related loss of resistance to *S. pyogenes* infection seen in humans to determine the impaired immune mechanism underlying the age-related infection susceptibility as well as to assess new strategies to boost immunity against this pathogen in the elderly. Young (2-3 months-old) and aged (<20 month-old) BALB/c mice were systemically inoculated with *S. pyogenes* and their capacity to control infection was compared. Aged mice showed rapid progression of disease, early morbidity and increased mortality when compared with young animals. Since macrophages are critical for host defence against *S. pyogenes*, we investigated whether susceptibility of aged mice may be due to an age-associated decline in the functionality of these cells. Our results showed that macrophages from aged mice were as capable as those from young animals to uptake and kill *S. pyogenes* but the number of resident tissue macrophages was significantly lower in the aged host. Treatment of aged mice with macrophage colony stimulating factors (M-CSF) significantly increased the number of resident macrophages and improved the response to infection. These results indicate that an age-associated reduction in the number of resident macrophages underlies the susceptibility displayed by aged mice to *S. pyogenes* infection. Treatment with M-CSF can restore, at least in part, this deficiency and enhance natural resistance of aged mice. In addition, aged mice become entirely resistant to *S. pyogenes* after prime-boost vaccination indicating that vaccines tailored to the needs of the aging immune system may constitute a highly effective means to protect the elderly from severe *S. pyogenes* infections.

IIP04

Instable bacterial translation products are required for the presentation of *Listeria monocytogenes*-derived CD8 T cell epitopes

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Instable viral translation products have been implicated as substrates for the rapid processing and presentation of virus-derived antigens in the context of MHC class I molecules. In contrast to viral protein biosynthesis which depends on the protein biosynthesis machinery of the host cells bacteria provide their own protein synthesis. In order to evaluate the role of putative instable bacterial translation products we studied the synthesis and antigen processing of the secreted p60 molecule from *Listeria monocytogenes*. Our data show that approximately 30-50% of the p60 secreted by intracellular bacteria is instable and rapidly degraded intracellularly. We show that degradation of this instable p60 fraction is required for the presentation of p60-derived CD8 T cell epitopes.

IIP05

Absence of CYLD is essential for survival during murine listeriosis

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In infectious diseases, activating as well as inhibitory transcription factors are required to enable control of the pathogen and to prevent over-shooting pathological immune reactions. CYLD is a deubiquitylating enzyme which plays a pivotal inhibitory role in immune responses.In macrophages, CYLD downregulates NF-κB activity by the proteolysis of K63-linked ubiquitin from TNFR, TLR2, TLR4 and CD40.In addition, CYLD inhibits the TLR2 pathway by deubiquitylating TRAF6 and TRAF7, which leads to the suppression of MKK3 and MKK6 and a reduced phosphorylation of $_{p}$38MAPK. To gain insight into the function of CYLD in listeriosis, we infected C57BL/6 CYLD$^{-/-}$ and wildtype (WT) mice with a lethal dose *Listeria monocytogenes*. Whereas all WT mice succumbed to the infection up to day 7 p.i., 68% of CYLD$^{-/-}$mice survived the infection indicating that CYLD inhibits protective host responses. Since colony forming units did not differ in liver, spleen and lung between CYLD$^{-/-}$ and WT mice,the improved survival of CYLD$^{-/-}$mice was not due to a more efficient control of *Listeria*.A macroscopic examination revealed that WT mice suffered from bleeding in liver and lung,whereas no bleeding was observed in CYLD$^{-/-}$mice. This suggests that CYLD inhibits the induction of factors,which prevent bleeding in listeriosis. Additional WB analysis showed an increased activation of NF-κB in CYLD$^{-/-}$ mice as indicated by an increased phosphorylation of IKBα.There was also a strong upregulation of the tissue protective factor plasminogen activator inhibitor (PAI-1)in the lung and to a lesser extent in the liver of CYLD$^{-/-}$mice. Based on these data, we currently hypothesise that the upregulation of PAI-1 leads to an inhibition of tissue plasminogen activator and an increased deposition of fibrin, which limits the spread of *Listeria*.but also bleeding in CYLD$^{-/-}$mice we currently analyse the molecular mechanisms leading to the activation of PAI-1.

IIP06

Formyl peptide receptor-dependent proinflammatory consequences of peptide deformylase in *Staphylococcus aureus*

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In bacteria but not in eukaryotes the start methionyl-tRNA is formylated by formyltransferase (Fmt) leading to formylated N-termini on all newly synthesized bacterial proteins. This formyl group can be subsequently split off by an essential bacterial enzyme called deformylase (Def). Inhibition of Def by antibiotics such as actinonin or related antibiotics is regarded as an attractive new approach for antimicrobial therapy of *Staphylococcus aureus* and other bacterial pathogens. Of note, bacterial formylated peptides (FPs) can be recognized by human innate immune system. Neutrophils sense FPs by the formyl peptide receptor (FPR) and respond by chemotactic migration and mediator release. It has remained unclear whether treatment of *S. aureus* with actinonin affects the release of FPs.
Our study demonstrates that subinhibitory concentrations of actinonin lead to a considerable increase in the proinflammatory capacity of *S. aureus*. Human PMN responded with higher levels of IL-8 release, chemotactic migration, intracellular calcium ion fluxes and CD11b upregulation to supernatants of actinonin-treated *S. aureus* cultures compared to non-treated cultures. Using a FPR-transfected cell line and a Fmt-deficient *S. aureus* mutant we confirm that the increased leukocyte response to actinonin-treated *S. aureus* depends on FPR-mediated sensing of FPs.
These data underscore the role of FPs as important pathogen-associated molecular patterns and indicate that inhibition of basic bacterial processes by antibiotics can have critical side effects on innate immunity and inflammation.

IIP07
Role of Candida albicans MAPK cascade in the interaction with macrophage

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C. albicans is the most important human fungal pathogen. This is at least partly due to its capability to adapt to different niches in the body. Pathways mediated by mitogen activated protein kinase (MAPK) cascades are key elements in regulating the response of cells to environmental conditions. In *C. albicans* the MAPK pathways Hog1, Mkc1 and Cek1 are known to be involved in the response to osmotic and oxidative stress, cell wall construction and morphogenesis. However, though different susceptibilities of deletion mutants of MAPKs for killing by phagocytes were reported, the role of the MAPK cascades in the first steps of the interaction with those immune cells is not understood. Thus, we focussed on the process of phagocytosis, which is the first step of the innate immune defence. Previously we had shown that deletion of the histidine kinase Chk1 led to increased phagocytosis, whereas deletion of the osmotic stress sensor protein CaSln1 had no effect. In line with these data phagocytosis of the corresponding MAPK mutant Δhog1 mutant was comparable to the reference strain CAF2-1. However, phagocytosis efficiency for the respective MAPKK ΔPbs2 was almost doubled compared to the reference strain, whereas deletion of the other MAPKs increased phagocytosis by more than a factor of three. To elucidate the underlying mechanisms we studied cell surface properties of the mutants but did not find any effects on cell surface hydrophobicity. However, the correlation between increased accessibilities of β-glucans, increased accessibilities of mannans and increased phagocytosis efficiencies was significant. Thus, the Hog1 pathway is not involved in cell wall construction, whereas both the other MAPK pathways are. Histidine kinases are considered to be sensor proteins for environmental conditions, i.e. primary elements of signaling cascades. Interestingly deletion of only the histidine kinase Chk1 resulted in the same phenotype as deletion of Mkc1 and Cek1.

IIP08
YopM from *Yersinia enterocolitica* penetrates host cell membranes and down-regulates transcription of pro-inflammatory cytokines

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Pathogenic Gram-negative bacteria frequently employ type III secretion systems (T3SS) to translocate effector proteins directly into target cells, where they compromise the innate immune response of the host. Here, we identified and characterized the ability of recombinantly expressed YopM of pathogenic *Yersinia enterocolitica* to penetrate host cells and to localize to the cytosol without the requirement for additional factors. After lipid raft-associated uptake YopM appears to be distributed all over the cytoplasm but accumulates around the nucleus of the target cell. Molecular analysis using several truncated versions of YopM revealed that the N-terminal α-helices of YopM mediate translocation into the host cell. Moreover, this domain is also capable of delivering heterologous cargo such as the green fluorescent protein (GFP) or YopE. Most interestingly, we found that after cell penetration, YopM down-regulates the transcription of several pro-inflammatory cytokines (TNFα, IL-12, IL-15, IL-18 etc.), while the mRNA level of anti-inflammatory IL-10 is not significantly altered. Analysis of a potential interference of YopM with signal transduction pathways involved in inflammatory responses revealed an inhibitory effect on NF-κB signaling. We found that YopM inhibits phosphorylation of the inhibitor of NF-κB α (IκBα) in HeLa cells. Consequently proteasomal degradation of IκBα and phosphorylation of the NF-κB subunit p65 is decreased. Moreover, to investigate potential therapeutic abilities of the anti-inflammatory effector protein, YopM was applied in an *in vitro* model for inflammatory bowel disease (IBD). For this, HIMEC (human intestinal microvascular endothelial cells) were preincubated with YopM and subsequently stimulated with poly (I:C). Analysis by qRT-PCR revealed a strong reduction in poly (I:C) induced VCAM-1, IL-1β and IL-12p35 transcription due to YopM. These findings strongly suggest potential therapeutic applications of YopM in inflammatory conditions.

IIP09
Subcutaneous infection with *S. aureus* in mice reveals association of resistance with influx of neutrophils and Th2 response

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S. aureus is the leading cause of bacterial skin infection. Once it overcomes the epithelial barrier it either remains locally controlled or spreads in the dermis causing soft tissue infection. These different courses depend not only on its virulence factors, but also on the immune response of the infected individual.
To elaborate host factors which influence different outcomes we inoculated different inbred strains of mice subcutaneously with *S. aureus* strain SH1000.
We found that C57BL/6 mice are more susceptible than BALB/c and DBA/2 mice. Higher susceptibility was reflected by significantly higher footpad swelling, bacterial load, and increased dissemination of bacteria into inguinal lymph nodes and kidneys. This susceptibility was associated with lower influx of polymorphonuclear neutrophils (PMN) but higher secretion of CXCL-2.
Since *S. aureus* infection persisted more than 2 weeks in our model we analysed T cell response to *S. aureus* antigen and found that infection with S. aureus induces an antigen-specific T cell response. Remarkably, resistance correlated with *S. aureus*-specific Th2 cell response in BALB/c and DBA/2 mice, while susceptible C57BL/6 mice generated a Th1 cell response.
Since Th1 cells are able to release significant amounts of CXCL-2 and since CXCL-2 is able to increase survival of *S. aureus* within PMN, interactions of PMN and Th1/Th2 cells need to be considered as important host determinants of resistance in murine soft tissue infection with *S. aureus*.

IIP10
Boosting of phagocyte extracellular traps as a novel therapeutic strategie against bacterial infections?

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Antimicrobial extracellular traps (ETs) have been recently discovered as a new paradigm in the innate immune function of leukocytes. ETs are released by different host immune cells such as neutrophils and mast cells upon stimulation. They consist of a nuclear or mitochondrial DNA backbone associated with antimicrobial peptides (AMPs) and histones that entrap and kill various microbes.
Here, pharmacological agents with known or suspected immunostimulatory activities were screened by immunofluorescence microscopy for their ability to induce formation of ETs in human blood-derived neutrophils or mast cells. Inhibitors of cholesterol biosynthesis, such as statins, and agonists of the transcriptional regulator HIF-1alpha, such as desferoxamine and compound A, enhanced formation of ETs *in vitro*. Induction of ETs correlated with enhanced binding and killing of various bacterial pathogens, e.g. *Staphylococcus aureus*. Bacterial killing was unaffected by treatment with the phagocytosis inhibitor cytochalasin D, confirming the extracellular site of action. Finally, a well-established mouse model of *S. aureus*-induced pneumonia was used to confirm the presence and antimicrobial role of ETs *in vivo*. *S. aureus*-infected mice treated with simvastatin showed significantly more ETs and reduced bacterial load and organ damage in lung tissue, compared to the control mice.
These data shed new light on the importance of ETs in host defense against bacterial infections, and provide new therapeutic opportunities to enhance the local innate immune response by boosting ET formation.

IIP11
The Role of Human DC-SIGN in Anti-fungal Immunity

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Life-threatening mycoses, mediated amongst others by the bimorphic fungus *Candida albicans*, are rising especially in immunocompromised patients. Whilst the hyphae form is linked to Th2 responses and pathogenesis, the yeast form is associated with Th1 T cell responses and clearance of the infection. Additionally, Th17 T cell responses have been associated with protection from fungal pathogens. The different immune responses are orchestrated by dendritic cells (DC) that recognise *Candida albicans* pathogen-associated molecular patterns via different pattern recognition receptors such as C-type lectins, of which the DC-specific intracellular adhesion molecule-3 grabbing non-integrin (DC-SIGN) is a prototypic member. Since no mouse homologue with the same expression pattern of human DC-SIGN is known, our laboratory generated a mouse line transgenic for the human receptor under the control of the murine CD11c promoter (hSIGN mice). Using a different ligand of DC-SIGN (*Mycobacterium tuberculosis*) we observed a decrease of IL-12p40 production by DCs. Since IL-12p40 is a subunit of IL-23, which is involved in Th17 differentiation, the question arises whether a possible decrease of Th17 responses has influences on the course of infection.
Therefore, this study endeavours to investigate the role of human DC-SIGN during fungal infections with a special focus on Th17 responses. We are comparing hSIGN mice with wild type mice upon *Candida* infection *in vitro* and *in vivo*. Using imaging technology, we are currently examining differences in the intracellular localisation of the fungus in wild type and hSIGN-derived DCs. Furthermore, the cytokines produced upon *Candida* infection by the respective DC cultures are assed to draw first conclusions of the induced T cell responses. A better understanding of the role of DC-SIGN in fungal infections could contribute to new vaccination protocols or immune modulation approaches.

IIP12
The role of TLR and DC-SIGN signalling in dendritic cell-mediated host defence against pneumococcal infection

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Infectious diseases are a major cause of mortality worldwide. *Streptococcus pneumoniae* causes more deaths from invasive infections than any other bacterium. Pattern recognition receptors (PRRs) like Toll like receptors (TLR) and C-type lectin receptors (CLR) recognise highly conserved molecular structures of pathogens and are found on immune cells such as macrophages and dendritic cells (DCs). Although extensively studied *in vitro*, the function of TLRs and CLRs expressed by DCs or macrophages has not been assessed *in vivo* due to the lack of genetic tools. Previous studies have shown that MyD88- and in particular TLR9-dependent signalling play a crucial role in the innate immune defence against pneumococci. Using the Cre/loxP recombination system under cell-specific promoters, we generated conditional DC- and macrophage-specific TLR9 knock-out and MyD88 knock-in mice to investigate the importance of TLR signalling especially in these cell types during pneumococcal infection *in vivo*.
The C-type lectin family member DC-SIGN (DC-specific intercellular adhesion molecule-3-grabbing nonintegrin) and the murine homologue SIGNR-1 detect the polysaccharide capsule of pneumococci. However, in primates there are highly conserved putative signalling and internalization motifs, which are missing in all murine homologues. By expressing the human DC-SIGN-cDNA under the control of a minimal murine CD11c promoter, we generated a humanized mouse model (hSIGN) and observed a prolonged survival of these mice and less IL-12 production by hSIGN-DCs in a mycobacterial infection model. To elucidate the role of human DC-SIGN on murine DCs during pneumococcal infection, we are currently analysing wt and hSIGN-DCs with regard to their cytokine production using ELISA assays and pneumococcal uptake via fluorescence microscopy. Deeper insights into the pneumococcal pathogenesis are of vital importance for designing more specific vaccination or immune modulation approaches.

IIV01
Characterisation of CD8 T cells against malaria liver stages

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The feasibility of vaccination against malaria has been demonstrated using irradiated sporozoites, currently considered as the "gold standard" vaccine, which protects rodents, non-human primates and humans against subsequent challenge with normal sporozoites. The potent protective immunity induced by irradiated sporozoite immunisation is thought to be multi-factorial, involving both antibodies and T cells. Nonetheless, CD8 T cells have been shown to be the primary mediators of protection in rodent models. In this study, we followed the evolution of the CD8 T cell response in Balb/c and C57Bl/6 mice immunised with irradiated sporozoites from the rodent malaria parasite, *Plasmodium berghei*. We analysed responses in the spleen and the liver of immunised mice. Strikingly, evaluation of liver-infiltrating cells induced after immunisation indicated a high frequency of CD8 T cells with an „effector" phenotype. However, further analysis of the CD8 T cell response revealed a limited contribution from currently known antigens. Characterisation of the specificity and functionality of CD8 T cells against malaria liver stages will not only further the current understanding of immune responses against the parasite, but also yield insights toward the rationale design of an effective vaccine.

IIV02
Regulatory T cell subsets in the course of *Plasmodium yoelii* infection

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It has been well established that CD4+CD25+Foxp3+ regulatory T cells (Tregs) play a critical role in maintaining immunological homeostasis. Moreover, Tregs have been described to interfere with acute and chronic infectious diseases. However, the precise role of Tregs in the course of infections is subject to some speculation. To gain further insights into Treg biology we have analyzed the frequency and the molecular phenotype of Tregs in different organs and at various time points post infection (p.i.) in mice infected with *Plasmodium yoelii* (*P.yoelii*). We could detect an increase in the frequency of CD4+CD25+Foxp3+ Tregs at early time points upon infection in the spleen. Interestingly, gene expression analysis of CD4+CD25+Foxp3+ Tregs as well as CD4+CD25-Foxp3- T cells revealed that Treg associated molecules like IL-10, CTLA-4, Lag3 and PD-1 are expressed in both T cell subsets in the course of infection. These results suggest that *P.yoelii* infection induces Tregs which lack Foxp3. However, functional analysis have to clarify if these CD4+CD25-Foxp3- T cells exhibit suppressive activity and thereby interfere with *P.yoelii* infection *in vivo*.

IIV03
Delivery of functional DNA and messenger RNA to mammalian antigen-presenting cells by recombinant yeast

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Among the different vaccination approaches, DNA/RNA vaccination represents a promising means in particular for the induction of effective cellular immune responses conferred by CD8-positive T lymphocytes. To achieve such immune responses, there is a need for novel delivery systems that allow the introduction of nucleic acids to the cytosol of immune cells. We show, for the first time, the delivery of functional DNA and messenger RNA to mammalian antigen-presenting cells including murine macrophages and human dendritic cells using the yeast *Saccharomyces cerevisiae* as delivery vehicle. After transfer of the particular nucleic acid, subsequent antigen processing and presentation were demonstrated in a human system. Remarkably, release of DNA/mRNA does not require additional "helper" proteins such as listeriolysin. In conclusion, the yeast-based system described here is superior to many bacterial and viral systems in terms of efficacy, safety and targeting suggesting "mycofection" as a promising approach for the development of a novel type of live vaccines.

IIV04
Influence of Salmonella colonization kinetics on the induction of antigen-specific CD8 T cells in a murine oral vaccination model

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Live vaccine carriers can be used to elicit both humoral and cellular immunity against viruses, intracellular and extracellular bacteria and even tumors. Our laboratory uses attenuated *Salmonella enterica serovar typhimurium* for oral immunization protocols in BALB/c mice. The model antigen p60 of *Listeria monocytogenes* is translocated by the type III secretion system (T3SS) of Salmonella directly into the cytosol of eukaryotic cells and induces p60-specific CD8 T cells via the class I antigen presentation pathway. This immunization strategy mediates protective immunity against a virulent *L. monocytogenes* challenge. In the present study, we applied the fluorquinolone ciprofloxacin to mice at different time points after oral immunization to reduce the colonization with the Salmonella vaccine strain and to determine the effect on antigen-specific CD8 T cell induction. The antibiotic was applied twice daily over a time period of four days. The effect of the ciprofloxacin treatment on the colonization was measured by analyzing the bacterial burden (colony-forming units, cfu) in the spleen, the cecum and feces from treated mice. As expected, the colonization studies revealed that after two days of ciprofloxacin treatment the cfu-counts in these organs were significantly reduced. In further experiments, p60-specific CD8 T cells in the spleen were detected 11 days after vaccination by using MHC class I tetramer technology and FACS analysis. Co-staining with CD62L (L-Selektin) and CD127 (Interleukin 7 receptor α-chain) was performed to determine subsets of these antigen-specific CD8 T cells. Co-application of ciprofloxacin and the Salmonella vaccine strain inhibited a significant p60-specific CD8 T cell induction. However, mice treated with antibiotics from day two after immunization revealed high frequencies of antigen-specific CD8 T cells similar to a control group that was immunized with Salmonella but did not receive ciprofloxacin treatment.

IIV05
Optimizing *Salmonella enterica* as live carrier for vaccination

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Salmonella enterica harbours two Salmonella pathogenicity islands (SPI), each encoding a type III secretion system (T3SS) for virulence proteins. In particular SPI2 is required for systematic infections and intracellular accumulation of Salmonella. SPI2 effectors are induced inside host cells and are translocated into macrophage and dendritic cells. Attenuated live bacterial vaccines can be administrated orally and induce long-lasting protective immunity in humans without causing major side effects. An attenuated *Salmonella enterica* serovar Typhi strain is registered as live oral vaccine against typhoid fever and has been in used for more than two decades. Recombinant attenuated strains are also an attractive means of delivering heterologeous antigens to the immune system, thereby, stimulating strong mucosal and systematic immune responses. We followed the strategy of fusion of heterologous antigens to effector proteins of the SPI2-T3SS for delivery into antigen-resenting cells. For designing efficient vaccination strategies, the choice of heterologous protective antigens and their expression under the control of appropriate promoters were evaluated. Moreover, the translocation efficacy of effectors, which confer the efficient presentation for heterologous antigens to the immune system, should be tuned by choosing the effector most efficiently translocated. Here we show that SPI2 effectors SseJ and SteC are the most efficient translocated effectors which can be used for vaccination against infections against as well as to treat cancer.

IIV06
Novel combined Multiple Subunit Vaccine protects against Extraintestinal Pathogenic *E. coli* (ExPEC)

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ExPEC are a frequent cause of sepsis, neonatal meningitis and urinary tract infections. They lead to significant mortality and morbidity and are an eminent burden for healthcare providers. With antibiotic resistance rates rising preventive vaccination against these pathogenic *E. coli* is greatly desired.
Based on data from genome analysis and *in vivo* transcription studies we selectively target virulence factors expressed during infection such as iron acquisition systems. Immunogenic regions, the three dimensional structure of these proteins as well as possible MHCI and MHCII epitopes were analyzed in silico.
This led to the design of two synthetic modular vaccine proteins each with eight epitope rich subfragments. For each a fully synthetic gene was constructed and optimized regarding sequence and codon bias. We evaluated two application routes in the mouse model. First we applied purified vaccine proteins for intranasal application, second we used a Salmonella T3SS for vaccine delivery. As it translocates the vaccine directly into the cytoplasm of immune cells *in vivo*, T-cellular response is greatly enhanced.
After vaccination specific immune responses were evaluated with IFN-γ ELISpot, FACS and sub-class sensitive antibody ELISA. Intranasally immunized mice demonstrated massive increases of specific serum IgG as well as vaginal fluid IgA antibodies against the both the recombinant vaccine protein and the purified full length virulence factors of ExPEC. Expansion of vaccine specific IFN-γ secreting T-cells could be proven in both immunization routes. The challenge model of peritonitis showed a significant reduction of bacterial load in immunized mice demonstrating the first successful combined T-cell vaccine against ExPEC in the mouse model.
Combined T-cell and antibody stimulating vaccines containing multiple epitopes are effective against ExPEC in the mouse. Further evaluation is needed to elucidate the potential for use in humans.

IIV07
Detrimental role of dendritic cells during *Y. enterocolitica* infection *in vivo*

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Yersinia enterocolitica (*Y. enterocolitica*) blocks T cell priming, required for the control of *Y. enterocolitica* infection, by inhibiting maturation and antigen uptake as well as by the induction of cell death of bone marrow-derived DCs (DCs). Here we investigated the role of dendritic cells during an infection with *Y. enterocolitica in vivo* using C57BL/6 wild type and transgenic mice expressing the diphtheria toxin (DT) receptor under the control of the CD11c promoter (CD11c.DOG mice). Repetitive application of DT led to a long term ablation of DCs. Analyzing the course of infection we observed less bacterial load in the spleen one to 7 days after infection in the CD11c.DOG mice compared to wild type mice. This led to a better survival of the CD11c.DOG mice compared to the wild type mice. In addition, we observed less viable intracellular *Yersinia* in $CD11b^+$ and $Gr\text{-}1^+$ cells one day post infection in the CD11c.DOG mice compared to the wild type mice. No difference in the cell populations in the spleens could be observed one day post infection between the two mouse strains. Later on the number of $CD11b^+$ and $Gr\text{-}1^+$ cells was doubled in the spleen of CD11c.DOG mice compared to wild type mice. One day post infection the amount of CCL2, CCL4, CCL7, and IFN-γ was increased 12 to 35 fold in wild type mice compared to CD11c.DOG mice as analyzed by RT-PCR. Furthermore, adoptive transfer of CFSE-labeled OVA-specific $CD4^+$ T cells into CD11c.DOG mice treated with DT and infected with *Y. enterocolitica* revealed reduced proliferation rates compared to these of infected CD11c.DOG mice not treated with DT. Taken together, our data demonstrate that DCs play an adversary role during infection with *Y. enterocolitica* by increasing cytokine and chemokine production as well as bacterial load.

IIV08
Analysis of host factors in a murine model of *Aggregatibacter actinomycetemcomitans*-induced periodontitis

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The Gram-negative bacterium *Aggregatibacter actinomycetemcomitans* is strongly associated with chronic and aggressive periodontitis. Periodontitis is an inflammatory process induced by periodontal pathogenic microorganisms and affects the supporting structures of the tooth. If untreated, periodontitis can cause severe alveolar bone erosion followed by tooth loss. The aim of this study was to assess genetic host factors of this disease in a murine model of periodontitis. C57BL/6 wild-type mice, inducible nitric oxide synthase-deficient (iNOS-/-) and NADPH oxidase-deficient (gp91-phox-/-) mice were orally inoculated with *A. actinomycetemcomitans* and the degree of alveolar bone destruction was quantified 5 weeks after infection by conventional morphometric assessments and 3-dimensional micro-computed tomography (micro-CT). Additionally, *A. actinomycetemcomitans* cultures were performed to investigate bacterial persistence in the various strains at different time points after infection. Differential persistence of *A. actinomycetemcomitans* in the murine oral cavity was documented among the three mice groups showing the highest rate in infected gp91-phox-/- mice in which the periodontal pathogen was still present even 38 days after inoculation. In accordance with *A. actinomycetemcomitans* persistence, significant alveolar bone loss could be detected in gp91-phox-/- mice in contrast to iNOS-/- and wild-type mice and uninfected controls by using morphometry and micro-CT. It is noteworthy that two mice of the infected gp91-phox-/- group even lost teeth during the course of the experiment. This study provides a sensitive method to quantify alveolar bone erosion and demonstrates that *A. actinomycetemcomitans* can induce significant alveolar bone destruction in NADPH oxidase-deficient mice compared to wild-type and iNOS-/- mice. Our results indicate a protective role of NADPH oxidase against periodontal infection.

IIV09
Generation and function of human CD8+Foxp3+ regulatory T cells by TGF-beta and retinoic acid in the light of inflammatory bowel disease

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The intestinal immune system is constantly challenged by foreign antigens and commensal bacteria but nonetheless is able to maintain a state of immunological homeostasis. Therefore, proper control of the intestinal microenvironment is required. One important arm of this regulatory network consists of regulatory T cells. Failures of these regulatory mechanisms contribute to the development of inflammatory bowel disease. In contrast to CD4+Foxp3+ regulatory T cells, which have been well characterized, immunomodulatory CD8+ T cells that express Foxp3 have not been well defined in terms of their generation and function. Here we show that chronic expression of intestinal antigen in a transgenic mouse model of intestinal tolerance leads to the peripheral induction of CD8+Foxp3+ T cells that exert an immunosuppressive effect on CD8+ and CD4+ T cells *in vitro*. In contrast, reduced numbers of CD8+Foxp3+ regulatory T cells are found in patients with ulcerative colitis. Interestingly, murine and even human CD8+Foxp3+ regulatory T cells can be induced by stimulating naïve CD8+ T cells in the presence of TGF-beta and retinoic acid. These T cells share common molecular features with CD4+Foxp3+ regulatory T cells: strong expression of CD25, CTLA-4, and Nrp1. CD8+Foxp3+ T cells induced *in vitro* exhibit a reduction in proliferative capability and display cell-cell contact–dependent immunosuppressive activity *in vitro* and *in vivo*. Taken together, our findings show that gut-related CD8+Foxp3+ regulatory T cells compose an immune regulatory pathway. The induction of these cells *in vitro* may be an effective therapy for inflammatory bowel disease.

INP01
Regulation of host cell miRNA by *Helicobacter pylori* in the context of innate immunity

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MicroRNAs (miRNAs) constitute an abundant class of small non-coding RNAs that regulate protein expression by either mRNA degradation or translational inhibition. MiRNAs are involved in many physiological processes including cellular development, but also in pathological processes like cancerogenesis.
Helicobacter pylori is a human pathogen living in the stomach of around 50% of the world´s population. Infection with *H. pylori* evokes an innate immune response in epithelial cells, macrophages, and other cell types. *H. pylori* induces signals in these cells mainly via the toll-like receptor (TLR) family as well as the nucleotide oligomerization domain (NOD) receptors thereby leading to the release of proinflammatory cytokines.
We are interested in the innate immune response to *H. pylori* with respect to the expression and the effect of miRNAs during this process. By performing miRNA micro-array, Northern blot and realtime-RT-PCR experiments we determined miR-155 being upregulated after *H. pylori* infection in all investigated cell types (gastric epithelial cells, T lymphocytes, macrophages). MiR-155 has been shown to be involved in different cellular processes and to be dysregulated in certain types of B-cell lymphoma. MiR-155 has been described as targeting important factors such as IKKε, FADD and Ripk1 which may show the putative impact on *H. pylori* infection.
We focused our study on the murine macrophage cell line J774.A as well as bone marrow derived macrophages (BMDMs). Analyzing BMDMs from mice deficient in certain receptors the regulation of miR-155 showed a strong dependence on TLR2 and TLR4 as well as other yet unknown extra- and intracellular receptor signaling. We are further interested in the upstream receptors and factors leading to the expression of miR-155 during *H. pylori* infection. In addition, identifying targets of miR-155 after infection with *H. pylori* will be important to understand downstream effects of miR-155 *in vivo*.

INP02
Differential macrophage response after infection with either virulent *B. pseudomallei* or avirulent *B. thailandensis*

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Burkholderia pseudomallei is the causative agent of melioidosis, an infectious disease endemic in Southeast Asia and northern Australia. Severe systemic infections are characterized by a dysregulation of the immune system including high plasma cytokine levels e.g. TNF-alpha. The closely related species *Burkholderia thailandensis* shares many similarities with *B. pseudomallei* such as intracellular replication within the cytosol and actin tail formation but is considered to be avirulent. Our previous work has shown that macrophages play an important role in the defense of *B. pseudomallei*. Therefore, the aim of the present study was to investigate possible differences in the interaction of both the virulent and avirulent *Burkholderia* species with macrophages. Experiments with bone marrow-derived macrophages (BMM) from susceptible BALB/c and more resistant C57BL/6 mice revealed no significant differences in macrophage invasion and intracellular replication between both species with the strains tested. To elucidate if any differences exist in bactericidal mechanisms which are responsible for the clearance of either intracellular *B. pseudomallei* or *B. thailandensis*, we further investigated the bacterial burden in iNOS-, gp91phox- and caspase-1-deficient BMM. These experiments revealed an important function of caspase-1 in anti-*B. pseudomallei* and anti-*B. thailandensis* activity whereas iNOS and NADPH oxidase did not seem to play a crucial role in both species. However, infection of macrophages with *B. pseudomallei* resulted in a significantly higher release of proinflammatory cytokines like TNF-alpha, IL-6 and IL-1beta compared to *B. thailandensis* infected macrophages. Thus, our data suggest that the much lower virulence capacity of *B. thailandensis* compared to *B. pseudomallei* is probably linked to a significantly lower capability to induce a cytokine response in macrophages.

INP03
The role of 4-1BB in LPS-activated macrophages

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4-1BB belongs to the TNF receptor superfamily and was originally identified on T-cells as a co-stimulatory molecule. It is now clear that 4-1BB is expressed on almost all types of immune cells. Increased 4-1BB expression was found in the inflammatory tissue of patients with Crohn's disease and other chronic diseases indicating that 4-1BB seems to be important for the maintenance of chronic inflammation. Lipopolysaccharide (LPS) is the major constituent of the cell wall of most gram negative bacteria and a very potent activator of macrophage (MΦ) functions. LPS signalling is mediated via Toll-like receptor 4 (TLR 4), but additional receptors also contribute to the early and late activation of genes by LPS. Very little is known about the function of 4-1BB in MΦ. In this study we used the murine MΦ cell line RAW264.7 to characterize the role of 4-1BB in LPS stimulated MΦ. For this we generated a 4-1BB receptor knock-down MΦ clone by stable transfection with a shRNA plasmid. Interestingly, MΦ deficient in 4-1BB displayed a substantially reduced late mRNA expression of a selected group of genes. For example, IL-6 and IL-1β expression was similar in wild type and 4-1BB knock down MΦ until 2 h after stimulation with LPS. However, whereas in the wild type MΦ the expression peaked 8 h after stimulation in the knock down clone reduced or no further increase of IL-6 and IL-1β mRNA levels were seen. This was especially evident 24 h after stimulation. TNFα mRNA expression was slightly lower in the knock down clone but showed a comparable time kinetic. To gain insights into the mechanisms, we focussed on IL-6 expression by performing luciferase reporter gene assays with deletions and mutations in the IL-6 promoter. These analyses revealed that IL-6 promoter activity in LPS stimulated 4-1BB knock down clone was only marginal induced whereas the IL-6 promoter activity in LPS stimulated MΦ was mediated through a NFκB binding site.
Together, our results suggest that signalling via the 4-1BB receptor is important for full activation of MΦ proinflammatory functions via NFκB.

INP04
S. aureus induces plasmacytoid dendritic cell activation by FcγRIIA-dependent and -independent mechanisms

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Little is known on the role of plasmacytoid dendritic cells (pDC) in the immune response against extracellular bacteria. Here, we asked whether pDC respond to stimulation with *S. aureus*. Our results revealed that *S. aureus* triggers human pDC-derived interferon (IFN)-α secretion by two distinct mechanisms: 1.) due to frequent exposure healthy individuals display anti-staphylococcal antibody titers, indicators of immune memory. Immune complexes of anti-staphylococcal IgG1 or IgG_3 with *S. aureus* promoted IFN-α secretion upon CD32 (FcγRIIA)-mediated uptake. Thus, pDC activation via this pathway may form part of a memory response. 2.) Additionally, CD32-independent pDC-derived IFN-γ was induced by individual strains of *S. aureus*. We found that this aberrant induction was dependent on the level of protein A (SpA) expression. Our data suggest that SpA-mediated TNF-R engagement contributes to pDC activation. However, recombinant SpA alone was not sufficient to trigger IFN-α release, since the presence of immunostimulatory DNA was a prerequisite for IFN-α induction. Moreover, our experiments demonstrate that, independent of the context, pDC activation can be blocked by inhibitors affecting endosomal uptake, endosomal maturation and acidification as well as protease (cathepsin B) activity. Thus, pDC-derived IFN-α induction by S. aureus is most likely mediated by endosomal nucleic acid-sensing Toll-like receptors (TLR).
In conclusion, our data suggest that pDC activation results from a sequence of events: 1.) *initiation* by engagement of CD32 or SpA-mediated activation of TNF-Rs, 2.) *bacterial uptake* required for access to endosomal TLR, and 3.) *sensing of nucleic acids*, thus eliciting 4.) *IFN-α secretion*.

INP05
Role of IRAK1 for the Establishment of Tolerance in the Intestinal Epithelium after Birth

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Cell membrane components of bacteria such as lipopolysaccharide (LPS) are amongst the most potent immunostimulatory molecules. Recognition occurs by innate immune receptors, such as Toll-like receptors (TLRs) and leads to the synthesis and release of proinflammatory cytokines that stimulate a proinflammatory response. In addition to professional immune cells such as macrophages, intestinal epithelial cells (IECs) have also been shown to express TLRs, including TLR4, the receptor for LPS. However, the intestinal tract is densely colonized by bacterial members of the physiological flora and an uncontrolled proinflammatory response may lead to chronic inflammation such as observed in inflammatory bowel diseases. Negative regulatory mechanisms must therefore exist that prevent inappropriate cellular activation and maintain mucosal homeostasis. A transient postnatal epithelial activation shortly after birth followed by a loss of TLR susceptibility has recently been described by our group. Intestinal epithelial innate immune tolerance is caused by posttranscriptional downregulation of the interleukin-1 associated kinase (IRAK) 1, an essential signalling mediator of the TLR4 pathway. Here, we provide a detailed analysis of the molecular mechanisms underlying this postnatal adaptive process of the neonate intestinal epithelium. Postnatal intestinal epithelial tolerance may contribute to the establishment of intestinal host-microbe homeostasis during bacterial colonization after birth.

INV01

Anti-*Burkholderia pseudomallei* defense mechanisms of hepatocytes

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The gram-negative rod *Burkholderia pseudomallei* is the causative agent of melioidosis, a potentially fatal disease which is endemic in tropical and subtropical areas. The facultative intracellular pathogen *B. pseudomallei* is able to spread from e.g. local skin lesions to almost any organ including the liver. Previously we have shown that the marked organotropism of *B. pseudomallei* for the spleen and liver in humans can also be observed after intravenous infection of mice. Electron microscopic analysis of the murine liver provided evidence, that *B. pseudomallei* containing phagosomes in hepatocytes fuse with lysosomes, leading to a degradation of bacteria. Thus, the aim of our study was to further elucidate the role of hepatocytes in restricting bacterial *B. pseudomallei* growth. *In vitro* studies using the human hepatocellular carcinoma cell line HepG2 as well as human primary hepatocytes revealed that *B. pseudomallei* was able to invade and replicate inside cells. Priming with IFN-gamma increased the antibacterial activity of hepatocytes against intracellular *B. pseudomallei* in the early and late phase of infection. Neither NADPH oxidase-derived superoxide nor iNOS-derived nitric oxide seemed to be essential for the host defense of *B. pseudomallei* within 24 hours, since specific inhibitors did not influence antibacterial activity. In contrast, inhibition of inflammatory caspase-1 led to an impaired bactericidal activity within 24 hours, although caspase-1-inhibited hepatocytes still exhibited substantial killing activity. However, the caspase-1-induced, but IFN-gamma-independent bactericidal mechanism is still unknown. In summary, our data suggest that the IFN gamma-dependent and IFN-gamma-independent elimination of virulent *B. pseudomallei* by hepatocytes might be an important mechanism in anti-*B. pseudomallei* host defense.

INV02

Lymphocytes modulate epithelial peptidoglycan recognition

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Innate immune recognition is facilitated by specific receptor ligand interaction. Bacterial ligands are conserved, essential molecular structures and receptor binding initiates a proinflammatory host reponses. The nuclear oligomerization protein NOD2/CARD15 is situated in the cytosol and recognizes fragments of the bacterial cell wall constituent peptidoglycan. Epithelial NOD2 expression has been shown to significantly contribute to intestinal mucosal homeostasis. Here we examined the expression of a secreted amidase with peptidoglycan-cleaving activity in various cells of the intestinal mucosa. Using primary cells isolated from GFP transgene mice and quantitative RT PCR, we demonstrate that lymphocytes within the lamina propria express high levels of this amidase. In addition, we characterize the amidase-positive lymphocyte population in great detail and show that coincubation of bacterial peptidoglycan with recombinant amidase leads to reduced NOD2-mediated epithelial cell stimulation. Our results indicate that lymphocytes within the mucosal tissue influence epithelial innate immune recognition and suggest a cooperative function of epithelial cells and professional immune cells for the maintenance of mucosal homeostasis.

INV03

Bacterial RNA is a potent activator of innate immunity

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Recognition of microbial nucleic acids by the innate immune system is achieved by various receptors including the endosomal Toll-like receptors (TLRs) 3 and 7-9 as well as cytosolic recognition systems such as RIG-I, MDA5 and AIM2. Activation by RNA of viral origin has gained much interest over the last years, yet, only little is known about the immunogenicity of prokaryotic RNA. Here we provide evidence that bacterial RNA is a potent activator of innate immunity. Interestingly, bacterial RNA triggers innate immunity in a cell-type specific manner: Human PBMCs showed strong induction of type I IFN upon bacterial RNA stimulation. Activation of human plasmacytoid dendritic cells was dependent on endosomal maturation, could be blocked by TLR7 inhibition and murine plasmacytoid dendritic cells from TLR7 deficient mice were unresponsive to bacterial RNA. Surprisingly, myeloid dendritic cells showed no dependency on TLRs for secretion of TNF and interleukin-12 upon triggering with bacterial RNA. Even non-immune stroma cells were able to mount a NFκB response upon stimulation with bacterial RNA. The viral RNA sensors RIG-I and MDA5 could be ruled out to be responsible for this reactivity. Interleukin-1β secretion could also be induced by bacterial RNA and was dependent on the NALP3-inflammasome adaptor protein ASC and a functional type I interferon receptor. However, both molecules were dispensable for TNF and interleukin-12 induction by cytosolic bacterial RNA in myeloid dendritic cells. The results show that (1) besides of activation of TLR7 in plasmacytoid dendritic cells and (2) the NALP3-inflammasome, bacterial RNA activates (3) additional cytosolic receptors similarly as has been reported for recognition of bacterial DNA.

INV04

Peroxiredoxin expression in macrophages after *Burkholderia pseudomallei* infection

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Peroxiredoxins are important components of the cellular antioxidant defense system due to their thiol-dependent peroxide reductase activity, but their role in intracellular host-pathogen interactions has rarely been addressed. Infection of macrophages with the gram-negative saprophyte *Burkholderia pseudomallei*, the causative agent of melioidosis, leads to an increased production of nitric oxide and reactive oxygen species, thereby resulting in oxidative cell stress. As a first step towards the analysis of the role of peroxiredoxins in intracellular infections, this study aimed to examine the regulation of peroxiredoxin (Prx 1-6) gene expression in IFN-gamma-activated bone marrow-derived macrophages (BMM) of highly susceptible BALB/c and more resistant C57BL/6 mice after infection with *B. pseudomallei*. Infection of macrophages with *B. pseudomallei* increased Prx 1, 5 and 6 gene expression in cells from both strains of mice, with Prx 6 more strongly induced in C57BL/6 BMM. *In vitro* experiments with iNOS- and gp91phox-deficient macrophages revealed a diminished upregulation of several peroxiredoxins after infection with *B. pseudomallei* indicating that iNOS-derived nitric oxide and NADPH oxidase-derived superoxide contribute to the regulation of certain peroxiredoxins. Furthermore, infection of activated C57BL/6 BMM with strains of the closely related but avirulent species *B. thailandensis* resulted in a lower iNOS expression than *B. pseudomallei* infected BMM and also in a significantly reduced induction of Prx 6 gene expression compared to virulent *B. pseudomallei*. In summary, our results show that peroxiredoxins are differentially expressed in BMM from different strains of mice and after infection with either *B. pseudomallei* or *B. thailandensis*. Future studies using peroxiredoxin knock out cells will further elucidate their role in intracellular infection.

INV05

Pathogen entrapment by Transglutaminase – a conserved early innate immune mechanism

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The innate immune system represents the first line of host defence against invading pathogens. Here, we present data to show that the coagulation systems in both insects and humans have beneficial effects during an infection.
Our results show, that Drosophila transglutaminase (TG) targets surface proteins of several invading gram-positive or gram-negative bacteria leading to pathogen entrapment within a hemolymph clot. After infection with Escherichia coli, Staphylococcus aureus or the entomopathogenic bacterium Photorhabdus luminescens, TG-knockdown Drosophila larvae show a higher susceptibility in comparison to wildtype animals.

Human Factor XIII (FXIII) is the vertebrate homologue of TG and, similar to the observations with Drosophila hemolymph, also targets surface proteins of several Gram-negative and Gram-positive pathogens. Electron microscopic analysis revealed that bacteria are entrapped inside a fibrin clot after exposure to human plasma. This bacterial immobilisation is dependent on thrombin-activated FXIII because fewer bacteria were entrapped when FXIII-deficient plasma was used or a specific inhibitor for TG/FXIII was added. Detection of a gold-labelled antibody against the FXIII crosslinking site displayed that bacterial proteins are covalently attached to fibrin by the action of FXIII.
After exposure of bacteria to purified FXIII or human plasma, FXIII activity was detectable on the bacterial surface as seen by fluorescence microscopy. No bacterial labelling was observed when FXIII-deficient plasma was used.
Taken together, our results show that TG in insects as well as FXIII in humans crosslink bacterial surface proteins to hemolymph or fibrin leading to pathogen entrapment and immobilization within a clot. Therefore we suggest a conserved early immune function for Drosophila TG and human FXIII.

INV06
Crosstalk between the long pentraxin PTX3 and the complement system

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The long pentraxin PTX3 is a multifunctional soluble pattern recognition molecule, which is produced and rapidly released by innate immune cells in response to inflammatory stimuli. PTX3 selectively recognizes certain pathogens and participates in defense against infections. PTX3 was shown to bind recognition molecules of different complement pathways and thus to activate the complement cascade, a powerful innate effector system, that is aimed to recognize and eliminate microorganisms. Because overwhelming complement activation could damage the surrounding host tissue, we analyzed whether PTX3 also interacts with complement inhibitors.
Binding of the complement regulators factor H (CFH) and C4b-binding protein (C4BP) to PTX3 was analyzed by ELISA and in serum adsorption experiments. A dose-dependent binding of CFH to PTX3 was observed. Inhibition experiments and peptide array analysis revealed a PTX3 binding site within the C-terminus of CFH. Incubation of human plasma on immobilized PTX3 and subsequent Western blot analysis of the eluted proteins demonstrated also a binding of the factor H family proteins CFHL1 and CFHR1, two complement regulators structurally related to CFH. A dose-dependent binding of C4BP to immobilized PTX3 was detected using both purified protein and human plasma as a source of C4BP. Results of blocking experiments excluded Protein S, which is complexed to C4BP in plasma, as a PTX3 ligand. Binding of CFH and C4BP to PTX3 was reduced in the absence of calcium and was inhibited by complement C1q. CFH and C4BP did not compete for PTX3 binding. Inactivation of complement by CFH and C4BP was not affected by PTX3, thus their regulatory activity is maintained when bound to PTX3.
In summary, in addition to complement activators, PTX3 also binds to complement inhibitors. This balanced interaction of PTX3 with activators and inhibitors may enable opsonization and removal of pathogens but at the same time prevent excessive complement activation.

KMP01
Emergence of OXA-48-producing Enterobacteriaceae in Germany

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Objectives: The slightly increasing carbapenem resistance in nosocomial Enterobacteriaceae is of special concern because of limited therapeutic options and higher mortality. This resistance is based on various resistance mechanisms like increased production of efflux pumps or loss of porins (outer membrane proteins, OMPs). Most prevalent is the acquisition of different carbapenemases like KPC and OXA-48 whose respective genes are localised on plasmids which enables the transfer between different enterobacterial species. OXA-48 was first described in *K. pneumoniae* from Turkey, France and last year from Belgium. Here we report on molecular analysis of different Enterobacteriaceae isolates from German hospitals with OXA-48 carbapenemase.
Methods: Carbapenem resistant isolates of *Klebsiella* spp. (n=4), *E. coli* (n=2) and *Citrobacter freundii* (n=1) were collected from three German hospitals in 2008 and 2009. Relevant resistance genes were identified by PCR and sequencing. Conjugation experiments and PFGE-typing were performed.
Results and conclusion:
The carbapenemase OXA-48 was identified in all strains. Analysis of the genetic environment revealed the location of insertion sequence IS*1999* upstream of $bla_{\text{OXA-48}}$. Furthermore ESBL genes $bla_{\text{SHV-12}}$ and $bla_{\text{CTX-M-15}}$ as well as beta-lactamase gene $bla_{\text{TEM-1}}$ were detected in several strains. All $bla_{\text{OXA-48}}$ genes were successfully transferred into recipients and plasmids of ca. 60kb size were isolated from transconjugants. The reduced carbapenem MIC values of transconjugants indicate the occurrence of further carbapenem resistance mechanisms in the clinical isolates. XbaI-macrorestriction analysis revealed that *K. pneumoniae* (n=3) from one hospital were identical.
Although OXA-48 is obviously rare in Europe it needs special attention because of the risk of wide horizontal dissemination among different enterobacterial species.

KMP02
Resistance to carbapenems and other beta-lactams in northern Germany is not mediated by Klebsiella pneumoniae carbapenemase

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Objectives: Emergence of beta-lactam-resistant Enterobacteriaceae producing Klebsiella pneumoniae carbapenemase (KPC) reduces therapeutic options in southern Europe and parts of the USA. To screen for KPC at a university hospital in northern Germany we tested *Klebsiella pneumoniae* strains with reduced susceptibility to 3rd generation cephalosporins and Enterobacteriaceae with reduced susceptibility to carbapenems by the new hyplex® KPC ID assay.
Methods: Strains isolated from November 2007 to November 2009 were identified by Vitek® 2 and MALDI-TOF MS. 71 *K. pneumoniae* strains positively tested for extended-spectrum beta-lactamases (ESBL) by Vitek® 2 and Etest® were included in the study. Additionally, one *K. pneumoniae* strain and 11 strains of Enterobacteriaceae (8 *Enterobacter cloacae*, 1 *Enterobacter aerogenes*, 1 *Citrobacter freundii*, 1 *Escherichia coli*) with reduced susceptibility to carbapenems were included. KPC-positive strains of 4 different species (*K. pneumoniae*, *Serratia marcescens* , *E. coli*, and *Salmonella Cubana*) were used as control. All strains were analyzed by the hyplex® KPC ID kit which comprises PCR with primers for multiple variants of blaKPC followed by reverse hybridization of the denaturated amplified DNA with specific single-stranded probes.
Results: All 72 *K. pneumoniae* strains and all 11 clinical Enterobacteriaceae strains were tested negative for KPC expression in contrast to all known KPC-producing control strains. Thus, the specificity and sensitivity was 100%, respectively. In contrast to culture-based methods like Vitek® 2 or Etest® hyplex® KPC ID takes less than six hours.
Conclusion: The result for 83 strains of Enterobacteriaceae with reduced susceptibility against wide spectrum beta-lactams including in some cases carbapenems suggest a low prevalence of KPC at a university hospital in northern Germany. The hyplex® KPC ID kit is a rapid, inexpensive, and easy-to-handle assay for detection of KPC in Enterobacteriaceae.

KMP03
Characterization of coagulase-negative Staphylococcus in the stool of newborns

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Background: Coagulase-negative staphylococci (CoNS) are the most prevalent pathogens causing late onset sepsis in neonates. They are often multiresistant to antibiotics and the abilities to form biofilms and to carry distinct insertion elements e.g. IS256 are considered their main virulence determinants. We asked whether neonates acquire clonal multiresistant CoNS after birth during their hospital stay. Therefore this study aimed to analyze whether newborns acquire pathogenic multiresistant CoNS directly after birth.

Methods: For this purpose we performed a prospective study on the presence of CoNS strains in the stool of newborns born at the department of gynecology at the Otto-von-Guericke-University in Magdeburg. CoNS were genotyped by terminal restriction fragment analysis, by pulsed-field-electrophoresis and by rep-PCR as well as by MLST. Resistance profiles, biofilm production, the presence of the icaABCD locus and the IS256 element were determined.
Results: From a total of 207 stool samples taken from 56 newborns CoNS were detected in 38% of the newborns, mostly on day 3 after delivery (62.5%). Among the CoNS isolates *S. epidermidis* was isolated most frequently (85.7%). Molecular epidemiology of *S. epidermidis* by rep-PCR divided the isolates into 3 main clusters each divided in 2 subclusters. No differences were observed between babies born vaginally or by sectio. Only minor antibiotic resistance was observed. Although >50% of the strains harboured the icaABCD locus, we could not detect biofilm formation using standard assays. IS256 was not detected as well.
Conclusion: In summary, our data indicate that the isolated *S. epidermidis* strains in the stool of babies born either vaginally or by sectio do not belong to a clonal group but are incidentially occurring strains. Thus, S. epidermidis strains acquired just after birth are not a risk factor for late onset sepsis in newborns.

KMP04
Occurrence of Resistance to Tobramycin and Colistin in Isolates of *P. aeruginosa* from chronically colonised Patients with Cystic Fibrosis under antimicrobial treatment

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Background: tobramycin and colistin represent two standard antimicrobial agents in the treatment of CF patients which are chronically colonised with *P. aeruginosa*. The objective of this study was to determine whether persistent resistance to tobramycin and colistin may occur in isolates of *P. aeruginosa* obtained from chronically colonised CF patients under continuous or intermittent treatment with nebulised tobramycin and colistin in turn.
Methods: In this study, we retrospectively determined the minimal inhibitory concentration (MIC) of tobramycin and colistin in 300 isolates of *P. aeruginosa* collected between 2004 and 2008 from sputum samples of 22 chronically colonised CF patients which received continuously or intermittently nebulised tobramycin and colistin in turn.
Results: Resistance to tobramycin was observed in 23.6% of all isolates of *P. aeruginosa* tested. With regard to the isolates obtained from single patients, a persistent resistance to tobramycin occurred in four patients (18%). Furthermore, none of the 300 isolates tested showed a resistance to colistin.
Conclusions: persistent resistance to tobramycin in *P. aeruginosa* isolates of chronically colonised CF patients under antimicrobial therapy may occur. In contrast, occurrence of colistin resistance is uncommon. These data confirm that colistin represents a valid alternative to tobramycin in the treatment of CF patients.

KMP05
Typing of *Aggregatibacter actinomycetemcomitans* in subgingival plaque samples

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Background: *Aggregatibacter actinomycetemcomitans* plays an important role in etiology of severe periodontitis. In Northern Africa, JP2 clone having a deletion in the promote region of the leukotoxin gene is found in cases of extremely progressive disease. Studies in U.S. associated the prevalence of serotype b with aggressive periodontitis, contrary in Asia serotype c is more prevalent in severe periodontitis.
Objectives: The purpose of our study was to analyze 102 *A. actinomycetemcomitans*-positive subgingival plaque samples obtained in four cities of Germany (29 Jena, 38 Leipzig, 17 Frankfurt, 18 Hamburg).
Methods: Detection of JP2 clone and serotyping was made by means of PCR technique.
Results: JP2 clone was only found in two patients with an ethnical background (at least one of the parents originated from Africa) in Frankfurt. In all samples, serotype a was detected 25 times, b and c each 21 times, d 7 times, e 8 times, f 10 times, five samples were not typeable. Interestingly differences between the regions were visible. In Frankfurt, serotype a was not detected. Serotype d was only found in samples from Leipzig.
Prevalence of serotypes seems to depend very much on the region and does not represent in first line virulence. JP2-clone keeps its affinity to Nothern Africans.
Conclusions: Serotyping of *A. actinomycetemcomitans* in clinical microbiological analysis does not seem to be very useful. Contrary, analysis for JP 2 should be made in samples obtained from patients with an ethnical background.

KMP06
Prevalence of rectal, genital and pharyngeal *Neisseria gonorrhoeae* and *Chlamydia trachomatis* infections in sexually active females

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Background: *Neisseria gonorrhoeae* and *Chlamydia trachomatis* are the most prevalent bacterial causes of sexually transmitted diseases (STDs) in the world. Most likely a high proportion of infections is asymptomatic especially in females. However the prevalence of asymptomatic gonorrhea and chlamydial infection rates among females in Germany is unknown. We investigated swabs of sexually active healthy females including prostitutes as a high risk group to evaluate the prevalence of rectal, genital, and pharyngeal gonococcal and chlamydial infections.
Methods: Participants attending regular STD consultations in two Bavarian local health authorities were enrolled. They were asked to complete a questionnaire regarding their age, current clinical complaints, STD history and country of origin. Swabs were tested for *Neisseria gonorrhoeae* (NG) and *Chlamydia trachomatis* (CT) by ligase chain reaction and culture using a special transport medium for NG. Susceptibility testing for NG was performed according to CLSI standards.
Preliminary Results. Currently, a number of 45 pharyngeal, 44 vaginal and 26 rectal swabs from 45 females were investigated. The prevalence of gonorrhea was 4% (2/45 persons). Rectal and vaginal swabs were positive for NG in both patients, one of these persons also harboured NG in the pharynx. In another two different cases CT was diagnosed in vaginal and pharyngeal swabs. All four patients were asymptomatic.
Conclusions: The pharynx is a possible asymptomatic reservoir for both gonorrhea and chlamydial infections in sexually active females suggesting the need for routine screening of nonurethral sites in persons at higher risk of STD infection such as prostitutes. Further investigations are certainly needed.

KMP07
Hypoxia affects the efficacy of antibiotics to intracellular Chlamydia

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Objectives: Chlamydiae are obligate intracellular pathogens that cause a wide range of human diseases. Currently available antibiotics are effective in treating acute chlamydial infections, but fail to eradicate persistent infections. We could show previously that a low oxygen microenvironment, which is found under physiological conditions, induces chlamydial growth and progeny. The aim of this study was to investigate the activity of different antibiotics on intracelllular chlamydial growth under hypoxic conditions.
Methods: We determined the MIC of doxycyclin, rifampin, erythromicin and moxifloxacin on chlamydial growth in HEp-2 cells under normoxic and hypoxic (2% O2) conditions. Kinetics of C14-labeled moxifloxacin uptake were monitored in hypoxia. To analyze antibiotic efficacy in different chlamydial developmental stages, time-kill curves were performed.

Results: MIC for doxycyclin, rifampin, erythromicin and moxifloxacin on *C. pneumoniae* and *C. trachomatis* did not differ between normoxia and hypoxia. Furthermore, uptake of C14-labeled moxifloxacin was not affected under hypoxic conditions, showing high intracellular concentrations of the drug within 5 min after stimulation. Interestingly, reduction in chlamydial growth within the early phase of infection was less effective in doxycyline (2 µg/mL) treated cells when cultured under hypoxia than under normoxia.
Conclusion: Low oxygen concentrations have a strong impact on the transcriptional activity and metabolism of host cells that have to be considered in testing antibiotic efficacy to intracellular pathogens. We suggest to perform time-kill curves instead of standard MIC testing to calculate efficacy of antibiotics under low oxygen concentrations. Further studies will show whether reduced antibiotic efficacy under hypoxia contributes to the emergence of persistent chlamydial infections in humans.

KMP08
Mastoiditis with a foreign body - a fatal combination

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In Oktober 2008 a 70 year old male was found unconscious in his flat in an assisted living institution. The evening before he had been fit and well. His recent medical history revealed a persistent MRSA-positive otitis media. The course of disease had been frustrating because the patient kept on sticking foreign bodies into his outer ear. Besides the patient had developed a tendency to fall without cause just recently.
An initial cranial CT revealed a mastoiditis on the right side and a paramedian infarction of the pons. On the day of admission the patient underwent mastoidectomy. A burnt match and plant residues were removed. Gram stain of the material did not show any bacteria and a therapy with ampicillin, ceftazidime and tobramycin was initiated. A day later a pressure hydrocephalus developed and a drainage had to be implanted. CSF showed a pleocytosis (>1000 cells/ml) and gram-positive cocci. Due to the patients MRSA+ history vancomycin was given intrathecally and linezolid was started systemically. Two sets of blood cultures were positive with oxacillin-resistant *Staphylococcus hominis* (4/4). Culture of the mastoiditis material revealed no bacteria but culture of the CSF revealed *Bacteroides fragilis*, *Peptostreptococcus sp.* and *Actinomyces odontolyticus*. Therefore ampicillin and tobramycin were stopped and metronidazole started.
Despite all intensive care the patient developed a thalamic infarction, high cerebral pressure and anisocor pupils. He passed away 7 days after admission.

KMP09
Retrospective analysis of the antibiotic susceptibilities of *Pseudomonas aeruginosa* from cystic fibrosis (CF) patients in Germany

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Pseudomonas aeruginosa is the major bacterial pathogen that infects and chronically persists in the lung of patients with cystic fibrosis (CF). In a retrospective analysis by the two consiliary laboratories for CF bacteriology in Germany (Medizinische Hochschule Hannover and the Max von Pettenkofer-Institut, Munich), susceptibility patterns of P.aeruginosa from CF patients of the last decade were analyzed. Interestingly and in contrast to the emergence of antibiotic resistances of *P. aeruginosa* from the non-CF-community, resistance patterns and multidrug resistance (MDR; definition: only one or none bactericidal antibiotic (ceftazidime, ciprofloxacin, meropenem) is found to be susceptible) of CF isolates showed only minor variations over the last years. Resistance rates of *P. aeruginosa* recovered from adult CF patients range from 39.4%-45.6% for ceftazidime, 57.4%-72.7% for tobramycin, 43.6%-47.9% for ciprofloxacin, 28.1%-36.7% for meropenem and 4.3%-6.3% for colistin, respectively. Lower resistance rates were generally found for isolates of patients less than 18 years of age. Since 2000, the rate of MDR among isolates from adults remains stable above 70%, indicating a permanent antibiotic selection pressure.

KMP10
Characterization of antimicrobial substances produced by *Bacillus pseudomycoides* DSM 12442

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The emergence of antibiotic resistance, such as vancomycin-resistant enterococci and methicillin-resistant staphylococci, has been of great concern in recent years, so there is a pressing need for development of novel antimicrobial agents. Lantibiotics are small microbial peptide antibiotics that are characterized by the presence of thioether amino acids lanthionine and methyllanthionine. Lantibiotics possess structural genes which encode inactive prepeptides. During maturation, the prepeptide undergoes posttranslational modifications including the introduction of rare amino acids as lanthionine and methyllanthione as well as the proteolytic removal of the leader. The structural gene *(lanA)* as well as the other genes which are involved in lantibiotic modification *(lanM, lanB, lanC, lanP)* , regulation *(lanR, lanK)* , export (*lanT(P)*) and immunity *(lanEFG)* are organized in biosynthetic gene clusters.
Genomic data mining results, identified new complete lantibiotic gene cluster in Gram positive bacteria *Bacillus pseudomycoides* DSM 12442. Antimicrobial activity was detected only in an isopropanol wash extract of the celll pellet of 24 hours incubated *B. pseudomycoides* DSM 12442 while not in culture supernatant. In agar well diffusion assays these substances showed activity against many Gram positive bacteria, including bacilli, sretptococci and staphylococci, whereas no activity against Gram negative bacteria. Also, MALDI-TOF predicted a mass of 2786.59 Da for the active peptide but these data have to be confirmed.
Further experiments will be directed towards characterizing the lantibiotic gene cluster of *B. pseudomycoides* DSM 12442 by identifying relation between the antibacterial activity and lantibiotic gene cluster by lanthionine detection and amino acid sequence analysis. Furthermore, stability assays will be performed to determine the influence of temperature, pH and various protease enzymes in antimicrobial substances produced by *B. pseudomycoides* DSM 12442.

KMP11
Thymidine-dependent *Staphylococcus aureus* small colony variants - human pathogens that are relevant not only in cystic fibrosis lung disease

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Thymidine-dependent small colony variants of *Staphylococcus aureus* (TD-SCVs) are well-known in the context of cystic fibrosis (CF) lung disease. Here, we report the isolation of TD-SCVs from clinical specimens other than CF respiratory samples. These patients suffered from a chronic soft tissue infection, a chronic tympanitis, a chronic bronchitis, a recurrent abscess with subsequent peritonitis, and septicemia and all of them had received prior antibiotic prophylaxis with trimethoprim/sulfamethoxazole (SXT). All TD-SCVs analyzed in this study had mutations in the thymidylate synthase-encoding thyA gene and four of five isolates were able to revert to the normal colony variant (NCV) phenotype. Sequence analysis of thyA of NCV revertants revealed that an SCV-inducing nonsense mutation can be compensated for by different missense mutations in the same codon. With the exception of SXT susceptibility, the NCV revertants showed antibiotic resistance patterns similar to that of the corresponding TD-SCVs. Interestingly, one fosfomycin-resistant TD-SCV isolate without any CF context showed a remarkably elevated mutation frequency. Furthermore, we provided direct evidence that thymidine and its metabolite dTMP are present not only in CF sputum, but also in various other human specimens. The availability of these growth factors will facilitate in addition to antibiotic selective pressure and hypermutability the long-term persistence of TD-SCVs. All, these observations emphasize the clinical significance of TD-SCVs in chronic infections and should alert microbiologists to look for TD-SCVs not only in the context of CF lung disease but in particular in the case of prior or ongoing SXT treatment.

KMP12
96-well plate based optical method for the quantitative and qualitative evaluation of *Pseudomonas aeruginosa* biofilm formation and its application for susceptibility testing

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The major reason for bacterial persistence during chronic infections seems to be that the bacteria survive within biofilm structures, which protect the cells from environmental stresses, the host immune response and antimicrobial therapy. Thus, there is concern that laboratory methods developed to measure the antibiotic susceptibility of planktonic bacteria may not be relevant to chronic biofilm infections and it was suggested that alternative methods should test antibiotic susceptibility within a biofilm. Here we describe a fast and reliable 96-well microtiter based method for the formation of *P. aeruginosa* biofilms, which is easily adaptable for antimicrobial susceptibility testing. This method is based on bacterial viability staining in combination with automated confocal laser scanning microscopy. The procedure simplifies qualitative and quantitative evaluation of late stage biofilms and proved to be a highly effective and rapid method for standardized determination of antibiotic efficiency on *P. aeruginosa* biofilms.

KMP13
ESBL in nosocomial Enterobacteriaceae from Germany – a one-year study

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Objectives: In recent years the rate of resistance to 3rd gen. cephalosporins in Enterobacteriaceae is increasing continuously. The main cause of resistance is the expression of Extended-Spectrum Beta-Lactamases (ESBL) whose respective genes are localised on plasmids which enables the transfer between different enterobacterial species. Since 2008, in the ARS-surveillance-system (Antibiotikaresistenz-Surveillance Deutschland) antimicrobial resistances of all nosocomial pathogens were collected and analysed continuously. In the scope of ARS the aim of this study was to investigate emergence and prevalence of distinct ESBL-types in a representative sample of Enterobacteriaceae from Germany.
Methods: All hospitals participating in the ARS-study were asked to send in the first Enterobacteriaceae isolates with ESBL-phenotype in 2008. A total of 154 *E. coli* (150 hospitals) and 66 *Klebsiella* spp. (53 hospitals) from the whole of Germany were analysed. Relevant resistance genes were identified by PCR and sequencing. Conjugation experiments, PFGE-typing and analysis of the genetic environment of plasmid-located ESBL genes were performed with selected isolates.
Results and Conclusion: More than 70% of all cephalosporin resistant isolates were additionally resistant to fluoroquinolones and sulfonamides. Among all ESBL the CTX-M-group enzymes were predominant (93% *E. coli*, 79% *Klebsiella* spp.) and CTX-M-15 (53% *E. coli* 63% *Klebsiella* spp.) was the most prevalent ESBL-type. Using the example of CTX-M-1, analysis of the genetic environment revealed a similar structure in 14 *E. coli* isolates whereby $bla_{\text{CTX-M-1}}$ was located between two copies of insertion sequence IS*26*.
CTX-M group 1 enzymes are wide-spread in Enterobacteriaceae. In addition to the successful dissemination by conjugative plasmids the distribution of $bla_{\text{CTX-M-1}}$ is probably supported by a specific IS*26* composite transposon.

KMP14
Comparative Analysis of the Resistance Patterns, Virulance Determinants and Antibiotic Sensitivity of German and Nigerian Enterococcal Isolates

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Background: Enterococci are usually part of the normal flora of the gastrointestinal tract of humans and animals. However, there are consistently cases of severe infections in patients with vancomycin-resistant enterococci (VRE) and other virulent enterococci. In this study we compared Nigerian and German enterococcal isolates according to their resistance and pathogenetic patterns.
Methods: Antibiotic susceptibility was tested by use of VITEK2. VREs were confirmed by Etest. Multiplex-PCR was performed for detecting the virulence factors (aggregation substance (*asa1*), gelatinase (*gelE*), cytolysin (*cylA*), enterococcal surface protein (*esp*) and E. faecium-hyaluronidase (*hyl*)) of 150 enterococcal isolates from Nigeria and 150 isolates from Germany.
Results: 70% of Nigerian isolates (n=105) expressed *gelE* and approx. 50% expressed *asa1*. One E. faecalis isolate expressed *esp* and another E. faecium isolate expressed *hyl*. More than 50% were erythromycin and quinudalfopristin resistant, and one third was tetracycline resistant. The German isolates (E. faecium, n=21; E. faecalis, n=129) expressed more frequently the genes *gelE* (85%, n=128), *asa1* (70%, n=105), *esp* (10%, n=15) and *hyl* (10%, n=15). The German and Nigerian isolates shared similar resistant profiles, especially according to erythromycin. 50% of the Nigerian and 75% of the German isolates were tetracycline-resistant. 12 German isolates were vancomycin-resistant (VRE), whereas none of the Nigerian isolates were VREs.
Conclusion: The resistance results are essential for the treatment monitoring and the epidemiology of the enterococci. The PCR-analysis revealed a high distribution of *asa1* and *gelE* among the five virulence genes in the samples of the two countries. None of Nigerian isolates expressed *esp* whereas approx. 10% of the German isolates were positive for this genes.

KMP15
First Report of Association of Integron-Borne Extended Spectrum β-Lactamases (OXA-10, TEM-106, VEB-1), an Amp-C β-lactamase (CMY-2) and a Plasmid-Mediated Quinolone Resistance (qnrA1) in a Clinical Strain of *Providencia rettgeri*, from a Tertairy Hospital in Nigeria, West-Africa

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Background: Infection by *Providencia spp*, gram-negative bacteria, belonging to the family Enterobacteriaceae is of recent, on the increase and more significantly are infections caused by multidrug resistant strains of this species.
Method: We investigated the presence of various β-lactamases and antibiotic resistance genes in a multidrug resistant *Providencia rettgeri* clinical strain isolated from the catheter-tip of a patient. Antibiotic susceptibility was carried out using VITEK 2. The double-disk synergy test (DDST) and ESBL E-test methods were used to confirm the production of ESBL enzymes. PCR and DNA sequencing was carried out to investigate the type of antibiotic resistance and ESBL genes present.
Result: The multidrug resistant Providencia rettgeri strain was found to harbour an „insertion sequence common region" (ISCR) orf 513 and IntI1 (integron class 1) on which four diffferent beta-lactamase genes (blaCMY-2 an AmpC β-lactamase; and Extended-Spectrum β-lactamases: blaOXA-10, blaVEB-1 and blaTEM-106) were located. This strain harboured, in addition, on the class 1 integron, a plasmid-mediated quinolone resistance gene qnrA. Tetracycline resistance genes L, A, E encoding tetracycline efflux pump and the dfrhA1, 15, 16 dihydrofolate reductase genes which confer resistance to trimethoprim were also detected in this strain.

Conclusion: This strain expressed a fatal combination of three different ESBLs and an AmpC β-lactamase. This is the first report of the occurrence of each of these β-lactamases and the qnrA gene in such association in any Enterobacteriaceae species from West-Africa. Without new methods for attacking them, potentially fatal bacterial infections could pose a serious threat to the world's population. With widespread international travel and the evolution of resistant strains of bacteria the situation could become worse than it was before the discovery of penicillin. To prevent the spread of these strains in the hospital, hospital-wide multi-factorial intervention needs to be put in place.

KMP16
Discrimination of erythromycin resistant and erythromycein sensitive *Francisella tularensis* with different molecular methods

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Francisella (F.) tularensis is a small, Gram-negative bacterium causing tularemia in humans and a wide range of mammals. *F. tularensis* is intrinsically resistant against all beta-lactam antibiotics and cotrimoxazole. Strains, which are resistant against macrolides have been also described. A study on a collection of German *F. tularensis* isolates revealed, that a large fraction (40%) is resistant against erythromycin and that a single nucleotide polymorphism (SNP) within the 23S rRNA gene mediates the resistance.
Here, we developed several molecular methods for the discrimination between sensitive and resistant *F. tularensis* strains based on the previously described SNP: Two different real time PCR assays (TaqMan® and Lightcycler®) targeting *F. tularensis* 23S rDNA as well as FISH probes targeting *F. tularensis* 23S rRNA. E-tests for determining the antibiotic phenotype were used as a reference method. A representative panel of 10 sensitive and 10 resistant *F. tularensis* strains was tested with all three molecular methods. The real-time PCR assays as well as the FISH assay correctly identified all resistant and sensitive strains and therefore could discriminate between both phenotypes.
Our assays establish the basis for the development of diagnostic tools for the discrimination of macrolide-resistant and macrolide-sensitive *F. tularensis* isolates. Such tools are of special interest in the light of recent recommendations to explore the use of macrolides in the treatment of tularemia and also the new FDA „Orphan Drug Designation" for the prophylactic treatment of tularemia with cethromycin.

KMP17
Exoproteomic characterization and comparison of *Staphylococcus aureus* strains isolated from human and bovine hosts

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Staphylococcus aureus can cause a range of illnesses from minor skin infections to life-threatening diseases such as endocarditis and mastitis in a variety of mammalian hosts. However the role of *S. aureus* virulence factors in the different clinical outcomes is far from being completely unraveled.
To address this we compared *S. aureus* strains isolated from human and bovine hosts contracted with endocarditis or mastitis by analyzing their 2-DE derived exoproteomes and genetic variations (MLST and Clondiag diagnostic microarrays).
We demonstrate that within the same host as well as causing a similar clinical picture the exoproteome composition can be diverse as a combined result of both genome plasticity and expression heterogeneity. Furthermore our results prove important differences between the genetic repertoire of virulence factors and the de facto expressed genes in the analyzed strains.
Our data imply that genomic studies comparing clinical isolates of *S. aureus* need to be complemented by exoproteomic analyses to assess the full virulence potential of this bacterial pathogen.

KMV01
Prevalence of *Chlamydia trachomatis* among adolescents and young adults in Southern Germany

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The *Chlamydia trachomatis* (*C. trachomatis*) Serotypes D-K are among the most common sexually transmitted bacterial organisms in industrialized countries, and typically cause urethritis in men and cervicitis in women. To prevent serious sequelae of infections such as pelvic inflammatory disease and tubal infertility a Chlamydia screening program was established in 2008 by the G-BA (Gemeinsamer Bundesausschuss). However, since *C. trachomatis* infections are not among the notifiable diseases reliable data about the prevalence of infections in Germany are sparse.
We therefore performed a prospective prevalence study including more than 1000 young sexually active people of Ulm and the surrounding area. First void urine samples were obtained and analyzed using an established PCR assay (Roche, COBAS®Amplicor) as well as a newly developed PCR assay (GenoQuick®CT Hain Lifescience GmbH) that also enables the detection of the so-called new Swedish *C. trachomatis* variant. In addition study participants completed a standardized questionnaire which gathered behavioural information as well as clinical symptoms.
The overall PCR prevalence of *C. trachomatis* was nearly 4%. Although the prevalences in women and men did not differ significantly the highest prevalence (8.5 %) was found in men aged between 25 and 35. More than 60% of infected women and 100% of infected men were asymptomatic. The risk of infection increased with increasing sexual activity including several sex partners and an early first sexual intercourse. Both PCR assays showed comparable sensitivities and specificities. Studies are on the way to clarify if a few discrepant samples can be explained by the presence of a new variant of *C. trachomatis* recently discovered in Sweden.
Our study demonstrates that asymptomatic sexually active men might be an important reservoir for the transmission of the organisms and poses the question if Chlamydia screening programmes should be restricted to women.

KMV02
Pigs as a source for toxigenic *Corynebacterium ulcerans* in diphtheria-like disease

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We would like to present this case report in the TED-session of Clinical Microbiology.
Toxigenic Corynebacterium ulcerans may cause a zoonotic infection similar to Corynebacterium diphtheriae-caused diphtheria. Previously, mainly dairy cattle, pet dogs and pet cats were described as *C. ulcerans* carriers. We report the first case of severe *C. ulcerans* diphtheria-like disease after pig contact in a female farmer. On admission to the university hospital, a previously healthy 56-year-old female farmer presented with a sore throat and progressive dysphagia for one week. Clinical examination of the oropharynx revealed thick whitish pseudomembranes on both tonsils, the uvula and the pharynx. A pharyngeal swab obtained from below the whitish pseudomembranes grew toxigenic *C. ulcerans*. Pharyngeal swabs of three family members, 19 pigs and the farm dog were obtained and analysed for *C. ulcerans*. While all family members and the dog were *C. ulcerans*-negative, one of the 19 asymptomatic pigs harboured a toxigenic *C. ulcerans* strain. Sequencing of rpoB and tox revealed 100 % homology between the human and the pig strain. Ribotyping confirmed this result suggesting the identity of both strains. The obtained ribotype is similar to the reported U1 ribotype profile found in both humans and cats.

KMV03
Case Report: Diagnosis of a rare microbial infection by detour

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We describe a case of a 50 year old male patient who suffered since nearly 20 years from chronic polyarthritis, thoracic myalgia, recurrent episodes of fever with increased CRP and morning stiffness. The patient was treated according to the presumptive diagnosis of a Still syndrome of the adult type. Over the years he was medicated with various immunosuppressive drugs including methotrexate, leflunomide, chloroquine, sulfasalazine and azathiprine without major amelioration of the symptoms. In the course of the disease therapy trials with different biologicals were performed. Steroid treatment up to 150 mg prednison was required to increase state of health. In 2006 a coloscopy produced no pathologic result and a gastroscopy showed an inflammatory process with increase in lymphocytes. Weight loss was not reported.
At the time of admission the patient had fever, leucocytosis, increased CRP and increasing pain in the back (with a history of herniated disc). Close examination revealed a small abscess in the lumbar spine which was punctured. Conventional microbial culture showed no growth of bacteria or fungi. To test for fastidious bacteria a 16S rRNA PCR analysis was performed which surprisingly identified DNA from *Tropheryma whippelii*. The diagnosis could be confirmed applying a specific semi-nested PCR on the sample. To further strengthen the suspected diagnosis of Morbus Whipple a former duodenal biopsy specimen taken in 2006 was obtained. Histology previously had shown lymphocyte infiltration but no typical PAS positive cells. However, *T. whippelii* PCR of the paraffin embedded material now detected the causative organism in the specimen. Immunosuppressive therapy was aborted immediately and antibiotic treatment (ceftriaxon followed by cotrimoxazol) was started. After two month of treatment fever and leucocytosis had disappeared, the general condition of the patient had improved considerably and steroid treatment could have been decreased steadily.

KMV04
Impact of performing multiplex real-time PCR and interleukin-6 determination in CSF for the rapid diagnosis of ventriculitis in neurosurgery patients with external ventricular drainage

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Background: The accurate and timely diagnosis of external ventricular drainage (EVD)-related infections in neurosurgical patients is highly important for clinical management because the early availability of information on the causative pathogen is crucial for targeted infective therapy. The goal of this study was to assess the clinically utility of a novel multiplex real-time PCR in conjunction with interleukin-6 (IL-6) measurements in cerebrospinal fluid (CSF) in neurosurgery patients with suspicion of EVD-related ventriculitis.
Methods: Overall, IL-6 and microbiological CSF cultures were analyzed from 62 neurosurgical patients with EVD. In addition, 1.5 ml of the paired CSF samples were used for a commercial multiplex real-time PCR assay (SeptiFast, Roche Diagnostics, Mannheim, Germany) which detects simultaneously 25 nosocomial-relevant bacteria and fungi within six hours. The PCR was performed according the manufactures' instructions using MagNA Lyser for extraction of DNA and LightCycler 2.0 for amplification and detection. The intrathecal IL-6 concentrations were correlated with the pathogen detection by the PCR assay.
Results: Concordance of CSF culture and PCR was established in 91% of the samples. In one case a pathogen could only be detected with the PCR assay. Four samples were CSF culture positive but PCR negative from which three isolates were considered to be contaminations. According to a cut-off value IL-6 of 4,050 pg/ml (WHO standardization) the OR was 9.66 [95% CI 2.3 – 39.1] for pathogen detection by PCR ($P < 0.01$). The median time for PCR results was 6.5 h compared to 42 h for identification by culture.
Conclusion: The data indicate that performing multiplex PCR in CSF samples with increased IL-6 concentrations is highly effective for the rapid etiologic diagnosis of EVD-related ventriculitis in neurosurgery patients.

KMV05
Rapid identification of single bacteria by means of micro-Raman spectroscopy

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Due to its high specificity Raman spectroscopy has proven to be a powerful tool for the analysis of isolated molecules, single cells or even tissue. Since water exhibit only week Raman signals even in-vivo spectroscopic information about cell components can be analysed. By means of micro-Raman spectroscopy a spatial resolution in the sub-micrometer range can be achieved allowing the investigation of single bacterial cells.
In this contribution we will present the identification of microorganisms without the need of any cultivation step. The combination of micro-Raman spectroscopy and different chemometric approaches allows for the identification of bacteria even on strain level [1] and in medical samples such as liquor [2]. However, for real samples a localization step is necessary prior to identification.
The localization of microorganisms in e.g. blood or food samples is often complicated, since many particles exhibit similar size and shape compared to microorganisms. Therefore, a localization routine has to be established which allows for the differentiation of biotic and abiotic particle. This localization step can be performed by active fluorescence staining as long as the fluorescence dye does not absorb in the range of the Raman excitation laser. Still the Raman spectra of fluorescence labeled bacteria can be used for identification. In addition, this approach allows for the differentiation between living and dead microorganisms which is essential for medical applications. [3]
Acknowledgement
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KMV06
Establishment of a MALDI-TOF-MS based typing scheme for rapid discrimination of Methicillin-Resistant *Staphylococcus aureus*

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Methicillin-resistant *Staphylococcus aureus* (MRSA) are a major cause of nosocomial infections. Early detection of potential nosocomial outbreaks and initiation of hospital infection control measures are important to limit MRSA dissemination. To reach this goal, timely determination of epidemiologic relatedness of clinical MRSA isolates is essential. Genetic typing methods like pulsed-field gel electrophoresis (PFGE), *spa*-typing, or multilocus sequence typing (MLST) have a high discriminatory power, however, these methods are time consuming and cost intensive, precluding short term typing and broad routine application. The aim of this study was to establish and evaluate a typing scheme based on matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS) based finger printing for clonal discrimination of clinical MRSA isolates. Mass spectra from 25 representative MRSA isolates belonging to the five major hospital-acquired (HA) MRSA clonal complexes (CC5, CC8, CC22, CC30, CC45) were used to identify specific peaks for each complex, resulting in a unique reference peak profile for each clonal complex. MALDI-TOF MS spectra of 61 isolates were matched against the reference spectra and allocated to putative clonal complexes. Comparison of MALDI-TOF MS grouping with clonal complexes deduced from *spa* typing data revealed a high level of concordance. Our results suggest that MALDI-TOF MS has the potential to become a valuable first-line tool for inexpensive and rapid typing of MRSA in hospital infection control.

TED-Fall 1

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TED-Fall 3

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TED-Fall 4

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LMP01

Prevalence of Salmonella among food handlers in *Owerri metropolis*

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A study of Salmonella amongst food handlers in *Owerri metropolis* was carried out between February and August, 2006. This was done with a view to elucidating the level of hygiene employed by food handlers. Of the 50 stool samples collected 36(72.0%) had growth on the selective media used. After biochemical and morphological characterization of the isolates were done, 19(52.8%) and 17(47.2%) had Salmonella and Shigella species respectively. The frequency of isolation of Salmonella from stool sample of food handlers obtained from different eating houses showed that open-air eateries had the highest prevalence (62.5%), followed by mobile food vendors (60.0%), while the least prevalence was observed amongst canteens, low-class hotels and fast food outfits (25.0%). No Salmonella species were isolated from middle class and high class hotels. The present result indicates that the prevalence of Salmonella amongst food handlers in Owerri is high and calls for proper health education package for these group of people. This study was therefore designed and carried out to ascertain the prevalence of Salmonella infection among food handlers in *Owerri metropolis*, with a view to elucidate the level of hygiene employed by food handlers and the danger inherent in poor handling of foods.

LMP02

Quorum sensing capacities of *Campylobacter spp*. in food matrices

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Numerous bacteria, including *Campylobacter (C.) jejuni* communicate via the small interspecies-specific signalling molecule autoinducer-2 (AI-2). This process (Quorum sensing) modulates physiological functions in many bacterial species, and specifically targets and affects bacterial growth and viability as well as the production of toxins.
Nonetheless, data are missing about the ability of other *Campylobacter spp*. to produce AI-2 and the inhibitory effect of food matrices on AI-2 activity.
In our study, Vibrio harveyi BB170, which is able to respond to AI-2, was used as a bioluminescence-reporter. We demonstrate that other Campylovbacter spp. such as C. upsaliensis, C. sputorum, *C. fetus spp. fetus* and *C. concisus* encode for the AI-2 synthase LuxS and are able to produce AI-2 with a maximal AI-2 activity during the late logarithmic-phase. For *C. lari* we could neither detect luxS nor AI-2 activity in a large number of strains screened.
Incubation of *C. jejuni* NCTC 11168 in different food matrices, like chicken juice and milk, resulted in an inhibition of AI-2 activity. Also the mutant strain Vibrio harveyi BB152 which is known to produce AI-2, shows a reduced AI-2 activity when incubated in these food matrices.The inhibitory effect of food matrices on AI-2 activity occurs without affecting bacterial cell viability. The observation that food matrices with a higher fat-level have a higher inhibitory effect, leads to the assumption that fatty acids might act as inhibitors in quorum sensing-systems of Campylobacter.
We demonstrated that the expression of luxS in *C. jejuni* is not regulated during incubation in chicken juice. The luxS expression and protein production was tested at the RNA and the protein level using Real-time-RT-PCR and Western Blot respectively. As the expression level of luxS is not affected during the incubation of *C. jejuni* in food matrices the aforementioned effects could be mediated by a direct interaction of the AI-2 and the food matrices.

LMP03

Rapid identification and characterization of Vibrio species using whole-cell MALDI-TOF mass spectrometry

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Vibrio identification by means of traditional microbiological methods is time consuming due to the many biochemical tests that have to be performed to distinguish closely related species. This work aimed to evaluate the use of MALDI-TOF mass spectrometry for the rapid identification of *Vibrio (V.) spp*. as an advantageous application to rapidly discriminate the most important *Vibrio spp*. and distinguish *Vibrio spp.* from closely related bacterial species like *Photobacterium damselae* and *Grimontia hollisae* and aquatic bacteria like Aeromonas spp.
Starting from sub-colony amounts of pure cultures grown on agar plates, a very simple sample preparation procedure was established and combined with a rapid and automated measurement protocol that allowed species identification within minutes. Closely related species like *V. alginolyticus* and *V. parahaemolyticus* or *V. cholerae* and *V. mimicus* could thus be differentiated by defining signatures of species-identifying biomarker ions (SIBIs). As a reference method for species designation and for determination of relationships between strains with molecular markers, partial rpoB gene sequencing was applied.
The MALDI-TOF MS based method as well as the rpoB sequence-based approach for Vibrio identification described in this study produced comparable classification results. MALDI-TOF MS – based grouping and phylogenetic classification based on rpoB sequences revealed a good degree of congruence. Our results suggest that whole-cell MALDI-TOF MS - based proteometric characterization represents a powerful tool for rapid and accurate classification and identification of *Vibrio spp*. and related species.

LMP04
Isolation and identification of biogenic amines producing lactic acid bacteria from German wines

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Biogenic amines are small nitrogen compounds which are widespread in food and beverages such as fish, meat, cheese, wine and milk. Usually, they are an indication for microbiological activities. Studies have shown that biogenic amines are responsible for several health problems. For example histamine can cause headaches, hypertension and digestive problems while tyramine is often associated with migraine. Their toxicological effect in wines is increased by the presence of ethanol which inhibits the mono-amine oxidase (MAO) and thereby the degradation of biogenic amines. Lactic acid bacteria (LAB) which play an important role in wine making are mainly responsible for the production of biogenic amines by decarboxylation of amino acids. Species of the genus Pediococcus, Lactobacillus and Leuconostoc as well as Oenococcus are potential biogenic amine producers in wine.
The present investigations have shown that more than half of the bacterial isolates from 53 German red and white wines were able to produce biogenic amines. Tyramine, histamine and phenylethylamine were mainly detected in the analysed wines. The isolated strains were identified by two independent methods. Firstly, a molecular fingerprint method (SAPD-PCR), based on the amplification of a certain gene sequence and secondly a multiplex-PCR system with LAB-specific primers were applied.
Our results give a general overview of the diversity of biogenic amines producing LAB in recent German wines.

LMP05
A PCR tool for the rapid detection and differentiation of bacteriophages infecting *Leuconostoc* flavour cultures in dairies

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Complex starter cultures used in dairies are composed of different types of bacterial strains. At the beginning of the fermentation, *Lactococcus lactis* strains with high proteolytic activity will decrease the pH by homofermentative lactic acid fermentation. These activities of the acid-producing cultures will stimulate the subsequent growth of *Leuconostoc* strains (which are either members of *Ln. mesenteroides* or *Ln. pseudomesenteroides*). *Leuconostoc* strains are usually minor components in these defined or undefined mixed-strain starter cultures. *Leuconostoc* strains play an important role as flavour producers but not as lactic acid producers. We have shown that *Leuconostoc* phages capable of infecting and lysing the flavour cultures are widely disseminated in dairy samples (whey samples, cheese brines, dairy products). Since *Leuconostoc* strains do not contribute significantly to lactic acid production, phage infection of these cultures cannot be detected concentionally by measuring disturbances of their acidifying activities. In order to develop appropriate phage control tools, an in-depth genomic analysis of these phages was performed. Phages could be subgrouped into two genotypes. Phages from both genotypes were genetically different except for a short DNA region within their gene modules coding for the structural proteins. A pair of PCR primers was designed for this conserved DNA region allowing the concomitant detection of all phages from both genotypes. Phages from the two genotypes revealed different genes for their major head proteins. Sets of primers amplifying a central DNA region from the two different major head genes could be used for the differentiation of *Leuconostoc* phages belonging to different genotypes. The PCR tool also allowed the detection of *Leuconostoc* phages in whey samples in cases, where phage-sensitive indicator strains were not available.

LMP06
Molecular effect of nitrite on *Listeria monocytogenes*

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The curing process in which sodium nitrite or sodium nitrate is added to the raw material is a common food preservation technique for meat products. It is postulated that the addition of sodium nitrite inhibits the growth of pathogenic bacteria.
Indeed, the addition of nitrite to raw sausages results in a better elimination of *L. monocytogenes*. *In vitro* growth analysis of *L. monocytogenes* at neutral pH revealed that the addition of nitrite results in diminished growth. This effect was not seen for the gram positive bacteria *Staphylococcus epidermidis* and *Bacillus cereus*. To investigate the inhibitory effect of nitrite on the growth of *L. monocytogenes*, global transcription analyses were performed on *L. monocytogenes* EGDe grown in the presence or absence of sodium nitrite. The most prominently regulated genes were the genes of the *pyr*-operon (~ 20 fold down regulation after sodium nitrite induction), whose products are involved in pyrimidine synthesis. A similar observation was described previously in the context of the identification of the nitrite regulon in *S. aureus*.
In contrast to *L. monocytogenes*, *Staphylococcus* spp. and *Bacillus* spp. harbour the *hmp* gene, encoding for a NO-detoxifying flavohemeprotein. Therefore it is tempting to speculate that even small amounts of NO, possibly derived by disproportionation of nitrite, could inhibit the growth of *L. monocytogenes*, whereas growth of *S. epidermidis* and *B. cereus* is not affected.
Further analysis will be performed to (i) clarify the regulatory mechanism behind the observed down regulation of the *pyr*-operon in the presence of nitrite, (ii) to analyze whether the down regulation of the *pyr*-operon is the major cause for the inhibitory growth effect and (iii) to investigate whether the nitrite sensitivity of *L. monocytogenes* can be ascribed to the absence of the *hmp*-gene.

LMP07
Transcriptional analysis of the lysogeny module of temperate *Streptococcus thermophilus<i> phage TP-34*

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Analysis of the DNA sequence of TP-J34 suggests that all except four of the phage genes are transcribed in one direction. The four genes are separated from the others by a genetic switch region and appear to be transcribed in one operon. They encode the following functions (in the order of transcription): repressor (<i>crh), potential metalloproteinase (*orf3*), lipoprotein mediating superinfection exclusion (*ltp*), and integrase (*int*). To elucidate the roles of Orf3 and Int in induction, we performed gene knock out and transcriptional analysis by Northern blot and RT-PCR. In addition, a non-inducible TP-J34 derivative (TP-J34-12) was analysed, which harbored just one mutation within the lysogeny module: a frame-shift mutation in *orf3*, causing premature termination of translation.
Knock out of *int* and of *orf3* of TP-J34, respectively, resulted in prevention of induction by mitomycin. Transcriptional analysis revealed that transcription of *int* was induced by mitomycin: no transcript was seen in uninduced cells. RT-PCR showed that *int* was exclusively transcribed from its own promotor, located in the intergenic region between *ltp* and *int*. For *ltp*, Northern blots revealed two transcripts: i) a few polycistronic transcripts comprising genes *crh*, *orf3* and *ltp* (also detected by an *orf3* probe), and ii) a majority of monocistronic transcripts comprising just *ltp*. Both transcripts were not affected by addition of mitomycin. As control, transcription of *ant* encoding the antirepressor involved in lytic development and of *orf44* encoding a structural phage protein were shown to be induced by mitomycin.
In non-inducible TP-J34-12 neither transcription of *ant* nor of *int* was detected, whereas transcription of *ltp* appeared to be basically unaffected.
From these results we conclude that Int is involved in excision during induction, while Orf3 apparently plays a role in regulation of induction.

LMP08
Antibiotic resistance of lactic acid bacteria isolated from Turkish dairy and meat products

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Increasing resistance of bacteria to commonly used antibiotics has become a serious problem. Commensal bacteria including lactic acid bacteria (LAB) may act as reservoirs of antibiotic resistance genes similar to those found in human pathogens and can transfer these resistance genes to pathogenic bacteria. The main objectives of this project are determination of antibiotic resistance in LAB isolates from Turkish dairy and meat products, characterization of resistance determinants and transfer (*tra*) genes carried by them and phylogenetic affiliation of LAB by 16S rDNA sequencing. Antibiotic resistance in 101 LAB isolates was detected against vancomycin (91% of the isolates), ciprofloxacin (77%), gentamycin (25%), erythromycin (9%) and tetracycline (4%) by the disc diffusion method. The LAB isolates were then screened for 13 different antibiotic resistance genes such as vancomycin, tetracycline, erythromycin, ampicillin, gentamycin and kanamycin, for Tra factors encoded by *virB1*, *virB4* and *virD4*-homologous genes from several broad-host-range Gram-positive conjugative plasmids (pIP501, pRE25, pSK41, pGO1, pSM19035, and pMRC01) and for relaxases of the pMV158 superfamily by PCR. In some of the isolates we found *tetM*, *ermB*, *ermG*, *aph3-III* (kanamycin resistance), *aph2-Ic* (gentamycin resistance) and *vanC* genes, respectively. In 85% of the isolates we could detect the relaxase gene of pSMA23, a 3.5 kbp plasmid of *Lactobacillus casei*. 23 LAB isolates harbouring transfer and/or resistance genes were applied to 16S rDNA sequence analysis. The LAB belong predominantly to the genera *Lactobacillus* (11), *Pediococcus* (11) and *Enterococcus* (1). The results of this project will aid in assessing food safety of these bacteria and lead to recommendations for the application of safe starter cultures.

LMV01
The unusual lifestyle of a foodborne pathogen: Listeria monocytogenes L-forms feature a novel mode of cell division

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Listeria monocytogenes is a foodborne pathogen that may cause serious invasive illness after ingestion by susceptible humans. To investigate the basic properties of cell wall-less L-form variants of *Listeria monocytogenes*, we produced non-reverting L-form lines from various strains by exposure to antibiotics that interfere with cell wall synthesis. The characterization of the resulting *L. monocytogenes* L-forms revealed that beyond surviving for a certain time period, they are able to further divide and multiply. This surprising observation implicates the question of how L-form bacteria manage to proliferate in the absence of a mature cell wall? To address this fundamental issue, growth and cell division of stable L-form lines (native and GFP-labeled) were investigated by time-lapse confocal laser scanning microscopy. Inspection of L-form growth revealed the development and maturation of internal vesicles within maternal L-form cells. Based on these observations we propose a hypothetic model for development of L-form *L. monocytogenes*, which takes into account their ability to divide in the absence of a rigid cell wall. Furthermore, global gene expression of parental and L-form *L. monocytogenes* was analyzed and compared by transcriptome analysis on whole genome macro-arrays. The results revealed a down-regulation of metabolism-related genes and a strong up-regulation of stress-related genes in L-forms compared to parental bacteria, indicating to a triggered adaptation of the L-form bacteria with respect to their new lifestyle. We currently study the pathogenic potential of *L. monocytogenes* L-forms to provide an answer whether these L-forms may maintain their pathogenic potential. Preliminary experiments suggested intracellular survival of L-form *Listeria monocytogenes* within macrophages, which would represent yet another surprise offered by these interesting bacterial life forms.

LMV02
The non-hemolytic enterotoxin of *Bacillus cereus*: role of NheB and binding order of the three toxin components

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The non-hemolytic enterotoxin (Nhe) is a putative enterotoxin produced by *Bacillus cereus* composed of three distinct proteins (NheA, NheB and NheC). Nhe acts as a pore-forming toxin inducing cell lysis and a structural and functional correlation between Nhe and the pore-forming haemolysin cytolysin A (ClyA) from Gram-negative enteric bacteria was identified. It is, however, unknown how the three components of Nhe interact and if there is any ordered sequence necessary for cytotoxicity. In this study we used *B. cereus* mutants lacking either NheA or NheC, recombinant proteins (NheA, NheC, and fragments of NheB covering the N-terminal and C-terminal part of the protein) together with monoclonal antibodies against NheB, which exhibit a nearly complete neutralization capacity on cytotoxic Nhe effects, to further elucidate the functional properties of Nhe. We provide immunocytochemical evidence that NheB binding to Vero cells depends on an intact N-terminal part of the molecule. In addition, we present data suggesting that formation of an active toxin complex is inhibited, when the C-terminal part of NheB is modified or blocked with antibody. This epitope, located in the helix equivalent to the αG helix of ClyA, is likely to play a pivotal role in toxicity. Finally, the results of Vero cell assays using consecutive and simultaneous incubation conditions indicate that Nhe induced cytotoxicity requires a specific binding order of the individual components.

LMV03
Long-chain polyphosphates inhibit cereulide toxin synthesis in emetic *Bacillus cereus*

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Bacillus cereus is the causative agent of two types of food poisoning: diarrhea and emesis. The emetic type of foodborne illness is caused by intoxication with the cyclic peptide cereulide, which is preformed in foods and elicits vomiting a few hours after ingestion [1]. Moreover, cereulide is immunomodulatory and has been implicated in severe clinical manifestations resulting in acute liver failures [2]. The 1.2kDa small, highly heat, acid and proteolytically stable dodecadepsipeptide is structurally related to the potassium ionophore valinomycin [3] and synthesized enzymatically by a non-ribosomal peptide synthetase (NRPS) [4]. Due to its small size and the remarkable stability, cereulide is not restrained by filtration processes or heating of the foods. Therefore, inhibition of toxin formation in the food itself is of outmost importance for food safety, however, strategies have not been developed since now.
Here we report that food grade, long-chain polyphosphates (polyPs) delay and decrease cereulide toxin synthesis in culture broth as well as in two model foods under simulation of temperature abuse conditions. PolyPs, which are generally recognized as save (GRAS), are widely used in the dairy and meat industry as food additives primarily for flavour protection and emulsification of the products. The inhibitory potential of three different polyP blends was monitored on transcriptional level via qPCR and visualized in food with a photon counting ICCD camera using a luciferase based *B. cereus* reporter strain. Levels of toxin production were assessed with a Hep2-cell based cell culture assay and via HPLC/ESI-TOF-MS analysis. In summary, tested polyP blends inhibited NRPS toxin formation in complex matrices, thereby displaying a high potential to contribute to the improvement of consumer protection and food safety issues regarding emetic *B. cereus*.

[1] Stenfors Arnesen *et al.*, 2008
[2] Dierick *et al.*, 2005
[3] Agata *et al.*, 1994
[4] Ehling-Schulz *et al.*, 2006

LMV04
Detection and quantification of *E. coli* and coliform bacteria in water samples with a new method based on Fluorescence *in situ* Hybridisation

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Monitoring of microbiological contaminants in water supplies requires fast and sensitive methods for the specific detection of indicator organisms or pathogens. The standard cultivation methods are too time-consuming to match the requirements of modern water safety management, i.e. approaching the equivalent of online measurements. Within the TECHNEAU project – an integrated project funded by the European Commission – we developed a protocol for the simultaneous detection of *E. coli* and coliform bacteria based on the Fluorescence *in situ* Hybridization (FISH) technology. The developed protocol consists of two different approaches. One approach allows the direct detection of single *E. coli* and coliform bacterial cells on the filter membranes. The second approach includes incubation of the filter membranes on a nutrient agar plate and the subsequent detection of the grown micro-colonies. Both approaches were validated using drinking water samples spiked with pure cultures and naturally contaminated water samples. The effects of heat, chlorine and UV disinfection on the FISH based detection of *E. coli* and coliform bacteria were also investigated. The micro-colony approach yielded very good results for all different samples and conditions tested, and thus can be thoroughly recommended for usage as an alternative method to detect *E. coli* and coliform bacteria in water samples. In contrast the single cell approach shows some limitations, especially with disinfected samples.

LMV05
Identification of a novel fructosyltransferase from the water kefir isolate *Gluconobacter frateurii* TMW 2.767

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Water kefir is a beverage based on the fermentation by a microbial consortium mainly consisting of yeasts and lactic acid bacteria. The matrix of the kefir grains mainly consists of a glucan produced by *Lactobacillus hilgardii*. Recently, we isolated an acetic acid bacterium (*Gluconobacter frateurii* TMW 2.767) from water kefir, which produces a water-soluble polysaccharide in high amounts (up to 20 g/l) when growing in sucrose-containing media. Exopolysaccharides (EPS) can be used to improve the rheological properties of functional foods and furthermore can serve as prebiotics due to their ability to stimulate bifidobacterial growth in the gut. The aim of the study was to identify the chemical nature of the *G. frateurii* TMW 2.767 EPS and to investigate the genetic organisation of the enzyme responsible for the biosynthesis of this polymer. Using HPLC analysis, 1H NMR and 13C NMR spectroscopy we suggest this EPS to be a levan-type homopolymer (consisting of β-2,6-linked β-D-fructofuranosyl units). Furthermore the nucleotide sequence of a putative levansucrase gene was identified from *G. frateurii* TMW 2.767 by using degenerate and inverse PCR techniques. The sequence encodes a putative 445 amino acid (aa) protein with a predicted molecular weight (Mr) of 49,48 kDa and a predicted pI of 4,97. Blast research revealed highest similarities with the fructosyltransferases (Ftf´s) of *Gluconacetobacter xylinus* (66% identities and 77 % positives in 416 aa) and *Gluconobacter oxydans* 621H (62% identities and 75% positives in 448 aa). Structurally, *G. frateurii levansucrase* belongs to the glycoside hydrolases 68 family and shares some conserved motifs (e. g. the catalytic centre that is involved in fructan polymerisation) with all other known Ftf´s.

LMV06
Isomaltulose formation using an isomaltulose synthase (PalI) expressed in Lactococcus lactis

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Isomaltulose (palatinose) is a structural isomer of sucrose. In nature it is contained in very little amounts of up to 1 % in honey and sugarcane. Isomaltulose is half as sweet as sucrose. Due to the fact that it is non-cariogenic and has a low glycemic index, its importance in the food sector is growing.

The formation of isomaltulose is catalyzed by an isomaltulose synthase (PalI; EC 5.4.99.11) from the Gram-negative bacterium *Protaminobacter rubrum* CBS 574.77. PalI of *P. rubrum* produces about 85 % of isomaltulose and small amounts of other mono- and disaccharides.
Isomaltulose synthase was heterologously expressed in Gram-positive *Lactococcus lactis* strains having GRAS status. For expression, the nisin inducible commercial two component NICE expression system was used. Additionally, the gene was cloned in the expression vector pSTS1a under the control of a constitutive bacteriophage promoter. In both cases, the region encoding the Gram-negative signal sequence of *palI* was removed.
In order to verify the expression of PalI proteins, polyclonal antibodies specifically raised in chicken were used. Expression was detected by Western blotting.
Enzymatic activity was indirectly detected by measuring the degradation of sucrose to reducing sugars. These reducing sugars could be measured by a rapid photometric test, the tetrazolium blue assay. Detailed information on activity of PalI and yields of isomaltulose after enzymatic conversion of sucrose were obtained by HPLC-RI, in which small amounts of the byproducts trehalulose, isomaltose, glucose and fructose were detected as well.

LMV07
Selection of potential starter cultures for gluten-free sourdough fermentation

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The pseudocereals amaranth and buckwheat as well as the gluten-free cereal millet are attracting increased attention for the manufacturing of bakery products, because they are suitable for consumption by people suffering from celiac disease, and they confer new aroma properties to the product. The flours need to be fermented prior to application in doughs due to technological and sensory reasons. No starter cultures tailored for these grains are commercially available yet. In order to isolate and characterize candidates for starter cultures, in this study these grains were evaluated as a source for potential starter culture strains. Spontaneous fermentations with different grains were carried out with daily back-slopping, and the fermentation parameters pH-value and total titratable acidity degree were assessed regularly over the whole 10 day fermentation period. The dominating lactic acid bacterial strains were isolated, differentiated by RAPD-PCR and identified by 16S rDNA-sequencing. Altogether, strains of the species *Pediococcus pentosaceus*, *Lactobacillus sakei*, *L. paralimentarius* and *L. plantarum* were identified as prevailing microflora. Selected strains were applied as starter cultures in further fermentation experiments and were found to dominate the microbiota. Two strains from amaranth were examined in more detail concerning their fermentation parameters at diverse incubation temperatures and during the first 24 hours. They were also compared to commercial starter cultures for cereals. They were assessed to their dominance over an established spontaneous microbiota and to their metabolic capacities by HPLC. A safety evaluation consisting of antibiotic resistance profiles as well as presence and transferability of plasmids was conducted. On account of the results, the two strains were considered interesting candidates for pseudocereal sourdough starter cultures.

LMV08
Characterization of bacteriophage populations infecting *Leuconostoc* flavour cultures in dairies

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Lactic acid bacteria of the *Leuconostoc* genus are used in mesophilic dairy starter cultures and are members of either *Ln. mesenteroides* or *Ln. pseudomesenteroides*. *Leuconostoc* strains are the minor components in these defined or undefined mixed-strain starter cultures and their number varies between 1 – 10 %. Lactic acid-producing strains (i.e., *Lactococcus lactis* strains) are the major components of these starter cultures. However, *Leuconostoc* strains play an important role as flavour producers as they metabolize citrate in milk, resulting in the production of flavour compounds (e.g. diacetyl). Furthermore, carbon dioxide is produced during citrate metabolism and also during heterofermentative lactose metabolism. This CO_2-production contributes significantly to the generation of an optimal open texture in soft ripened cheeses and to eye formation in cheeses. Infection of these important flavour starter cultures by lytic bacteriophages will cause variation of product quality and defects of texture and flavour. We therefore analysed the distribution of *Leuconostoc* phages in 26 German dairies. It is notable that *Leuconostoc* phages were detected in 11 of the 26 dairies. Phages were found in samples of whey, cheese brine and also in the corresponding dairy products in low or average titers (i.e., 10^2-10^7 plaque-forming units per ml). Phages could also be isolated in similar titers from product samples collected from the local market. All phages revealed the same basic morphology and were isometric-headed *Siphoviridae*-phages with non-contractile tails and either non-structurated or complex base plates with globular fine structures. They were differentiated into two subgroups, and the DNA homology between phages from the two different subgroups was low. Hence, populations of virulent *Leuconostoc* phages represent a remarkably homogeneous phage pool.

MPP01
Screening for novel virulence determinants of *Salmonella typhimurium* using an amoeba infection model

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Salmonella typhimurium possesses in addition to its pathogenic potential a high degree of adaptability to different hosts and for survival within the environment. On passage through the environment or life within the intestine, *Salmonella* meets protozoa (e.g. amoebae) usually feeding on bacteria. Many pathogenic bacteria, however, developed strategies to resist amoebal predation and digestion. Exploitation of such environmental hosts may be beneficial for *Salmonella* in several ways: 1) intracellular localization within amoebae protects from external dangers, 2) amoebae are vectors of bacterial spread, and 3) passage through amoebae renders bacteria more invasive and virulent even for human cells. Although *Salmonella* has not yet been isolated from environmental amoebae, it has been shown that they indeed survive and multiply within as they do in mammalian cells and some virulence traits are conserved (Bleasdale *et al*., 2009, AEM). Since many aspects of *Salmonella* infection of potential environmental hosts are still unkown, we here aimed to identify novel determinants needed for amoeba infection. We therefore developed an agar plate assay, designated the scatter screen, which allowed screening of 36.000 *S. typhimurium* clones for mutants deficient in infecting *Acanthamoeba castellanii*. Mutants with sensitivity towards amoebal grazing were isolated and further investigated. We found both known and novel genes, including genes associated with potential metabolic, regulatory chaperoning, transport, or hypothetical function. In addition, we analyzed the mutants with respect to defective infection of macrophage and amoebal cells and by comparing the secretion level of known *Salmonella* outer proteins. Our study shows that the scatter screen used for identification of clones with a defect in defence against amoebal predation, is an easy and rapid procedure to find novel *Salmonella* virulence genes as well as determinants for bacterial establishment within the environment.

MPP02
Chromosomal transfer of the *Enterococcus faecalis* pathogenicity island

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Enterococci are the third leading cause of hospital associated infections, and have gained increasing importance due to its fast adaptation to the clinical environment by acquisition of antibiotic resistance and pathogenicity treats. *E. faecalis* possesses a 153 kb pathogenicity island (PAI) bearing the enterococcal surface protein (*esp*) gene among other pathogenicity factors (Shankar, 2002). This PAI is distributed among isolates of different origins, clonal types and complexes (McBride, 2009). Until now only fragments of the PAI have been demonstrated to transfer (Oancea, 2004. Coburn, 2007). Here we demonstrate horizontal transfer of the entire PAI from an *E. faecalis* donor into *E. faecalis* and *E. faecium* recipients and evaluate phenotypic changes induced by the PAI aquisition.
The *E. faecalis* PAI was horizontally co-transferred to *E. faecalis* and *E. faecium* by filter mating conjugation. The transferred fragment contains several deletions and insertions as compared to the original PAI; however, the structure was maintained after transfer. SmaI-, I-CeuI- and S1-nuclease PFGE analysis resolved transfer of a 200 kb chromosomal fragment and a conjugative *ermB* plasmid. The whole PAI excises precisely from the chromosome, forms a circular intermediate and integrates in *E. faecalis* into an intergenic region previously described and in *E. faecium* into a tRNA-lys. Biofilm formation, an important treat for colonization and the establishment of infections was seen to be enhanced in *E. faecalis* by acquisition of the PAI. The *esp* gene is expressed on the surface of donor and transconjugants as seen by FACS analysis and TEM imaging.
Conclusions: Pathogenicity islands and other mobile elements can be transferred horizontally with the help of conjugative elements like conjugative resistance plasmids among enterococci supporting the role of horizontal gene transfer and antibiotic selective pressure in the successful establishment of certain enterococci as nosocomial pathogens.

MPP03
Generation and functional characterisation of truncated *Bartonella henselae* BadA mutants

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Human pathogenic *Bartonella henselae* cause cat scratch disease and vasculoproliferative disorders (e.g., bacillary angiomatosis). Expression of *Bartonella* adhesin A (BadA) is crucial for bacterial autoagglutination, adhesion to host cells, binding to extracellular matrix proteins and proangiogenic reprogramming via activation of hypoxia inducible factor (HIF)-1. Like the prototypic *Yersinia* adhesin A (YadA), BadA belongs to the class of trimeric autotransporter adhesins and is constructed modularly consisting of a head, a long and repetitive neck-stalk module and a membrane anchor. Until now, the exact biological role of these domains is not known. Here, we analyze the functions of the BadA head and stalk domains in greater details. For this purpose, we produced deletion mutants by truncating the repetitive neck-stalk module and deleting of different head domains of BadA. Like wildtype bacteria, a mutant with a nearly completely truncated stalk (*B. henselae* HN23) showed autoagglutination, adhesion to collagen and endothelial cells (ECs) and induced activation of HIF-1 in host cells. Remarkably, *B. henselae* HN23 did not bind to fibronectin (Fn), whereas a mutant with a longer stalk than *B. henselae* HN23 (*B. henselae* HN2R20) showed Fn binding. The recombinantly expressed BadA head domain itself adhered preferentially to ECs. Our data suggest that the head domain represents the major functional domain of BadA responsible for host adhesion and angiogenic reprogramming, whereas the stalk plays a crucial role in Fn binding.

MPP04
Distinct invasion mechanisms trigger different intracellular trafficking of streptococci

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Group A streptococci (GAS) and Group G streptococci (GGS) are prominent Gram-positive pathogens that cause a wide spectrum of human diseases. Both adhere to and invade into non-phagocytic epithelial host cells. In GAS the surface associated streptococcal fibronectin-binding protein I (SfbI) plays a key role in the invasion process through fibronectin (Fn), which acts as a bridging molecule, and binds to $\alpha_5\beta_1$-integrins, resulting in integrin-clustering and caveolae-mediated uptake. In GGS the group G Fn-binding protein A (GfbA) has been identified, however, the underlying invasion mechanisms are still unknown. Both proteins show equal modular structures with similar C-terminal Fn-binding repeats and proline-rich repeats, whereas the N-terminal aromatic amino acid-rich domains (Aro domain) differ significantly. We could demonstrate that in contrast to SfbI, GfbA mediates bacterial uptake via massive cytoskeleton rearrangements. Protein-expression on the surface of *S. gordonii* showed that after truncation of Aro in GfbA (GfbApro) the invasion process was altered into a caveolae-mediated pathway. Expression of a chimeric protein (SfbIGaro), in which the Aro domain of SfbI was replaced by that of GfbA, also triggered cytoskeleton rearrangements. Additionally, we observed integrin-clustering on the HUVEC surface after exposure to recombinant SfbI or GfbApro coated gold-nanoparticles and a subsequent caveolae-mediated uptake. Neither GfbA nor SfbIGaro coated gold-nanoparticles showed a clustering, instead cytoskeleton rearrangements were induced. This is the first evidence of a biological function for the aromatic domain of a Fn-binding protein. Furthermore, we could show that intracellular trafficking of streptococci is mediated by the invasion mechanism. After internalization via cytoskeleton rearrangements streptococci follow the classical endocytic pathway and fuse subsequently with lysosomes, whereas caveolae-mediated invasion bypasses fusion with lysosomes.

MPP05
Fatty acid resistance in *Neisseria meningitidis* – independent of the highly specific transcriptional regulator FarR

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The transcriptional regulator FarR is highly conserved yet fulfills divergent roles in the two *Neisseria* species that are closely adapted to the human host. In *N. meningitidis*, FarR negatively regulates expression of the adhesin NadA (Schielke *et al.*, 2009), whereas the homologous regulator in *N. gonorrhoeae* is involved in fatty acid resistance (Lee *et al.*, 2003).
Here we show that the intrinsic resistance of meningococci to long chain fatty acids is due to lipopolysaccharide (LPS) composition and independent of the FarR controlled FarAB efflux-pump system. Testing stepwise LPS-truncated mutant strains we demonstrate that addition of the core oligosaccharide as well as lipid A hexaacylation are mainly responsible for this intrinsic resistance. We show that a subset of clinical strains (serogroup Y, ST-23) are sensitive to fatty acids due to naturally occurring inactivating mutations in the *lpxL1* gene, which is responsible for addition of the sixth acyl chain on lipid A (Fransen *et al.*, 2009). The transcriptional regulator FarR, however, shows no contribution to fatty acid resistance. Consequently we examined the differentially expressed genes in the wild type compared to a *farR* deletion strain by whole genome DNA microarrays. Astonishingly, after qRT-PCR validation of five borderline values, the only highly downregulated gene in the wild type strain was the adhesin *nadA*. Therefore, FarR seems to be a very specific transcriptional repressor. Homology modeling using MarR-like transcriptional regulators with annotated crystal structure revealed that FarR probably acts as a dimer, binding to the target DNA with the flexible wing domains. Additionally, we present evidence by reporter gene assays that expression of this regulator is growth phase dependent, being highest in late exponential phase.

MPP06
Glycosylation of wall teichoic acid in *Staphylcoccus aureus* by TarM

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Wall teichoic acid (WTA) glycopolymers are major constituents of cell envelopes in *Staphylococcus aureus* and related Gram-positive bacteria with important roles in cell wall maintenance, susceptibility to antimicrobial molecules, biofilm formation and host interaction. Most *S. aureus* strains express poly-ribitolphosphate (Rbo-P) WTA substituted with D-alanine and N-acetylglucosamine (GlcNAc). WTA sugar modifications are highly variable and have been implicated in bacterial phage susceptibility and immunogenicity. A *S. aureus* mutant 52B2 lacking the WTA GlcNAc residues has been described in the 1960s but the pathway and enzymes of staphylococcal WTA glycosylation have remained unknown.
Revisiting the structure of *S. aureus* RN4220 by ^{1}H NMR analysis revealed the presence of canonical Rbo-P WTA bearing only α-linked GlcNAc substituents. A RN4220 transposon mutant resistant to WTA-dependent phages was identified and shown to produce altered WTA, which lacked the WTA α-GlcNAc residues completely. Disruption of a gene of previously unknown function was shown to be responsible for this phenotype and was renamed *tarM*. Recombinant TarM was capable of glycosylating WTA *in vitro* in a UDP-GlcNAc dependent manner thereby confirming its WTA-GlcNAc transferase function. In agreement with these findings, the WTA GlcNAc deficient phenotype of mutant 52B2 was found to result from a premature stop codon in *tarM* . While several genomes of WTA-producing bacteria contained *tarM*-related genes many others did not encode TarM homologues thereby supporting the notion that WTA glycosylation is a very variable and often strain-specific trait. Our study represents a basis for dissecting the biosynthesis and function of glycosylated WTA in *S. aureus* and other bacteria.

MPP07
Fibronectin-dependent cellular invasiveness clusters with molecular markers, but does not differ substantially between colonizing and infecting MRSA isolates

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Background: We have shown previously that fibronectin-dependent cellular invasiveness of MRSA strains clusters with molecular markers (spa type, SCCmec type and expression of Pls) in a defined collection of MRSA strains. Here, we tested the hypothesis whether cellular invasiveness predicts the propensity to cause infections in a prospective cohort.
Methods: MRSA isolates from the nose and and infection site (n=109) from a single center prevalence study (J. Elias et al., in preparation) were prospectively collected and identified by standard biochemical and molecular methods. Additional 13 infection isolates were used as a comparator group. SCCmec typing was performed by multiplex PCR (K. Boye, 2007). Respective spa types were assigned using the spa server (D. Harmsen, 2003). Cellular invasiveness was determined by a flow cytometric invasion assay for 293 cells (relative to reference strain Cowan I).
Results: Parallel control cultivation of MSSA isolates during part of the study yielded an overall S. aureus nasal colonization rate of 20.5 %. MRSA isolates with spa type t003 were most frequent, thus 1/3 were assayed further. Cellular invasiveness clustered according to molecular markers (spa, SCCmec, pls). This was reproducible for previously assayed markers. Cellular invasiveness of 59 colonizing and 16 infecting isolates was 100 ± 37 % and 89 ± 42 % (means + SD) of Cowan I ($p = 0.48$), respectively, whereas 13 comparator isolates derived from infection had a mean invasiveness of 109 ± 34 % ($p = 0.35$; $p = 0.24$ between both infection groups).
Conclusion: Cellular invasiveness of MRSA isolates appears to be reproducible with regard to molecular markers. However, cellular invasiveness did not differ statistically significantly for colonizing versus infecting isolates in this study. This suggests that cellular invasiveness (and thus fibronectin binding capacity) may not be used as a global surrogate parameter for the virulence potential of a given isolate.

MPP08
Changes in the proteome of *Neisseria meningitidis* during biofilm formation

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Neisseria meningitidis is a human pathogen that causes meningitis and sepsis. Nevertheless, asymptomatic nasopharyngeal carriage is crucial for the transmission of the bacterium and its interaction with the human host. Previous observations led to the assumption that meningococci persist asymptomatically in tonsillar tissue in a biofilm-like state (Sim *et al.*, 2000). We therefore established an *in vitro* biofilm model employing modified Neisseria Defined Medium (NDM, Lappann *et al.*, 2006) to study bacterial adaptation relevant to the carrier state. In this study the alteration of protein expression during biofilm growth in a flow system was investigated. The proteomes of *N. meningitidis* biofilms grown for 24 or 48 h, respectively and of exponentially grown planktonic cultures were compared by 2D-gelelectrophoresis. Differentially expressed proteins were identified by mass spectrometry. The results were confirmed by spectral counting and, if available, with specific antibodies.
The protein patterns revealed significant differences between biofilm and planktonic growth in 17 protein spots. Most of the changes were observed already after 24h. 12 protein spots could be identified by MS and confirmed by spectral counting. The four down-regulated proteins were assigned to carbohydrate metabolism and cellular processes (cell division). Up-regulated spots are involved in amino acid metabolism, carbohydrate metabolism and detoxification processes to evade oxidative stress (*sodC*, *mntC*). To study the effect of *mntC* on biofilm formation, a comprehensive set of mutants has been established. Biofilm formation in a *mntC* mutant was abrogated. This finding can only partly be explained by a slightly delayed growth in planktonic cultures and points to a central role of prevention of oxidative stress during biofilm growth.

MPP09
Rho-modifying bacterial toxins-induced inhibition of wound healing is based on inhibited cell proliferation and cell migration

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Low molecular weight GTP binding proteins of the Rho family regulate the actin cytoskeleton and play an essential role in cell migration and proliferation. RhoA, Rac and Cdc42 are mono-glucosylated (i.e. inactivated) by C. difficile Toxin A (TcdA) and Toxin B (TcdB). Treatment of cultured cells with TcdB results in a loss of cell shape and inhibited cell migration. Cytotoxic necrotizing factor 1 (CNF1) from E. coli deamidates and thereby activates RhoA, Rac and Cdc42. CNF1 treatment of Hela cells results in pronounced cytoskeletal features (stress fibres, lamellipodia and filopodia) and increased cell motility. Furthermore, either TcdB or CNF1 results in inhibited cytokinesis and cell division, as contractile ring formation is prevented in cells treated with either toxin. Wound healing requires both cell migration and cell proliferation. In this study, the consequences of inhibited cell proliferation on wound healing were investigated exploiting the scratch assay, a cell culture model of wound healing. In untreated Hela cells, the lesion was closed within 16 h. Thymidin treatment of Hela cells resulted in blocked cell proliferation but not cell migration. Thymidin-treated Hela cells migrated into the lesion but with reduced cell density. CNF1 treatment resulted in reduced wound healing, as CNF1-treated Hela cells were unable to proliferate and to migrate into the lesion, although they were highly motile. Cell migration requires distinct activities of the Rho proteins at the leading edge and at the rear of the cell. Following the hypothesis that constitutive activation of Rho proteins by CNF1 impairs cell polarization required for cell migration, the polarization of cells present at the edge of the lesion was analysed for the orientation of the microtubule organizing centre (MTOC). Untreated (migrating) cells were polarized towards the lesion, while CNF1-treated (non-migrating) cells appeared to be fully non-polarized. In conclusion, either inhibition of Rho proteins by TcdB or constitutive activation of Rho proteins by CNF1 blocks wound healing.

MPP10
Adaptation of the transcriptome of *Neisseria meningitidis* to the biofilm life style

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Carriage of *N. meningitidis* induces protective immunity. Meningococci reside within tonsillar tissue as microcolonies, which are considered to be the basic unit of microbial biofilms. The biology of meningococcal microcolonies *in vivo* is not well understood. In the present study, the transcriptomes of biofilms grown under gentle agitation in cell culture flasks (24, 48, 72 or 96 h) and of planktonically grown cultures were compared by micro-array analysis. Deregulation of genes was confirmed by Northern Blot hybridization. In 24 h old biofilms 13 genes were at least 2-fold up-regulated while 143 genes were down-regulated. The up-regulated genes mostly were involved in amino acid metabolism or displayed yet un-identified functions. Down-regulated genes among others were involved in energy metabolism and pilus expression. Immune-fluorescence microscopy verified the down-regulation of pilus-expression. Up-regulation of the oxidative stress regulated genes *bfrA/bfrB* was observed starting from 24 h to 96 h in biofilms. *BfrAB* encodes for bacterioferritin, and we could demonstrate that in meningococci, a *bfrAB* mutant showed reduced resistance to oxidative stress and was more efficiently killed by the macrophage cell line U-937. We finally studied the effect of biofilm formation on complement deposition, since unencapsulated bacteria might be especially vulnerable even towards low amounts of complement. A reduced deposition of complement factors (C3b, MAC) on biofilm cells was detected by immune-fluorescence microscopy suggesting a protective effect of biofilm formation towards complement. Taken together, the adaptation of meningococci during biofilm formation involved pili, necessary for initial attachment to cells, but down-regulated after bacterial aggregation. An oxidative stress response was elicited, which might train the biofilm cells against effector mechanisms of cellular defense mechanisms.

MPP11
Towards identification of substrate interaction sites on the *Escherichia coli* periplasmic chaperone SurA

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The periplasmic peptidyl-prolyl isomerase (PPIase) SurA is a chaperone for outer membrane proteins (OMPs) that also affects virulence of uropathogenic *E. coli*. SurA preferentially interacts with non-native OMPs by specifically recognizing peptides that display OMP-characteristic features [1, 2]. SurA consists of a N-terminal region, an inactive and an active PPIase domain (domains I and II, respectively), and a short C-terminal tail. Domain I has recently been proposed to carry the specific peptide binding activity [3]. Our previous studies have shown that this site may also act as a substrate binding site *in vivo*. However, analysis of the interaction between SurA and OMP-derived binding peptides [2] by electron paramagnetic resonance spectroscopy as well as the fact that domain I can be deleted without significant loss of SurA *in vivo* function suggests that additional sites in SurA may interact with substrates.
To elucidate the molecular mechanisms of SurA´s function not least with a view to its role in virulence, we are now investigating the interactions of SurA and newly-synthesized OMP polypeptides by site-specific photo-crosslinking. We use a modified tRNA/aminoacyl-tRNA synthetase pair to incorporate photoreactive *p*-benzoyl-phenylalanine (*p*Bpa) at specific sites of SurA through amber codon suppression [4]. Upon UV-exposure, *p*Bpa can covalently crosslink to nascent polypeptides in the immediate vicinity. Based on available structural information, we have chosen sites in each domain of SurA, including domain I, for *p*Bpa incorporation. First results show that the amber codon is suppressed and full-length single site SurA-Bpa proteins are produced. The SurA-Bpa proteins are tested for their function by *in vivo* complementation. Ongoing work will show if purified SurA-Bpa proteins can be specifically crosslinked to *in vitro* newly-synthesized LamB, an OMP that efficiently co-precipitates with SurA, possibly revealing further substrate interaction sites on SurA.

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MPP12
Inhibition of mitotic entry by *Clostridium difficile t*oxins

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Low molecular weight GTP binding proteins of the Rho family regulate the actin cytoskeleton and play an important (but less investigated) role in the regulation of the cell cycle. Mitotic entry is suggested to be regulated by Rho in a proteinkinase C-related kinase (PRK)-dependent manner and by Rac/Cdc42 in a p21-activated kinase (PAK)-dependent manner. The Rho proteins transduce mitotic stimuli induced by growth factors. The critical role of Rho proteins in mitotic entry was analyzed using Toxin B from the *C. difficile* that glucosylates and thereby inactivates Rho, Rac, and Cdc42. TcdB was added to G2 phase Hela cells synchronized using the thymidine double block technique. TcdB caused a delay in mitotic entry (about 2 h) that was analysed in terms of phosphorylation of the mitotic kinase AuroraA and histone H3. Furthermore, delayed activation of the CyclinB/Cdk1 complex was shown using a CyclinB/Cdk1 kinase assay. Expectedly, glucosylation of Rac/Cdc42 by TcdB resulted in PAK dephosphorylation. The association of PAK to the centrosomes is likely critical for G2-M transition and further cell cycle dependent processes. PAK association to the centrosomes, however, was reduced but not fully abolished, explaining why glucosylation of Rho proteins caused delayed mitotic entry but not a G2-M arrest. Mitotic entry was further analyzed in G2 phase Hela cells treated with isomeric Toxin B from the *C. difficile* serotype F strain 1470 (TcdBF) that glucosylates Rac/Cdc42 but not Rho. TcdBF caused a 2 h delay in mitotic entry comparable to TcdB, suggesting that the mitotic entry is regulated by Rac/Cdc42-PAK rather than by Rho-PRK signaling. These findings suggest that TcdB/TcdBF block mitotic signaling regulating G2-M transition at the level of Rho proteins. The modulation of cell cycle progression represents a new aspect of the pathogenic activity of the *C. difficile* toxins.

MPP13
Effects of Rho-inactivating clostridial toxins on F-actin polymerization and the formation of focal adhesion complexes

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Low molecular weight GTP-binding proteins of the Rho family are the master regulators of the actin cytoskeleton. RhoA regulate actin stress fibres, while Rac and Cdc42 regulate lamellipodia and filopodia, respectively. Both Rac and Cdc42 are further involved in the formation of focal adhesions (FAs). Rho proteins are inactivated by either C3-like mono-ADP-ribsoyltransferases (e.g. *C. botulinum* exoenyzme C3, C3-bot) or by clostridial mono-glucosyltransferases (e.g. Toxin B from *C. difficile*, TcdB). Either C3-bot or TcdB induces a loss of cell shape ("cell rounding"), considerably based on F-actin depolymerisation.
In this study, the effects of Rho-inactivating toxins on the cellular F-actin level are re-investigated in fibroblasts and Hela cells using the rhodamin-phalloidin-based F-actin ELISA. Rho inactivation by either C3-bot or TcdB induces F-actin depolymerisation comparable to actin depolmerisating toxins such as latrunculin B or *C. botulinum* C2 toxin. Lethal toxin from *C. sordellii* (TcsL) or variant Toxin B from *C. difficile* (TcdBF), that inactivate Rac1/Cdc42 but not RhoA, did not cause F-actin depolymerisation. Following the hypothesis that disassembly of FAs is sufficient for the loss of cell shape, FAs were visualized by fluorescence microscopy using vinculin and paxillin as marker proteins. Rac/Cdc42-inactivating as well as RhoA-inactivating toxins, however, induced paxillin dephosphorylation and the disassembly of FA. In contrast, constitutive activation of Rho proteins by CNF1 strongly increased the level of phosphorylated paxillin and increased the number of FAs, confirming their regulation by Rho proteins. Combined treatment of cells with CNF1 and latrunculin B results in a disassembly of FAs, showing that F-actin is required for the formation of FAs. These observations suggest two mechanisms on how Rho-modifying toxins induce loss of cell shape: RhoA-inactivating toxins cause F-actin depolymerisation and subsequently FAs disassemble. Rac/Cdc42-inactivating toxins directly induce the disassembly of FAs without F-actin depolymerisation.

MPP14
Detection of *Clostridium sordellii* Lethal Toxin-induced (H/K/N)Ras glucosylation by the glucosylation-sensitive Ras antibody Mab27H5

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Lethal Toxin (TcsL) from *Clostridium sordellii* is regarded as the major pathogenicity factor of *C. sordellii*-associated diseases including myonecrosis in obstetric patients and necrotizing fasciitis in injection drug users. TcsL inactivates low molecular weight GTP-binding proteins of the Rho- and Ras-families by mono-glucosylation at Thr-35 in Rac, Cdc42, and (H/K/N)Ras. In cultured cell lines, TcsL-catalyzed glucosylation of Rac/Cdc42 results in actin reorganization („cytopathic effect"). (H/K/N)Ras glucosylation is suggested to block critical survival signaling pathways, resulting in apoptotic cell death („cytotoxic effect"). One yet unsolved problem in studies on TcsL is the lack of a method allowing the specific detection of (H/K/N)Ras glucosylation. In this study, we identified the Ras(Mab 27H5) antibody as a glucosylation-sensitive antibody capable for the immunoblot detection of (H/K/N)Ras glucosylation in TcsL-treated cells. Alternative Ras antibodies including the K-Ras(Mab F234) antibody or the v-H-Ras(Mab Y13-159) antibody recognize Ras proteins regardless of glucosylation. (H/K)Ras are further shown to be more efficaciously glucosylated by TcsL than Rac1 in rat basophilic leukemia cells as well as in a cell-free system. This is the first study showing that the clostridial glucosylating toxins modify their cellular substrate proteins with distinct kinetics, a yet not addressed aspect of their biological activity.

MPP15
The twin-arginine translocation system promotes intracellular infection of murine macrophages and protozoan host cells by *Legionella pneumophila*

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Legionella pneumophila, the causative agent of Legionnaire's disease, is a facultative intracellular pathogen of amoebae and ciliates in fresh-water environments. Upon inhalation of contaminated water droplets, L. pneumophila is able to replicate within alveolar macrophages and epithelial cells, causing pneumonia. In Gram-negative bacteria, the twin-arginine translocation (Tat) system mobilizes proteins across the inner membrane to the periplasm. Our previous mutational analysis has shown that the Tat pathway promotes secretion of phospholipase C activity, formation of a respiratory complex, growth under low-iron conditions and optimal intracellular growth of *L. pneumophila* within human U937 macrophage-like cells [1].
As a next step to investigate the significance of Tat for *L. pneumophila*, we studied replication of the tatB mutant in murine macrophages and protozoans. In bone marrow derived macrophages from A/J mice and in the alveolar macrophage cell line MH-S, the *tatB*-negative strain exhibited a 3- to 10- fold reduction in growth, confirming that the Tat system facilitates optimal intracellular replication in macrophages. Whereas the *tatB* mutant is not impaired in growth within the protozoan host *Hartmannella vermiformis*, it exhibited a 65-fold defect within *Acanthamoeba castellanii*, thereby suggesting that the Tat system promotes intracellular replication of protozoa in a host-dependent manner.
To identify substrates of the Tat system in *L. pneumophila*, we have started to compare extracellular and periplasmic proteins of wild-type and *tatB*-negative strains. Major differences in the periplasmic protein profiles were observed upon entry into stationary phase. Further analysis by two-dimensional gel electrophoresis and mass spectrometry will aim to identify the different proteins whose localization is influenced by the Tat system of *L. pneumophila*.

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MPP16
Insights into the unique virulence gene regulation of *Yersinia enterocolitica subsp. palearctica* O:3

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Most yersiniosis in Europe and North America are caused by *Yersinia enterocolitica subsp. palearctica* O:3. To gain information about the high prevalence of serotype O:3 we investigate the ability of clinical isolates of *Y. enterocolitica subsp. palearctica* O:3, O:5,27 and O:9 to infect different animal and human cells *in vitro*. When grown under moderate temperature all isolates produce high amounts of invasin. Surprisingly, all tested O:3 isolates do not adhere and invade into intestinal epithelial cells. We show that the lipopolysaccharide O-antigen inhibits efficient invasion but is not the only factor that leads to the non-invasive phenotype. The O-antigen expression is regulated by temperature. During growth at 37°C only low amounts of O-antigen but still high levels of invasin are found in all O:3 isolates. As *Yersinia* typically does not express invasin at 37°C we analyzed this unique behaviour in more detail. An insertion sequence (IS) element in the promoter region of invasin leads to higher expression rates at 25°C and 37°C. Furthermore, the transcriptional regulator RovA is more expressed at 37°C. We postulate that the IS element prevents binding of the histone-like protein H-NS, which was shown to repress *rovA* expression. In addition, the LysR-type regulator RovM, also known to inhibit *rovA* transcription could not be detected by Western blot analysis. Therefore, we hypothesize that lack of RovM results in higher RovA levels and invasin expression. Higher amounts of invasin and reduced levels of LPS leads to the unique invasive phenotype of *Y. enterocolitica subsp. palearctica* O:3 at 37°C.

MPP17
Helicobacter pylori DNA uptake – exception to the rule?

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Natural transformation is widespread among prokaryotes and enables genetic adaptation to changing environments. All known transformable bacteria implement homologues of type IV pili/type II secretion systems for acquisition of external DNA. In contrast, a type IV secretion system is involved in natural transformation of the gastric pathogen *H. pylori*.
We established novel fluorescent methods for the localization of DNA molecules in bacterial compartments. Using single cell fluorescent analysis we revealed that the T4SS ComB mediates outer membrane DNA uptake. We characterized DNA single molecule import into the periplasm at real-time resolution, showing that the direction of DNA transport can be reverted at high external forces. This implies a quite robust outer membrane ComB transport complex, which is in loose contact with the DNA substrate. Furthermore, the fate of periplasmic DNA was addressed in more detail. All competent bacteria known so far exhibit a ComEC inner membrane channel protein, reported to mediate DNA uptake into the cytoplasm of *Bacillus subtilis*. Monitoring the fate of dsDNA in the periplasm, we provided evidence that ComEC is directly implicated in DNA transport across the cytoplasmic membrane in *H. pylori*.
I will discuss our results in respect to DNA uptake systems in other bacteria, giving insight into differences and commons of prokaryotic natural transformation systems.

MPP18
Characterization of factors involved in regulation of colibactin polyketide expression in *Escherichia coli*

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Bacterial toxins which interfere with eukaryotic host cell cycle have been characterized and their mode of action has been determined during the past few years. They affect either the G1 (CagA, VacA, CNF) or the G2 checkpoint (Cdt, Cif) of the cell cycle thereby actively stimulating or inhibiting its progression. A 55-kb genomic island encoding multiple putative hybrid polyketide synthases (PKS) and non-ribosomal peptide synthetases (NRPS) associated with cell cycle arrest has been discovered by comparative genomics in several extraintestinal pathogenic and non-pathogenic *E. coli* strains. Knowledge on the structure and function of the encoded polyketide colibactin is scarce. Here we report on regulatory aspects of colibactin expression and its impact on global gene expression in *E. coli* to gain a deeper insight into the integration of this genomic island into regulatory networks of *E. coli* and its biological function.

MPP19
Characterisation of a novel genomic island in multidrug-resistant monophasic *Salmonella typhimurium* strains

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Since 2006 monophasic multidrug-resistant *Salmonella typhimurium* strains have become one of the predominant serovars causing foodborne gastroenteritis in humans in Germany. In 2008 the monophasic variant represented 42.2% of all human *S. typhimurium* isolates sent to the Robert Koch Institute and was associated with 9 large diffuse outbreaks and increased need for hospitalisation. This trend continued in 2009. Meanwhile the same kind of strains has been detected in various European countries. The emerging strains mainly belong to phage type DT193 according to the Anderson phage typing scheme, show a consistent PFGE pattern (STYMXB.0131) and exhibit at least tetra-resistance towards antibiotics including ampicillin, streptomycin, sulfamerazine and tetracycline. Genome-based assays revealed an 18.4 kb fragment adjacent to the thrW tRNA locus in the vast majority of the monophasic strains. Subsequent sequence analysis identified 17 potential open reading frames. On nucleotide level some showed a high degree of similarity to other enterobacterial species like Escherichia and Shigella, indicating that the insert was acquired via horizontal gene transfer, as does the significantly lower G+C content compared to the closely related *S. typhimurium* LT2 genome (47.4% vs. 52.2%). For some ORFs extensive similarity was found also on protein level, e.g. to an integrase, putative phage injection / DNA transfer and membrane proteins, a transcriptional regulator and a predicted secreted protein. Transcripts have been shown for 13 of the 17 ORFs. One step deletion of the whole island resulted in changes in the protein expression of secreted as well as membrane proteins. The aim of our study is to reveal the biological function of the novel island with respect to virulence properties and metabolic functions.

MPP20
Characterization of a novel iron uptake system from uropathogenic *Escherichia coli* strain F11

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In silico analysis revealed a putative novel dicistronic iron uptake operon in uropathogenic *E. coli* strains such as F11. Upstream of the first gene a highly conserved Fur box was identified, indicative of iron dependent regulation. The first gene, *EcolF_01003470* (*fetM*), codes for a protein of the lead (Pb2+) uptake porter (PbrT) family, a subgroup of the larger iron/lead transporter (ILT) TC 9.A.10 superfamily. This iron permease possesses REXXE motifs similar to the distantly related EfeU iron-uptake proteins from strains Nissle 1917 or O157:H7 (Grosse *et al*., 2006). In contrast to EfeU, the FetM transporter possesses a large (387 aa) N-terminal periplasmic extension, possibly dedicated to metal binding. The second gene of the operon, *EcolF_01003469* (*fetP*), encodes a small periplasmic protein, which exhibits putative metal binding motifs. This protein might therefore act as a periplasmic metal chaperone.
Several results suggested that these two genes encode a functional iron uptake system. First, a single-copy operon integration into the chromosome of an *E. coli* W3110 derivative strain lacking all known iron-uptake systems led to improved growth under iron depletion. Second, *fetM* and *fetP* were specifically induced in medium supplemented with iron chelators. Moreover, it was shown by FURTA, a Fur titration assay, that the iron uptake regulator modulates expression of the F11 operon. Last, overexpression of both genes resulted in increased accumulation of iron in cells. Although iron uptake was mainly mediated by the permease, the additional presence of FetP resulted in higher growth yields. Fluorescence spectroscopy indicated that the purified FetP homodimer binds manganese as an iron analog and copper which seems to have structural function. In the current model the FetP protein shuttles periplasmic iron to the FetM permease for subsequent uptake into the cytoplasm.

MPP21
Comparative small non-coding RNAome Analysis in *Streptococcus pyogenes*

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In recent years, small non-coding RNAs (sncRNAs) have attracted attention as a new class of gene regulators in eukaryotes and in bacteria. Whereas in Gram-negative bacteria sncRNA genome-wide screening methods have been successfully applied to identify sncRNA regulators, little is known about sncRNAs in Gram-positive pathogens. In this study, two complementary genome-wide approaches have been used to investigate the functional role of sncRNAs in the pathogenicity for *Streptococcus pyogenes* (group A streptococcus, [GAS]). GAS is an important human pathogen, which causes diseases ranging from mild superficial infections of the skin and mucous membranes of the naso-pharynxs to severe toxic and invasive diseases. Initially, we identifiend sncRNA candidates using a modular bioinformatic approach combining compositional analysis, sequence based and structural homology comparison, and secondary structure prediction (molecular sequence suite, MOSES). From those putative sncRNAs four highly probable candidates were manually selected based on RNAz prediction and presence of a peak in the RNAfold profile. In a second approach, we analysed a temporal expression profile of potential sncRNAs for *S. pyogenes* serotype M49 grown in laboratory media using tiling arrays representing the intergenic regions of the M49 genome. Candidates from both screens were characterized and validated by reverse transcriptase-PCR and Northern-blot analysis. The expression of selected sncRNA candidates was verified in M49 batch culture. In order to investigate their potential role in virulence factor expression, sncRNA occurrence was determined during adherence to and internalisation into human laryngeal carcinoma cells (HEp-2) and skin keratinocytes (HaCaT) as well as after growth in whole human blood. For the detailed analysis of interesting sncRNA species, we will generate null mutant strains and perform overexpression studies.

MPP22
Dual roles of CRASP-1 orthologs in protection of Lyme disease spirochetes from complement-mediated killing: Recruitment of soluble human complement regulators and complement inhibition

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Borrelia burgdorferi, B. afzelii, and *B. spielmanii*, the etiologic agents of Lyme disease developed sophisticated means by which these bacteria evade the destructive attack by complement. Binding of complement regulators factor H (CFH) and factor H-like protein 1 (FHL1) via so-called complement regulator-acquiring surface proteins or CRASPs efficiently protects spirochetes from complement-mediated lysis. As each borrelial isolate expresses a combination of at least two CFH-binding proteins, the contribution of a single CRASP in complement resistance is still under debate. To further elucidate the role of CRASP-1 orthologs toward complement resistance, a serum-sensitive *B. garinii* strain unable to produce any CFH-binding proteins was transformed with plasmids directing production of either *B. burgdorferi, B. afzelii* or *B. spielmanii* CRASP-1. In contrast to the *B. garinii* strain, all transformants producing CRASP-1 orthologs bound CFH and FHL1. High levels of deposited complement components could also be detected on the surface of the wild-type *B. garinii* strain but not on CRASP-1 positive cells. More interestingly, CRASP-1 producing spirochetes converted the phenotype of the wild-type *B. garinii* strain to a serum-resistant phenotype. Furthermore, CRASP-1 of *B. burgdorferi* attenuate complement activation by an additional mechanism. This protein shows regulatory activity which is independent of CFH and FHL1 binding. The protective role of CRASP-1 in inhibiting complement-mediated killing was also assessed by incubation of human serum with purified CRASP-1. CRASP-1-treated serum did not affect growth of serum-sensitive cells indicating that soluble CRASP-1 is able to directly interact with the complement system of the human host.

These data strongly suggest that CRASP-1 orthologs represent important serum resistance factors of Borreliae which protect spirochetes from the destructive attack of human complement.

MPP23
Non-coding RNA detection methods combined to improve reproducibility, feasibility, and precision

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Non-coding RNA gain more attention as their diverse roles in many cellular processes are discovered. At the same time, the need for efficient computational prediction of ncRNA increases with the pace of sequencing technology. Existing tools are based on various approaches and techniques, but none of them provides yet a reliable ncRNA detector. Most existing tools require customized scripts and a combination of tools is hampered by non-uniform input and output formats.
We developed an Java framework to integrate existing tools and methods for ncRNA detection. This framework allows to construct reproducible and transparent detection workflows, and to combine and compare different methods efficiently. We demonstrate the effectiveness of combining detection methods (secondary structure prediction using RNAFold and RNAz) using known ncRNA in the genomes of Escherichia coli and *Streptococcus pyogenes*. Comparing the best individual method with the combined method, an increase of 9-16% was achieved in terms of precision and smaller improvements in sensitivity. New ncRNA in *S. pyogenes* were predicted using a combination of four methods, aided by manual inspection to identify the most probable candidates. All four of the selected candidates were verified experimentally using RT-PCR.
We have created an extensible framework for practical, transparent and reproducible combination of ncRNA detection methods. We have proven the effectiveness of this approach in tests and by predicting new ncRNA. The software is available at www.sbi.uni-rostock.de/moses along with source code, examples and tutorial material.

MPP24
RovA dependent membrane proteome of *Yersinia pseudotuberculosis*

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The MarR-type transcription factor RovA controls gene expression in *Yersinia* - in response to temperature. As a thermosensor it participates in the regulation of virulence genes and also contributes to the stress resistance in the host niche. However, a detailed characterization of the bacterial membrane that is expected to constitute the corresponding RovA-dependent molecular processes is still missing. Here we have established a robust method to purify and quantify membrane and membrane-associated proteins, using sucrose-step gradients, tandem mass spectrometry and quantitative iTRAQ™ labelling. Comparative proteome analyses of a rovA deletion mutant and the wildtype of *Yersinia pseudotuberculosis* were used to determine RovA-dependent expression and localization of proteins at the bacterial membrane. Nearly 700 proteins associated to the bacterial membrane could be identified, whereof about 50 RovA-regulated proteins were discovered. Interestingly, RovA seems to affect the VirF-regulon, which controls the expression of the *Yersinia* outer proteins (Yops), the Ysc (a type III-secretion system) and an adhesin protein (YadA). The VirF-controlled expression levels of these proteins are decreased in comparison to their production in the wildtype. Furthermore, several so far unknown membrane proteins were found to be regulated by RovA indicating their role in the physiological adaptation or directly in virulence.

MPP25
Involvement of the variable Xr repeat region of *Staphylococcus aureus* protein A in inflammatory host response

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The Gram-positive human pathogen *Staphylococcus aureus* expresses a variety of virulence factors that contribute to the pathogenesis of this bacterium. Among them are cytotoxins, like α-hemolysin and Panton-Valentin leukocidin (PVL), and the microbial surface components recognizing adhesive matrix molecules (MSCRAMMS). One of the most powerful means to evade the adaptive immune response is the cell wall anchored surface component protein A (SpA). Protein A consists of an N-terminal IgG-binding domain, an Xr or short sequence–repeat region (SSR) encoded by variable numbers of 24-bp repeated DNA sequences, and a C-terminal cell wall anchor. The variable Xr repeat region, which is generated by deletions, duplications, and point mutations of the DNA sequence, provides the basis of the *spa* typing system. Protein A interferes with opsonization by binding to the Fc portion of immunoglobulins, can stimulate cytokine release, and activate proinflammatory the TNFR1 signaling cascade. It has been shown that the Xr region encodes highly conserved peptides with homology to oncoprotein LMP1 of Epstein-Barr virus, a potent activator of type I IFN signaling. The type I IFN cascade is a major immune effector critical for the systemic response to viral infection and the coordination of innate and adaptive immune responses. It has been demonstrated that *S. aureus* activates components of the type I IFN cascade in airway epithelial cells through the Xr domain of protein A. Our aim was to investigate the role of the Xr region in stimulation of cytokine expression and activation of the TNFR1 signaling of airway epithelial cells. We cloned and recombinantly expressed different protein A fragments of *S. aureus* strain 8325-4 with diverse repeat length and stimulated an airway epithelial cell line. The cytokine release was measured by specific ELISA. We could demonstrate that the value of cytokine expression and release was associated with the length and composition of the repeat region.

MPP26
A cell biological approach to study *Listeria monocytogenes* pathogenicity

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Listeria monocytogenes is a Gram positive pathogen causing foodborne infections with a high lethality especially in the elderly. In the course of infection these bacteria run through multiple intracellular passages and they even have the remarkable capacity to directly spread from cell to cell within tissues. Most if not all of the known *Listeria* virulence factors are secreted proteins. Some of them are not simply presented on the bacterial surface but accumulate at certain areas of the envelope such as the polar actin nucleating protein ActA. Distinct localisation patterns of superficial proteins are often established by protein protein interactions with internal cytoskeleton proteins. Secretion systems and the cytoskeleton provide suitable targets of new antibacterial compounds. These considerations have prompted us to initiate a project on the contribution of accessory protein secretion systems and the bacterial cytoskeleton on listerial virulence. We have constructed several deletion mutants in specialized protein secretion systems such as the two YidC/Oxa/Alb3 family protein genes *spoIIIJ* and *yqjG*, the FliI ATPase of the flagellar transport apparatus or the YukA ATPase of the WXG100 secretion system. Furthermore, we are generating deletion mutants in various genes associated with cell division and the bacterial cytoskeleton. Among others, this includes the polar marker protein DivIVA and the three bacterial actin homologues encoded by *L. monocytogenes*, MreB, Mbl and MreBH. The phenotypic analysis of these mutant strains in infection experiments, by proteomic and cell biological methods will help us to understand their role in the infection process of *L. monocytogenes*. Current results of this ongoing project will be presented on this poster.

MPP27
Sec- and Tat-dependent export of two β-lactamases in *Yersinia enterocolitica*

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Yersinia enterocolitica are gram-negative, rod-shaped bacteria that are known to cause a variety of gastrointestinal syndromes. Most *Y. enterocolitica* strains are resistent to β-lactam antibiotics due to the production of one or two chromosomally encoded β-lactamases. Previous studies have reported that *Y. enterocolitica* WA314 (serotype O:8) harbors an *ampC* and a *blaA* gene. BlaA is a class A constitutive broad spectrum penicillase whereas AmpC is a class C inducible cephalosporinase. Mature β-lactamases are located in the periplasmic space where they cleave the amide bond in the β-lactam antibiotics. In this study we investigated the translocation mechanism of both yersinial β-lactamases across the cytoplasmic membrane. First we have shown that the twin arginine translocation (Tat) pathway is functional in *Yersinia enterocolitica*. β-lactamase N-terminal signal sequence-GFP fusions also revealed that BlaA β-lactamase is exported via the Tat system whereas AmpC is most likely Sec-dependent translocated. This is the first report of a Tat-dependent β-lactamase translocation process in *Yersinia*. Previous studies have reported this only in the genus *Mycobacterium* and *Stenotrophomonas*.

MPP28
Yersinia enterocolitica O:3 specific genes with a potential role in pathogenesis

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The zoonotic agent *Yersinia enterocolitica* causes severe diarrhoea in humans. *Y. enterocolitica* serogroup O:3 is the most prevalent serogroup in raw pork and reported cases in Europe, with rising global relevance. Comparing genomes of "American" serogroup O:8 (biotype 1B) with "European" isolate Y11 serogroup O:3 (biotype 4), we address reasons for O:3 serogroup niche adaptation and worldwide dissemination.
Genome comparison revealed a 10 kb unique region encoding RTX-like protein machinery in the Y11 genome. This gene cluster was restricted to "European" isolates and found in serogroups O:9 and O:5,27 that are closely related to serogroup O:3. The group of RTX multifunctional toxins comprises normally pore-forming, cytotoxic and haemolytic exotoxins of gram-negative bacteria, with a common thread of repeated nonapeptide motifs. RTX-like toxins were shown to be associated with the pathogenicity of *Vibrio cholerae*. They are described as autoprocessing cysteine proteases containing domains responsible for actin-crosslinking and inhibition of small Rho GTPases.
To construct the *rtxA* mutant in Y11 we applied different approaches, including Red/ET recombineering. Homologous recombination using a chloramphenicol-resistance cassette inserted into the *rtxA* gene following conjugation was the most successful method.
A pertactin-like protein, which could function both as an autotransporter and/or adhesin is another promising O:3 specific marker. The 3.7 kb pertactin-like gene was deleted using homologous recombination. Both obtained mutants were proven via PCR amplification of the whole region of recombination and complemented with plasmids containing the functional genes. Mutants and their complemented derivatives will be investigated in cell culture and animal models.

MPP29

Carbon metabolism of normal and SCV phenotypes of *Staphylococcus aureus* characterized by ^{13}C isotopologue profiling

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Staphylococcus aureus small-colony variants (SCVs) characterized by auxotrophisms for thymidine, menadione and/or hemin represent a naturally occurring, slow-growing subpopulation causing chronic infections. Since the metabolic and genetic backgrounds leading to this altered phenotype are only partly understood, the carbon metabolism of a clinical isogenic strain set comprising both the normal and the hemin-auxotrophic SCV phenotype were analyzed by ^{13}C isotopologue profiling. The bacteria were grown under aerobic conditions to late-exponential growth phase in a complex medium containing 2.5 g/l [U-$^{13}C_6$]-glucose as main carbon source. The labeling patterns of 14 amino acids were analyzed by gas chromatography/mass spectrometry. Isolates displaying the normal and the SCV phenotype differed substantially in their results in terms of ^{13}C enrichment and labeling patterns of amino acids directly linked to central metabolic intermediates. As expected, glucose served as main carbon source under aerobic *in vitro* conditions. All isolates irrespective of their phenotype showed high-efficient labeling of alanine. The predominant alanine isotopologue was the $^{13}C_3$ species indicating that the majority of the glucose is shunted through the glycolytic and/or Entner-Doudoroff pathway. Also, small amounts of $^{13}C_2$-alanine species were detected reflecting the ability of *S. aureus* to use the pentose phosphate pathway and/or malic enzymes as alternative pathways. The labeling patterns of aspartate and glutamate and their decreased ^{13}C enrichments in the SCVs indicated their reduced tricarboxylic acid cycle activity with concomitant increased relevance of anaplerotic reactions. ^{13}C enrichments and isotopologue patterns allowed the comparative reconstruction of the complex network of catabolic and anabolic processes of isogenic *S. aureus* isolates displaying different phenotypes.

MPP30

Staphylococcus saprophyticus: Structure analysis of the A domain of SdrI

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Staphylococcus saprophyticus is a gram-positive and coagulase-negative pathogenic staphylococcus causing urinary tract infections in young women. It is hydrophobic, able to bind fibronectin, laminin and collagen and hemagglutinates sheep erythrocytes. Some of its surface proteins have been characterised in the early past. This includes the lipase Ssp (*S. saprophyticus* surface protein), the autolysin/adhesin Aas, the uro-adherence factor A (UafA) and the collagen-binding protein SdrI. This serine-aspartate repeat protein contains the longest SD repeat region described so far (854 aa). It is a member of the MSCRAMM protein family and shows a typical ABB domain structure but also has n-terminal repeats.
The A domain of SdrI mediates fibronectin binding but contains none of the known fibronectin binding motifs. To find out more about the domain structure crystallization of the A domain will be attempted. The A domain was divided into three parts and these PCR amplificates (A2, A3 and A2+3) were cloned into the vector pQE30Xa for overexpression.
The proteins will be purified using a NiNTA matrix to be analysed by CD spectroscopy and crystallized under appropriate conditions. Finally an X-ray structure analysis of the protein crystalls will give some more information about the fibronectin binding motif in SdrI of *S. saprophyticus*.

MPP31

Knock out of the L-lactate-dehydrogenase has an impact on the virulence of *Streptococcus pyogenes* M49

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Streptococcus pyogenes (group A streptococci, GAS) is an important human pathogen causing a wide variety of diseases ranging form mild superficial infections of skin and throat up to sever systemic and invasive diseases and sequelae. GAS is euqipped with many virulence factors allowing the bacteria to infect and persist within the host cell. During infection GAS are exposed to different host environments with changing nutritional conditions potentially affecting the central metabolism of the bacteria. Like other lactic acid bacteria (LAB) *S. pyogenes* in general preferably applies the energetically rather inefficient homolactic acid fermentation pathway (2 ATP per molecule of glucose) although the genome encodes for the complete enzymatic setup for mixed acid fermentation (3 ATP per molecule of glucose). For determining the im pact of homolactic acid fermentation on the growth performance and virulence of *S. pyogenes* M49 we constructed an L-lactate-dehydrogenase (LDH) negative mutant and compared it with the corresponding wild type strain. The knock out hardly affected the growth rate in nutrient rich media but led to mixed acid fermentation in the resulting strain. Analysis of virulence-associated abilities of the *ldh*- strain revealed an increased biofilm formation ability but a decreased adherence to and internalization into human keratinocytes and a decreased survival rate in human blood. Survival in blood could be restored to wild type level by addition of lactate to the blood samples. In human serum neither the *ldh* knock out nor the addition of lactate caused changes in the bacterial survival, indicating an impact of homolactic acid fermentation on the cellular components of the blood. These results show that homolactic acid fermentation is not crucial for successful growth of GAS M49 in nutrient rich media but represents an advantage for survival in human blood.

MPP32

Heterogeneous expression of surface molecules within clonal subpopulations of *Staphylococcus aureus*

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Staphylococcus aureus is equipped with two gene clusters, *cap*A-P and *ica*ADBC, encoding enzymes necessary for the synthesis of the capsular polysaccharide (CP) and the polysaccharide intercellular adhesin (PIA), respectively. CP is antiphagocytic but also inhibits the binding of the underlying cell wall proteins to their specific target molecule. For instance fibrinogen binding via Clumping factor A (ClfA) is inhibited by CP expression. We could show that only a part of a given *S. aureus* population expresses CP, which in turn is not able to bind to fibrinogen or to endothelial cells in contrast to the non-encapsulated sub-population. In recent years it became evident that identical cells exposed to the same environmental conditions can show significant variation in phenotypic characteristics probably due to stochastic gene expression of regulatory molecules. Here, we characterized the heterogeneity of the capsule within a *S. aureus* population using single cell assays.
The capsule is regulated by a multitude of regulators including *agr*. The relationship between PIA and CP was analyzed, since both compete for the same substrate, namely N-Acetylglucosamine. Suprisingly, high expression of *ica* from a tetracycline-induced promotor resulted in an increase of capsular synthesis. Subpopulation differing in the expression of CP were separated and analyzed with regard to differential expression of regulatory genes by RT-PCR. The separated populations differed significantly in the *cap*AP mRNA level which correlated to the expression of RNAIII, the effectors molecule of the *agr* system. Thus, the stochastic expression of RNAIII within a culture presumably is a major factor driving heterogeneity of surface molecules. The splitting of the population during colonization may serve as a survival advantage in order to provide diversity for the needs to scope with the different host-environment during colonization and infection.

MPP33

Role of the *Escherichia coli* K1 capsule O-acetylation during systemic infection and gut colonization of White Leghorn-chicken

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Escherichia coli K1 is a commensal of the human and animal gut, occasionally causing disease. The major virulence factor of the bacterium, the polysialic acid capsule, can be phase-variably O-acetylated via the O-acetyltransferase NeuO (Deszo et al., PNAS, 2005).

Recently we demonstrated a tight association of *neuO* with the *E. coli* K1 sequence type (ST) complex 95. Furthermore, we provided evidence that the K1 capsule O-acetylation enhances extraintestinal survival of the bacterium, but adversely affects its ability to produce biofilms (Mordhorst *et al.*, Environm Microbiol, 2009). The relevance of capsule O-acetylation for *E. coli* K1 during systemic infection of the chicken is unknown, as well as its impact on the colonization of the chicken gut. Therefore, we compared genetically engineered mutant strains in an *in vivo*-systemic infection model using five-week-old White Leghorn-chicken (Antao et al., Microbial Pathogenesis, 2008). Furthermore, we conducted gut colonization experiments. Neither avian pathogenic strain IMT5155 (O2:K1:H5; ST140, ST complex 95) nor avian pathogenic strain TK3 (O1:K1:H7; ST95) depended on capsule O-acetylation in the systemic infection model as evidenced by bacterial loads of spleen, brain, and lungs. However, first results suggest that capsule O-acetylation is detrimental during the chicken gut colonization. We postulate that O-acetylation is negatively selected for in the guts, possibly also by its negative effect on biofilm formation, and is positively selected in the inanimate environment after shedding. This scenario provides an example for the versatility achieved by employing phase variation as means of adaptation.

MPP34
The role of the two-component sensor histidine kinase (BPSL0201) in *Burkholderia pseudomallei* virulence

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The Gram-negative soil bacterium *Burkholderia pseudomallei* is the causative agent of melioidosis, a tropical infectious disease with high mortality rates. *B. pseudomallei* can invade host cells, multiplies within the cytosol and induces the formation of actin tails, leading to direct cell-to-cell spreading. Clinical manifestations are extremely variable ranging from acute septicaemia to chronic infections with abscess and granuloma formation in various organs. During the latter conditions, *B. pseudomallei* is likely to encounter an anaerobic environment. Therefore, the aim of this study was to investigate the relationship of virulence and anaerobic growth conditions of *B. pseudomallei*. Initially, 2,300 transposon mutants were screened for defects in anaerobic growth but with preserved aerobic growth. Among 16 mutants with defects in anaerobic growth one mutant with a defect in a putative sensor histidine kinase mutant (BPSL0201) was detected and chosen for further characterization. Sensor histidine kinases are part of a two-component regulatory system in prokaryotes controlling gene expression in response to environmental changes. The BPSL0201 mutant was unable to grow under anaerobic conditions but displayed normal aerobic growth behaviour. In plaque assays the mutant revealed a significant reduction of plaque formation indicating a decreased capability of cell-to-cell spreading under these conditions. Moreover, the mutant was highly attenuated in a murine model of infection with reduced mortality and bacterial burden in various organs compared to the wild-type. Our data indicate that the sensor histidine kinase BPSL0201 plays an essential role in *B. pseudomallei* pathogenesis as well as in its anaerobic metabolism. Ongoing experiments aim to further investigate the molecular basis of the *in vivo* attenuation of the putative sensor histidine kinase (BPSL0201) mutant.

MPP35
Isolation and characterization of *Streptococcus pyogenes* biofilm mutants

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Next to *Streptococcus pneumoniae* and *Staphylococcus aureus*, *S. pyogenes* is one of the most important Gram-positive pathogenic bacteria, associated with various diseases in humans. Disease manifestation of GAS infections ranges from superficial infections of skin (impetigo) and pharynx (pharyngitis and/or tonsillitis) to highly invasive life-threatening illnesses with high mortality and morbidity rates (necrotizing fasciitis and toxic shock syndrome). In GAS, the biofilm phenotype is a novel described feature essential for the pathogenicity of many clinically important serotypes. The production of biofilm is associated with protection against host defences, antibiotics and increased resistance against various stress conditions.
In search for novel genes important for biofilm formation in GAS, we have generated a representative mutant library in GAS M18 serotype, comprised of 8170 GAS mutants, by means of TnSpc-based mutagenesis system. A large-scale screening for biofilm formation in 96-well plate format by using safranin staining was developed and applied for the whole mutant bank. This screening led to the identification of around 30 potential candidate transposon mutants influenced in their biofim abilities. Biofilm phenotype formation of the selected mutants under static conditions using polystyrene well plates, uncoated or coated with different matrix proteins (human collagen I, fibronectin etc.) was studied and compared. Direct sequencing on genomic DNA was carried out for identification of the TnSpc integration site. Strains deficient in proteins involved in cell envelope biogenesis, DNA/protein synthesis, regulators and several with unknown function were among those with altered biofilm formation abilities. Interestingly, the inactivation of Nra in M18 is associated with increased biform formation.
To identify the link between biofilm phenotype and *S. pyogenes* virulence, further studies applying different virulence-associated assays are underway.

MPP36
Caries animal model revisited: colonization patterns of *Streptococcus mutans* in Sprague-Dawley rats and clinical significance

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Animal models were capable of describing the infective nature of caries but also for establishing preventive agents and strategies. Rats are preferentially used because the locations of caries development are the same in rats and humans. Instead of using primarily sterile (gnotobiotic) rats, the aim of this study was to establish a more realistic and robust situation, in which the principal caries agent *Streptococcus mutans* has to interact with co-colonizers.
A total of 16 pathogen-free (SPF) Sprague-Dawley rats (age: 22 days) were treated with chlorhexidine (0,18%) and amoxicillin-clavulanic acid subsequently to reduce their indigenous flora below detection level and were then inoculated with *S. mutans*UA159 on four consecutive days and with $2x10^9$ cells per day. A caries inducing diet (sucrose content 63%) was provided *ad libitum*. Two rats were kept with their indigenous flora and on regular diet and two rats were not pre-treated with anti-infectives but inoculated with *S. mutans*. Three out of 16 rats were treated with a fluoride varnish and four were subjected to a caries-preventive laser-treatment. Over the next 50 days the numbers of *S. mutans* were monitored with oral swab samples (taken at t=1,5,12,20,25,30,40,50 days) using species specific primers targeting the glucosyltransferase gene (*gtf*) and real-time quantitative PCR.
Of the about 10^{10} *S. mutans*-cells administered during inoculation between 10^2 and 10^6 were consistently recovered from the standardized oral swabs, indicating successful colonization. *S. mutans*-levels in the control animals were below detection level of RTQ-PCR (<100 cells per swab). Variability of *S. mutans* colonization was lowest in the animals treated with fluoride, while the group subjected to laser treatment showed highest variability. In conclusion, our caries model system is suitable in reflecting the impact of different anti-caries treatment strategies.

MPP37
Mycobacterial ATP synthase: function, adaptations and interaction with a novel anti-tuberculosis drug

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Infections with *Mycobacterium tuberculosis* lead to nearly 2 million deaths per year with about 2 billion people latently infected. Multi-drug resistant strains as well as mycobacteria staying dormant within the host strongly demand development of drugs with new targets.
Previously, we validated ATP synthase as the target of Diarylquinolines (DARQs) [1], a novel class of antibiotics highly active against *Mycobacterium tuberculosis*. DARQs inhibit ATP synthase at nanomolar concentrations, binding studies with DARQ-coated chips revealed strong binding for purified ATP synthase. ATP synthase in mycobacteria is not only essential for growth, but also required in the physiologically dormant state associated with latent tuberculosis. Dormant mycobacteria are active in ATP synthesis and their ATP production is potently blocked by DARQs, leading to bacterial killing [2].

In contrast to typical inhibitors of ATP synthase (e.g. Oligomycin or DCCD), DARQ lead compound TMC207 displays an astonishing selectivity fro mycobacterial ATP synthase, with a Selectivity Index >20,000 as compared to human mitochondria [3]. These results indicate that respiratory ATP synthesis may be a critical weakness of (dormant) mycobacteria and suggest that inhibition of energy production, although the enzymes involved are strongly conserved between prokaryotes and eukaryotes, is a promising approach for antibacterial drug discovery.
Recently, we investigated the function of ATP synthase and its interaction with DARQs more in detail. Biochemical experiments indicate that DARQs do not act as competitive inhibitors of proton transport, but may interfere with conformational changes of ATP synthase [4]. Furthermore, ATP synthase in fast growing as well a in slow growing mycobacteria is active in ATP synthesis, but cannot invert its function to hydrolyze ATP and establish a proton motive force [4]. This feature may constitute an important mycobacterial adaptation life under to low-oxygen tensions, as found during infection in human macrophages.

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[2] Koul *et al.* (2008) J. Biol. Chem. 283, 25273-80
[3] Haagsma *et al.* (2008) Antimicrob. Agents Chemother, 53, 1290-1292
[4] Haagsma *et al.*, unpublished

MPP38
Heme transport

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Modified tetrapyrroles are complex macrocycles and the most abundant pigments found in nature. Tetrapyrroles play a central role in electron transfer-dependent energy generating processes such as photosynthesis and respiration. They further function as prosthetic groups for a variety of enzymes, including catalases, peroxidases, cytochromes of the P_{450} class and sensor molecules. Heme is a hydrophobic molecule and associates nonspecifically with lipids and proteins in aqueous solution where it promotes peroxidations. Due to its hydrophobicity und toxiticity, free heme has to be transported to its target proteins by different mechanisms, e.g. transport by transmembrane proteins, heme binding proteins and heme chaperones.
Bacterioferritin from *Pseudomonas aeruginosa* is known for its heme binding characteristics and it was shown that the absence of bacterioferritin lowers catalase activity and resistence to H_2O_2. The aim is to identify heme-binding and/or heme-transporting proteins *in vivo* using the *P. aeruginosa* Bacterial Adenylate Cyclase Two-Hybrid system and *in vitro* using the method of crosslinking with biotin lable transfer. The interaction between the probable candidates for heme-binding and/or heme-transport and their traget proteins are then further analysed by *in vitro* translation.

MPP39
Differential induction of LOX-metabolites in human and bovine macrophages by *Mycobacterium avium subsp. paratuberculosis*

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Paratuberculosis in cattle, caused by *Mycobacterium avium subsp. paratuberculosis* (MAP), and Crohn`s disease (CD) in humans, an incurable intestinal inflammatory disease with unknown cause, share striking similarities concerning histopathology and clinical symptoms. A putative pathogenic role of MAP in CD is controversially discussed. Leukotrienes, synthesized from arachidonic acid via Lipoxygenase (LOX) pathway, play a major role in the pathogenesis of inflammatory bowel diseases. Induction of LOX-metabolites in human macrophages by MAP would support the hypothesis that MAP is involved in the pathogenesis of CD. The objective of this study was to quantify formation of LOX-metabolites in human and bovine macrophages challenged *in vitro* with viable MAP bacteria.
The human monocyte cell line THP-1 and primary bovine monocyte-derived macrophages (MDM) were incubated with MAP reference strain ATCC19698. Cells and their supernatants were hydrolyzed with sodium hydroxide, extracted by solid phase extraction and measured by LC/MS/MS with negative electrospray ionisation in multiple reaction mode.
Increased intracellular concentrations of 15-HETE (Hydroxyeicosatetraenoic acid), 5-HETE and 12-HETE were induced in both, MDM and THP-1 cells, to similar levels. However, while MDM released these metabolites into the supernatants, THP-1 cells did not. The intracellular concentration of 15 HpETE (Hydroxyperoxy-eicosatetraenoic acid) was higher in THP-1 cells than in MDM. The time course of formation also differed in both cells. Whilst higher concentrations of LOX-metabolites were determined in THP-1 cells 24 h after challenge, levels decreased from 8 h to 24 h in MDM.
Induction of LOX-metabolites in human macrophages argues in favour of the potential of MAP to cause inflammation in human tissues. Striking differences between human and bovine macrophages with respect to kinetics and efficacy of secretion of the metabolites, however, need to be addressed in further investigations.

MPP40
Role of *pbp1b* and *murM* in high beta-lactam resistant *S. pneumoniae* transformants

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The development of beta-lactam resistance of the human pathogen *S. pneumoniae* involves acquisition of altered penicillin binding proteins (PBPs) by intra- and interspecies horizontal gene transfer. Resistant transformants containing altered PBP genes were obtained from *S. pneumoniae* R6 with chromosomal DNA of the high level resistant *S. mitis* B6 [1]. We now investigated the role in the resistance process of two proteins which were apparently altered in some high level resistant transformants: PBP1b and MurM.
One transformant (CCC) contained a mosaic *pbp1b* due to recombination with *S. mitis* B6 *pbp1b*, and PBP1b could not be detected in PBP assays [1]. We discovered recently that *S. mitis* B6 contains an authentic stop codon 568 in *pbp1b* within the transpeptidase domain leading to a non-functional domain (unpublished data), whereas the N-terminal transglycosylase domain remains intact. This was the first example of an altered PBP1b in a resistant strain. The PBP1b gene of *S. mitis* B6 could be transferred to low level resistant *S. pneumoniae* R6 derivatives; however, this did not result in increased resistance, and its disruption had also no effect.
Microarray-analysis of one last step transformant obtained with *S. mitis* B6 DNA (R6 CCCB) revealed in addition to PBP changes the presence of an altered *murM*. MurM and MurN catalyze the synthesis of L-Ala and L-Ser to the pneumococcal murein. The presence of MurM is required to express PBP-mediated resistance in *S. pneumoniae* [2,3], and contributes to resistance in some resistant *S. pneumoniae* clones. We now show that the *S. mitis murM* mediates beta-lactam resistance in *S. pneumoniae* R6 containing altered PBPs. The data suggest that interspecies gene transfer of *murM* is also important during the development of penicillin resistance in clinical isolates.

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[3] Weber *et al.* (2000). FEMS. Microbiol. Lett. 188: 81-85.

MPP41
The ESX-1 secretion pathway in *Streptococcus oralis* Uo5 and its substrates – EsxA/B

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Antibiotic resistance in *Streptococcus pneumoniae*, a major human pathogen, is the result of horizontal gene transfer events involving closely related streptococcal species. The search for sources of resistance determinants revealed *S. oralis* Uo5, a multiple and highly resistant isolate from Hungary, as possible donor strain in addition to resistant *S. mitis* [1]. In order to determine similarities between *S. pneumoniae* and oral streptococci, and to gain insights into the accessory genome of these species, several genomes have been sequenced. During analysis of the *S. oralis* Uo5 genome, the specialized protein secretion system ESX-1 was discovered. This system has been described in other Gram positive bacteria, but is absent from published *S. pneumoniae* genomes.
ESX-1 is involved in pathogenesis of *Mycobacterium tuberculosis* [2] and *Staphylococcus aureus*. The ESX-1 gene cluster of *S. aureus* is identical in terms of genetic arrangement to that of *S. oralis* Uo5, and consists of eight genes. ESX-1 products include EsxA and EsxB which are representatives of a protein family found in many Gram-positive bacteria, all comprising approximately 100 amino acids and a WXG signature motif. EsxA and EsxB form homodimers which are secreted by the flanking membrane-bound proteins EsaA, EssA, EssB and EssC, an ATPase displaying an FtsK/SpoIIIE like domain [3].

Since this is a rare example of the presence of putative virulence factors in a commensal bacterium, the putative ESX-1 secretion system of *S. oralis* Uo5 was examined on the genetic and biochemical level. We will describe gene expression analysis, and the production of antibodies specific for EsxA, which were used to detect secreted peptides.

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[3] Burts *et al*. (2005) Proc Natl Acad Sci USA. 102(4): 1169-1174

MPP42
Antibiotic resistance mechanisms under urinary tract-like conditions

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Pseudomonas aeruginosa is an ubiquitous Gram-negative bacterium isolated from water and soil. As an opportunistic human pathogen it is able to cause severe acute and chronic infections, as lung-, wound- and urinary tract infections (UTI). It has been reported as a causative agent in 12 % of complicated UTIs and moreover, 35 % of catheter-associated UTIs. Because of its enormous adaptability and its high intrinsic antibiotic resistance, infections with *P. aeruginosa* are difficult to eradicate.
To investigate the antibiotic resistance mechanisms of *P. aeruginosa* under urinary tract-like conditions we cultivated *P. aeruginosa* as colony biofilms using an artificial urine medium (AUM) as nutrient source. Proteome analyses revealed several proteins in increased concentrations in AUM, which might be involved in antibiotic resistance mechanisms. The transcriptional regulator AlgR, controling alginate biosynthesis, the polyamine transporter SpuE, the ribosome modulation factor Rmf and PprB, which is part of a two-component regulatory system. Furthermore, the fumarate hydratase FumC1, involved in the tricarboxylic acid cycle and the superoxid dismutase SodA were identified. Further investigations using transposon mutants of the corresponding genes indicated that they play a role for antibiotic resistance of *P. aeruginosa*. We showed that the physiologic changes of *P. aeruginosa* in the urinary tract enhance the antibiotic tolerance of the bacterium.

MPP43
Pathogenicity mechanism of an emerging pathogen, *Simkania negevensis*

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Simkania negevensis is an emerging gram negative obligate intracellular pathogen initially discovered as a bacterial contaminant in cell cultures. *S. negevensis* has been associated with community acquired pneumonia in adults, bronchiolitis in infants, acute rejection of lung transplants and with exacerbations of chronic obstructive pulmonary disease. The organism has an unusually slow developmental cycle of 6-12 days. The pathogenic strategies that Simkania has evolved to promote its survival are unknown. Here, we investigated the mechanism underlying the control of host cell death in the course of bacterial replication and development. We report that Simkania infected cells resist apoptosis induced with tumor necrosis factor alpha. Activation of downstream caspase 3 and cleavage of poly ADP-ribose polymerase (PARP) were inhibited in Simkania infected cells. In contrast, Caspase 8 activity in response to TNF-α was not affected and the activation of Caspase 9 was blocked. Cytochrome c release in induced cells was prevented by Simkania infection indicating apoptosis inhibition at the mitochondrial level. Further, the infected cells showed MEK/ERK activation, IkB degradation, nuclear translocation of nuclear factor kappa B (NF-kB) and a strong up regulation of the inhibitor of apoptosis (cIAP-1) and Akt. These results indicate that *S. negevensis* inhibit apoptosis through IAP and p-bad based mechanism. These results reveal a novel way by which this intracellular pathogen can interfere with the host cell apoptotic machinery. The induction of apoptosis resistance is thought to be an important immune escape mechanism allowing Simkania to complete its life cycle in the host cell.

MPP44
Adhesion of *Salmonella enterica* sv. typhimurium to polarized epithelial cells is dependent on the function of SopE

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Salmonella enterica sv. typhimurium is a broad-range host intestinal pathogen that causes gastroenteritis in humans. *S.* Typhimurium gains access to the host cells by coordinated action of *Salmonella* Pathogenicity Island 1 (SPI1), SPI4 and SPI5, and prophage-encoded effectors *sopE* and *sopE2*. While SPI1 encodes the type 3 secretion system 1 (T3SS-1) and effector proteins required for invasion of epithelial cells, SPI4 encodes the large non-fimbrial adhesin SiiE, involved in the adhesion to and invasion of polarized epithelial cells such as MDCK cells. In order to gain insight into the cooperation of SPI1 and SPI4 during invasion, we performed adhesion and invasion assays. The invasion and adhesion rates were determined following the infection of MDCK and HeLa cells with the strain SL1344 and its derived mutant strains Δ*sopE*, Δ*sopB*, Δ*sipA* and M712 (Δ*sipA sopA sopB sopE sopE2*). We found that the Δ*sopE* strain was non-invasive in MDCK cells but invaded HeLa cells. The other mutant strains were still invasive but reduced compared to the WT strain. We investigate the contribution of SPI1 effector proteins to adhesion. Surprisingly, the Δ*sopE* and M712 strains were not adherent after 25 min. of infection. We reasoned that these strains may initially adhere but are not able to remain attached at the cell surface. Therefore, we followed the adhesion kinetics from 2 to 25 min. after infection of MDCK cells. The results revealed that both Δ*sopE* and M712 strains were attenuated for the adherence at the same level during the whole experimental period. In contrast to the mutant strains, the WT strain exhibited increased adhesion. Interestingly, complementation of the Δ*sopE* strain could not restore the reduced adherence and invasion phenotypes. On the other hand, although not to the WT level, complementation of M712 strain with *sopE* restored adhesion and invasion. These findings suggest that SopE is required for full invasion of polarized cells. In addition, adhesion was modulated by effector proteins translocated by T3SS-1 like SopE.

MPP45
Serovar diversity of *Actinobacillus pleuropneumoniae*: A genomic perspective

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Actinobacillus pleuropneumoniae (*A. pp.*) is the causative agent of Porcine Pleuropneumonia which leads to high economic losses in the swine industry worldwide. *A. pp.* isolates have been differentiated into 15 serovars which to some extent vary profoundly in their virulence in animal experiments with pigs. A major hindrance for the successful introduction of a vaccination strategy against the pathogen is the limited crossprotection of conventional bacterin vaccines. We successfully demonstrated that a DIVA (differentiating infected from vaccinated animals) subunit vaccine containing outer membrane-associated and secreted proteins of serovars 2, 5, and 9 is highly protective in pigs upon both homologous and heterologous challenge and identified the main antigenic components using proteomics and mass spectrometry. After sequencing the genome of our laboratory *A. pp.* serovar 7 strain AP76, we used the open source maximal unique matching subsequence decomposition program (MUMmer version 3.20) for a whole genome comparison of 3 fully sequenced *A.pp.* chromosomes (3, 5 and 7). Though the multi-alignment of the chromosomes delivered similarities above 90% for the core genome, we could show several substitutions of amino acids in the main antigenic components of the vaccine when comparing serovar 5 with the less virulent serovars 3 and 7. Furthermore, a pairwise comparison of the open reading frames (ORF) identified 49 ORFs in serovar 5 which were missing in serovar 7, including mobile genetic elements, which might contribute to the differences in virulence between the serovars.
Based on the available sequence information of the three serovars of *A. pp.*, we developed a custom expression array on the Affymetrix platform encompassing all 2208 orthologous and serovar specific ORFs of serovar 3, 5 and 7, which can be used to further study the differences between these serovars under different growth conditions.

MPP46
Genomics of *Propionibacterium acnes*

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The anaerobic Gram-positive bacterium *Propionibacterium acnes* is a human skin commensal, residing preferably within sebaceous follicles. Despite its omnipresence, the interaction between *P. acnes* and human tissues is not yet understood. Some features of *P. acnes* point to a mutualistic relationship; others suggest that *P. acnes* is an opportunistic pathogen.
Properties of different *P. acnes* strains vary in many aspects and strongly suggest that *P. acnes* isolates may possess distinct virulence factors; however the genomic basis for such differences has not been uncovered. By classical typing of *P. acnes* strains based on serological agglutination test and cell wall sugar analysis, together with sequence analysis of two genes (*recA/tly*) distinguish the phylogenetic groups Ia, Ib, II and III are distinguished. Here we report the genome sequence of a type Ia strain, designated strain 266, which was isolated from a pleuropulmonary infection. Comparison of this genome to the previously published sequence of a type Ib skin isolate, and to the not completed genome of another type Ia strain (SK137) was undertaken in order to assess the genome plasticity. Island-like genomic regions encoding a variety of putative virulence-associated traits that differ between type Ib and type Ia strains were identified. In addition, subtle differences in known genes involved in *P. acnes* immunoreactivity existed also between the two Ia strains. These results suggest that the current typing scheme does not fully reflect the observed phenotypic differences. The genomic data provides the basis for further investigations on virulence factors and host-interacting properties of *P. acnes* and will increase our understanding of the role of *P. acnes* in health and disease.

MPP47
Identification of novel virulence factors of *S. pyogenes* serotype M18 and M49

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Streptococcus pyogenes is one of the most important gram positive pathogens in the human population responsible for high morbidity and mortality rates in developing nations. It´s pathogenicity is caused by an orchestral expression of different virulence factor subsets. To identify novel virulence factors important for survival under oxidative stress as a part of the host defence, transposon-based (Tn*Spc*) insertional mutants of serotype M18 and M49 were screened for their susceptibility to the oxidative agents menadionsodiumbisulfit (MSBS) and hydrogen peroxide (H_2O_2). Of 7500 insertional mutants in serotype M18 we observed 6 being more sensitive to H_2O_2 and 17 to MSBS. For serotype M49 where 6700 mutants were screened 52 revealed an increased sensitivity to H_2O_2, five to MSBS and three to both substances.
Additionally, all mutants with a distinct phenotype were screened for their sensitivity to the antimicrobial peptide polymyxin E as well as their ability to accumulate detectable amounts of H_2O_2. For 38 % of the M18 insertional mutants an enhanced and for 25 % a reduced susceptibility to polymyxin E was observed. For serotype M49 69 % of tested mutants were more sensitive and 1.7 % more tolerant than the wildtype to the antimicrobial peptide. Furthermore we were able to observe a correlation between H_2O_2 sucseptible M18 and M49 strains and the increased disposal of the compound itself.
Transposon insertion site was identified for a set of 17 mutant strains of both serotypes. Beside several known virulence factors like Epf, SagB and C also genes for DNA/protein synthesis, regulators, metabolic proteins, transporters, cell envelope biogenesis and hypothetical proteins were found. Promising candidates for novel virulence associated factors were found in serotype M49. Strains with an increased sensitivity to MSBS, polymyxin E and/or H_2O_2 were affected in the same gene regions the topoly regulator FlaR and dipeptide permease operon. Defined mutants are currently investigated in virulence assays to elucidate their role in adaptation of *S. pyogenes* to the human host environment.

MPP48
The role of the bacterial SOS response in the evolution of resistance against antibiotics in *Staphylococcus aureus*

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An alarming observation of the last years is the continuous development of *Staphylococcus aureus* strains with resistance against a wide range of antibiotics. In the last years increasing evidence has been published that indicates that the treatment of bacteria with antibiotics may lead to induction of stress mechanisms which then in turn stimulate the evolution of antibiotic resistance. In Gram-negative bacteria this development is mediated by the SOS response.
The SOS response is induced by DNA damage and is regulated by two major players, RecA and LexA. The LexA repressor binds to a specific gene sequence ("SOS-box") and thereby prevents the expression of the genes of the SOS regulon. Among others, this regulon comprises the genes of the error-prone DNA polymerases IV and V, thus affecting the bacterial mutation frequency. In the case of DNA damage, RecA binds to the single-stranded DNA and serves as a co-protease to support autocleavage of LexA.
In order to judge the influence of the SOS response on the mutation frequency of *S. aureus* cultured in the presence of antibiotics we constructed mutants which are deficient in this response employing *S. aureus* NCTC 8325 RN1-HG and the Northern German epidemic strain *S. aureus* SA1450/94, which is characterized by a mutator phenotype caused by insertion of IS*256* in *mutS*. The mutants constitutively express a non-cleavable LexA repressor. The experiments showed that the presence of subinhibitory concentrations of ciprofloxacin clearly increased the mutation frequency of both strains. This effect was inhibited by the presence of the non-cleavable LexA repressor and therefore is caused by the SOS-response. *S. aureus* SA1450/94 showed this effect as well, indicating that it cannot be solely mediated by downregulation of the mismatch repair enzymes [1].

[1] Cuirolo *et al,* J Antimicrob Chemother. 2009 Jul; 64(1):37-45

MPP49
Influence of antibiotics on the transposition frequency of IS*256*r in *Staphylococcus aureus*

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The first IS element was described forty years ago and since then more than 500 insertion sequences have been described in 159 bacterial species. IS elements have been shown to create mutations by insertion into and excision from the genome, to confer genome plasticity and to confer resistance against antibiotics by insertion into promoter sequences or open reading frames.
In this context we have studied the resistance mechanisms of an intermediately vancomycin resistant *S. aureus* (VISA) strain pair. In the strain that displays higher resistance, a part of the decreased susceptibility to vancomycin is caused by insertion of IS*256* into the gene *tcaA*.
In order to test whether the presence of antibiotics leads to the mobilisation of IS elements in *S. aureus*, a test system that measures the transposition frequency of IS*256* was employed [1]. This system comprised an IS*256* element that is tagged with an erythromycin marker (IS*256*r) and a non-transposable mutant for control purposes.
Treatment with subinhibitory concentrations of clinically relevant antibiotics (linezolid, ciprofloxacin and vancomycin) resulted in increases of transposition frequency of IS*256*r which was highest in the presence of ciprofloxacin in *S. aureus* RN1-HG (restored *rsbU*). In conclusion, there seems to be a correlation between antibiotic stress and mobilisation of IS*256*.
To understand the mechanism behind the activation of transposition, site directed mutations as well as the measurement of transposition frequency of IS*256*r in a *rsbU*-mutant strain, that shows lower SigmaB activity, are currently under investigation.

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MPP50
Structural and Biochemical Characterization of a Pathogenicity Island Integrase-Mediated Site-Specific Recombination

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Pathogenicity islands (PAIs) are a distinct class of genomic islands containing virulence genes. They are considered as discrete mobile genetic elements that can excise from the host chromosome. The excision of PAIs occurs by site-specific recombination between the flanking Direct Repeats and is mediated by PAI associated integrase. However, the mechanism of the PAI site-specific recombination is not known. The aim of the current study is to investigate the PAI site-specific recombination using structural and biochemical approaches. The integrase from PAI II of E. coli strain 536 was over-expressed and purified to homogeneity. The purified protein was used for studying the specific interactions with the Direct Repeat DNA present flanking the PAIs. Gel mobility shift assay was performed to characterize the protein-DNA interactions. Our results demonstrate a specific interaction with a 40-bp DNA stretch covering the Direct Repeat. Our future studies will be focused on co-crystallizing the integrase protein-DNA complex to get insights into the molecular mechanism of PAI integrase mediated recombination.

MPP51
Tools for investigation of oral mixed-species-biofilm formation and growth by using fluorescence protein expression vectors

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The oral cavity with its aerobic area and anaerobic niches, constant temperature and wet conditions, is a perfect habitat for many bacterial species primarily existing in biofilm structures. Beside the physiological flora these cohabitates contain several pathogens responsible for chronic inflammatory diseases. The major goal of this project is to elucidate the interaction and complexity in such biofilms. Therefore we selected *S. mutans, S. mitis, S. intermedius, S. sanguinis, S. salivarius* and *A. actinomycetemcomitans* representing an assortment of oral streptococci and facultative anaerobic oral pathogens. To study the architecture of mixed species biofilms by fluorescence microscopy and CLSM we recently constructed fluorescence protein expression vectors inserted into our investigated bacteria. Additionally this enables the determination of bacterial numbers of different species and their cell-ratio by FACS analysis. We used the fluorochromes HcRed, DsRM, EYFP, EGFP, ECFP and EBFP2 cloned into pAT18. For the best output we tested several promotors: P_{recA} and P_{gyrA}, taken from *S. pyogenes*, P_{tet} from pASK-IBA3 and P_{blaZ} from *S. aureus*. In a time laps movie we could show the growth and biofilm formation of *S. mutans* pAT18-P_{recA}-DsRM with a stable red fluorescence over time. For the investigation under flow conditions we engineered a cheap and useful new flow chamber which matches perfectly into the Keyence fluorescence microscope incubation chamber. With this tool we could demonstrate the growth of biofilms under flow conditions. Furthermore this new chamber enables us to study the biofilm formation upon various materials with different flow rates over the time. The complete setup will allow us to study 3D structures and the temporal development of such oral biofilm communities.

MPP52
New luxS-mutants and the role of AI-2 in oral mixed-species-biofilm growth and development

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Biofilm communities are an ubiquitous life style of many bacteria, which allows colonisation of boundary surfaces. After biofilm formation the bacteria are characterised by an increased resistance against chemical and mechanical force. This is a critical attribute with regard to a multitude of diseases because of the decreased sensitivity against antibiotics and immune response.
In our studies we focused on several commensals and pathogens of the oral cavity. They can cause infectious diseases like periodontitis or in some cases serious illnesses like heart disease in susceptible and immunocompromised human hosts. We selected *S. mutans, S. mitis, S. intermedius, S. sanguinis, S. salivarius, M. micros, P. gingivalis* and *F. nucleatum* representing an assortment of oral streptococci and anaerobic oral pathogens. These bacteria are able to form mixed species biofilms. But how is life organized in these mixed species settings?
AI 2 is known as a key player of interspecies QS system. To study the influence of the AI 2, *luxS* mutants were needed. Mutants of *S. mutans* and *P. gingivalis* were kindly provided by other laboratories. For *S. mitis, S. intermedius* and *S. salivarius* we had to extract gene locus and sequence information of the *luxS* loci by an optimized sequence finding PCR approach. Based on these sequences new *luxS*-mutants were generated and shown to be hampered in their biofilm phenotype.
Furthermore we optimised an existing assay with *V. harveii* for the detection of AI-2. This assay revealed a growth phase and species dependent AI-2 release. This might correlate with our findings in transwell analysis, where we could show a changed biofilm mass for several bacteria under *S. mitis*. The complete setup will give us an insight into the interspecies communication especially by AI-2 based communication and its connection to the development of multi-species biofilms.

MPP53
Hypoxia-inducible Factor (HIF)-1 regulated genes in infections with *Staphylococcus aureus*

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Hypoxia-inducible factor (HIF)-1 is the key transcriptional factor involved in the adaption process of cells and organisms to hypoxia. There is recent evidence that HIF-1 also plays a crucial role in inflammatory and infectious diseases. We demonstrated previously that activation of HIF-1 is a general phenomenon in infections with human pathogenic bacteria. To further investigate the role of HIF-1 in bacterial infections, we analyzed the regulation of HIF-1 dependent genes after *S. aureus* infections of Hep-G2 cells. For this purpose, Hep-G2 control cells and Hep-G2 cells with a HIF-1 kd were infected with *S. aureus* and total RNA was isolated after 4 hours of infection for microarray-analysis. First results show that various important genes in inflammatory and infectious diseases are regulated by HIF-1 after *S. aureus* infections and lead to the conclusion that HIF-1 plays a role in the inflammatory response of host cells upon bacterial infection.

MPP54
Studies on the contribution of the SurA chaperone to virulence of uropathogenic *Escherichia coli*

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Uropathogenic *scherichia coli* (UPEC) are the most common cause of community-acquired urinary tract infections (UTI) and also account for a high portion of nosocomial UTIs. UTI may be acute, symptomatic with a varying severity and localization, but may also be sporadic, recurrent, or chronic, a disease diversity that relates to the marked genetic and phenotypic variability of UPEC strains. The conserved periplasmic chaperone SurA for β-barrel outer membrane proteins has recently been shown to support virulence of the cystitis strain UTI89, mainly by affecting the biogenesis of the adhesive type 1-pili [1]. However, since UPEC strains display considerable differences in the repertoire and expression levels of virulence factors, we have set out to examine if and how SurA contributes to virulence of other UPEC pathotypes, specifically of *E. coli* 536 (pyelonephritis) and CFT073 (urosepsis), two generally accepted UPEC model strains. Our present data show that lack of SurA in these strains compromises type 1-piliation as well as adhesion to cultured bladder epithelial cells but in contrast to what has been reported for UTI89 [1], rather supports than impairs invasion under our assay conditions. This finding is under close examination. In addition, we are identifying proteins of UPEC that are influenced by SurA and contribute to pathogenesis. Currently we study the impact of a *surA* deletion on the release and the protein composition of outer membrane vesicles (OMVs), which act as vehicles for the delivery of virulence-associated proteins, such as α-hemolysin, to the host [2]. Electron microscopic and gel electrophoretic analysis shows that UPEC *surA* cells release an increased number of OMVs that dramatically differ from wild-type OMVs in their protein profile. Preliminary evidence also suggests a reduced hemolytic capacity of a UPEC *surA* mutant. These studies will provide new insights into the mechanisms underlying the pleiotropic effects of SurA on UPEC virulence.

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MPP55
Coagulation factors FXI and FXII do not influence *Yersinia enterocolitica* mouse infection, abscess formation or spleen and liver colonization

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The involvement of the coagulation system in *Yersinia enterocolitica* infection has previously only been shown by the presence of fibrinogen and fibrin deposits around *Yersinia* microabscesses and intertwining strands in small granulomas. Factor XI (FXI) and Factor XII (FXII) are surface sensitive coagulation factors which are activated by contact surfaces and amplify tissue factor mediated plasma coagulation. In contrast to other coagulation factors (e. g. Factor X or fibrinogen), the loss of either FXI or FXII is compatible with mouse survival. This allowed us to test the influence of FXI and FXII on *Yersinia enterocolitica* mediated mouse virulence. Intraperitoneally infected FXI knockout (ko), FXII ko mice, and C57BL/6 mice were compared 5 days after infection with a wildtype, a YadA negative, or a pYV plasmid negative strain. Interestingly, the wildtype strain efficiently colonized spleen and liver in all three mouse lines, while the YadA negative and the pYV plasmid negative strain were almost completely eliminated in all three. Furthermore, no significant quantitative changes in spleen or liver colonization were observable for the wildtype strain. Fluorescence microscopy showed comparable abscess formation and neutrophil recruitment in all three wildtype-infected mouse lines. These results show that neither loss of FXI nor of FXII leads to differences in *Yersinia enterocolitica* colonization or abscess formation.

MPP56
Elucidation of the complete genome sequence of *Escherichia coli* acute bovine mastitis isolate 1303: implications for host-pathogen interactions

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Bovine mastitis is in most cases an inflammation process in the cow udder triggered by the invasion of pathogenic bacteria. This process leads to the deterioration of milk secretion tissue and adversely effects milk quantity and quality. The result is a billion dollar economic loss worldwide. Environmental mastitis caused by *Escherichia coli* has increased in many countries at the same time as contagious mastitis, e.g. by *Staphylococcus aureus*, was successfully controlled. The outcome of the disease is most likely influenced by environmental factors, pathogenic species and their virulence determinants and the cow's immune response, so called "cow factors". *E. coli* usually causes an acute onset of mastitis with occasional severe systemic clinical symptoms like sepsis, but may also cause subclinical mastitis. The host response might be an important factor for different courses of disease. The bacterial traits underlying this difference are not known. Thus, coliform mastitis *E. coli* isolates can be a good model system to learn more about human ExPEC infections. Implications can be made concerning the genomic differences in clinical and subclinical ExPEC strains.
In this context, the genome sequence of an *E. coli* isolate of a case of acute bovine mastitis was elucidated.
The *E. coli* strain 1303 harbors a 5.1 Mb genome with approximately 4500 ORFs. Several phages have been found as well as two plasmids, one being an F-plasmid, indicative of active horizontal gene transfer. The strain's genome sequence reveals several fitness traits, most likely important in the infection and colonization of the udder. Further characterization of these traits will be done to understand their role in virulence and induction of the host's immune response.

MPP57
YadA mediated complement evasion of *Yersinia enterocolitica*

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The *Yersinia* adhesin A (YadA) is a trimeric autotransporter adhesin with multiple functions. YadA is essential for complement evasion *in vitro* and for virulence in the C57BL/6 mouse model. So far, we were not able to analyse the role of YadA in complement evasion *in vivo*. Now, we have generated several *Yersinia enterocolitica*(*Ye*) strains expressing point mutated versions of YadA carrying a single amino acid exchange within the membrane anchor domain. We have already shown that with increasing side chain size the YadA trimer stability decreases. The mutants *Ye* G389A and G389S and a strain expressing the YadA wildtype protein were used to test the importance of YadA for *Yersinia* virulence *in vivo*. 6 hours after systemic infection we could not detect differences in the bacterial count in the spleens of infected mice. One day after infection the CFU in the spleen of mice infected with (*Ye*)G389A or G389S was significantly lower than in the mice infected with wildtype *Yersiniae*. We conclude that the YadA mutant strains are rapidly killed by the complement system *in vivo*. To test which pathways of complement activation are decisive for the fate of (*Ye*)we used mice lacking the complement factor B (BfH2) or C1q or both. In these mice we could not observe differences in bacterial counts between *Ye* G389A and wildtype *Yersiniae* on either timepoint we checked, whereas the *Ye* G389S mutant was eliminated 3 days after infection. Taken together, our data suggest that (i) YadA protein stability is decisive for survival of *Yersinia enterocolitica in vivo* (ii) the reduction of virulence in YadA G389 mutated *Yersinia* strains appears to be not due to a reduced infiltration of the host organs but due to increased killing by the host (iii) both the classical and the alternative pathway of complement activation are involved in the killing of *Yersinia enterocolitica*.

MPP58
Characterization of the surface proteome of the Gram-positive pathogen *Streptococcus pneumoniae* using gel-free proteomics

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Pneumococcal cell surface proteins play a central role during the infection process and are therefore considered to be important virulence factors of the pathogen. They are known to mediate bacterial adhesion to host cells, contribute to colonization and invasion and are involved in immune evasion. Different clusters of proteins decorate the cell surface of pneumococci. There are lipoproteins, LPxTG cell-wall anchored proteins and choline binding proteins. Choline binding proteins are anchored to the cell wall via non-covalent attachment to phosphorylcholine. In addition, there are non-classical surface proteins so-called ‚moonlighting' proteins, which lack a typical signal sequence for crossing the phospholipid membrane or a classical anchoring motif. A method for the analysis of the pneumococcal surface proteome using a biotinylation approach in combination with gel-free proteomics (1D-Gel-LC-MS/MS) will be presented. Briefly, surface-exposed proteins are labeled using a biotinylation reagent. Biotinylated proteins are then solubilized by detergents and subsequently purified by affinity-chromatography. After separation in a regular SDS-Gel and an in-gel digest of the proteins with trypsin the resulting peptide mixtures are loaded onto an LC-MS system. In initial experiments with a non-encapsulated pneumococcal strain almost two thirds of the predicted lipoproteins along with a number of choline binding proteins and LPxTG proteins were identified by using CHAPS as a detergent. In the future the method could be applied for the evaluation of the virulence potential of new pneumococcal isolates.

MPP59
Structural characterisation of the *Yersinia enterocolitica* type III secretion system component LcrD

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The inner membrane protein LcrD is a core component of the type III secretion system (T3SS) that enables the pathogenic species *Yersinia enterocolitica* to deliver a set of effector proteins into host cells to escape from immune responses. The type III secretion apparatus can be divided into a basal body and the filament. The basal body consists of several integral membrane proteins that are evolutionary related to components of the bacterial flagellum. Regarding the structure of these components and their function in the process of substrate secretion not much is known. LcrD is an essential part of the basal body and comprises an N-terminal transmembrane domain, with eight putative membrane-spanning segments, and a C-terminal cytoplasmic domain.
Our goal is to elucidate the structure and function of LcrD. Therefor we expressed the cytoplasmic domain of LcrD in *E. coli*. After purification from inclusion bodies we could refold a considerable portion of the protein.
As a next step we are planning to characterize secondary structure and oligomerization state of the refolded protein by means of CD- (circular dichroism) and IR- (infrared) spectroscopy and crosslinking experiments, respectively. We also hope to identify interacting partners by analysing the *in vitro* interaction between refolded LcrD and T3SS effector proteins.

MPP60
Characterization of lipoproteins in *Staphylococcus aureus*

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Staphylococcus aureus is a Gram-positive human pathogen causing a wide range of infectious diseases. Recent studies showed lipoproteins to be an important component for activating the immune system of the host. Therefore, the consequent analysis of lipoproteins could reveal new virulence factors and, thus, provides new insights into host-pathogen interactions. The lipoprotein diacylglyceryl transferase (Lgt) catalyzes the transfer of a diacylglyceryl moiety to the N-terminus of a prolipoprotein which causes the anchoring of the lipoprotein to the membrane. Deletion of *lgt* in *S. aureus* leads to an increased release of lipoproteins into the culture supernatant. By means of the proteomics approach it was possible to identify these lipoproteins. Altogether 28 lipoproteins in five different *S. aureus* strains (COL, Newman, SA113, SH1000, and RN4220) have been identified by mass spectrometry. As expected among these proteins there are a lot of substrate-binding components of ABC transporters. Moreover, many lipoproteins with so far unknown function were detected. Among the substrate-binding components of ABC transporters there are some which are important for binding and transport of iron. An efficient iron transport is essential for *S. aureus* to survive in the host. Currently, we are analyzing the role of lipoproteins under iron-limited conditions.

MPP61
Interaction between hypoxia and IFN-γ mediated persistence of *Chlamydia pneumoniae*

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Infection with *Chlamydia pneumoniae* (*Cpn*) are detected in 5-10% of community acquired pneumonia. The immune effector molecule IFN-γ arrests the intracellular growth of *Cpn* in a persistent state via the induction of the Jak-Stat signalling pathway and the tryptophan catabolising enzyme indoleamine 2,3-dioxygenase (IDO). Persistent *Cpn* are thought to play a role in the pathogenesis of chronic obstructive pulmonary disease (COPD) and asthma. We wondered whether hypoxia (2% oxygen), e.g. as a consequence of pneumonia, has an effect on IFN-γ mediated *Cpn* persistence. Using Western Blot analysis we found a decrease of the IFN-γ induced IDO expression under hypoxic conditions. Furthermore we could show that in response to IFN-γ the phosphorylation of STAT1 on tyrosine 701 is decreased during *Cpn* infection in hypoxia. In contrast, the phosphorylation of STAT1 on serin 727 was not reduced under hypoxic conditions. Nevertheless, the co-stimulatory p38 was affected. *Cpn* induced p-p38 in the presence or absence of IFN-γ, whereas phosphorylation of p38 was reduced under hypoxic conditions 24h p.i.
In conclusion, our data indicate that during an infection with *Cpn* under hypoxic conditions and treatment with IFN-γ the tryptophan catabolism is reduced as well as the JAK/STAT and MAPK pathway. We now started to establish a human lung tissue culture model to further elucidate the mechanism between persistent growth under normoxic and acute infection under hypoxic conditions.

MPP62
Interplay between bacterial infection and low oxygen sensing contributes to epithelial-mesenchymal transition in lung epithelial cells

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Inadequate epithelial repair mechanisms and dysbalanced epithelial to mesenchymal cell transitions (EMT) are supposed to be key factors in the development of chronic lung disease like COPD and asthma. We wondered whether the infection of alveolar epithelial cells (A549) with *H. influenzae* (*Hi*) and *C. pneumoniae* (*Cp*) induces vasoactive factors with known effects on airway remodelling. We further wanted to know whether hypoxia, as a consequence of reduced ventilation of the small airways during pneumonia, might potentiate the release of these factors. We could show that *Hi*-induced expression of VEGF (vascular endothelial growth factor) and ET-1 (endothelin-1) in A549 cells significantly increased under hypoxic conditions ($p<0,05$). Similar results were observed in *Cp* infected A549 cells. Using siRNA against the HIF-1α (hypoxia-inducible factor-1α) we could almost completely block bacteria-induced VEGF and ET-1 expression under hypoxia. Interestingly, normoxic HIF-1α stabilisation with DMOG (Dimethyloxaloylglycine) resulted in VEGF upregulation but failed to induce ET-1. Using a human lung tissue culture model the bacterial effect to induce VEGF and ET-1 expression could be confirmed under normoxia. To further analyse bacteria-induced EMT in A549 cells, we investigated the expression of epithelial and mesenchymal cell markers by western blot. Thus, bacterial infection resulted in downregulation of E-cadherin in normoxic but even more pronounced under hypoxic conditions.

In contrast, vimentin was slightly upregulated in *Hi* infected cells under normoxia but significantly increased under hypoxia. Interestingly, bacterial infection of A549 cells had no impact on the expression of TGF-β, which is the best described mediator of EMT in lung tissue so far. Taken together our data indicate that respiratory bacterial infections in an environment of reduced oxygen availability induce vasoactive factors that trigger EMT in lung epithelial cells.

MPP63
Characterization of the SPI4-encoded adhesin SiiE and its role in infection

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The infection with *Salmonella* is maintained by various interactions between the host and the pathogen, which are mainly controlled by the functions of *Salmonella* pathogenicity islands (SPI). It has recently been shown that, in addition to SPI1, SPI4 plays a prominent role in adhesion to and invasion of polarized epithelial cells which are the predominant form of enterocytes. SPI4 encodes the components SiiC, SiiD and SiiF of a type I secretion system (T1SS) and its substrate protein SiiE. SiiE serves as a secreted adhesin that is only temporarily anchored to the bacterial envelope. Furthermore, there are two accessory proteins encoded by SPI4 genes, SiiA and SiiB, whose functions are not defined so far. To understand the binding process during SPI4-dependent adhesion, we set out to characterize SiiE and its various domains more precisely. Secretion of proteins by T1SSs commonly dependents on a C-terminal signal sequence. We investigated the C-terminal part for its ability to mediate secretion of SiiE. By truncating the C-terminus of SiiE, it could be shown that the signal sequence for the recognition and secretion of SiiE by the T1SS is located maximal 9 residues apart from the C-terminus of SiiE and that the ultimate residues of the signal sequence were essential for the secretion. Besides bearing the signal sequence, another function of the C-terminal part has been suggested: the recognition and binding of the host cell. Whereas the addition of antiserum raised against the C-terminal part of SiiE could decrease invasion of polarized epithelial cells by *Salmonella*, this effect was not observed during the infection of non-polarized epithelial cells. These data indicate an outstanding role for the C-terminus of SiiE. It mediates both its secretion to the surface of the bacterium and probably the interaction with the host cell.

MPP64
Molecular characterization of suilysin and its involvement in *Streptococcus suis* invasion of epithelial cells

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Streptococcus suis (S. suis) is a world wide important invasive swine pathogen causing infections such as meningitis, septicaemia and pneumonia. Furthermore, it is a zoonotic agent associated with human cases of meningitis and other diseases. *S. suis* produces a haemolysin called suilysin (SLY) which belongs to the family of Cholesterol-Dependent Cytolysins. It shows similarity to other members of this family, in particular to pneumolysin of *S. pneumoniae*. SLY has cytotoxic effects on epithelial- and endothelial cells and erythrocytes. It is a virulence-associated factor of *S. suis*, but its role in *S. suis* host-cell interactions is still unclear. Our preliminary results showed that a *sly*-positive virulent serotype 2 strain and its non-encapsulated mutant had a higher rate of invasion in comparison to the respective *sly*-negative mutants. Furthermore, inhibition of Rho-GTPases led to reduced bacterial invasion of the *sly*-positive strain. Therefore, we hypothesized that activation of the actin cytoskeleton is involved in SLY-mediated invasion of epithelial cells. To elucidate the molecular mechanism of SLY host-cell interaction a point mutation was introduced in the Trp-rich region of the conserved domain 4 of SLY, which might be responsible for the pore-forming function. Furthermore, to study hypothetical interaction with integrins, the RGD motif of SLY was mutated. The role of Rho-GTPases in the SLY-mediated phenotypes was investigated by pull-down analysis of activated Rho-GTPases and colocalization-studies. The mutated derivates of wild-type SLY, W_{433}→F and RGD→SVD, had a 10^1-fold and 10^2-fold reduction in haemolytic and cytotoxic activity, respectively. Pull-down assays demonstrated a time-dependent activation of Rho-GTPases, in particular Rac1. In accordance, colocalization experiments showed SLY associated with F-actin and Rac1 in epithelial cells. These results suggest that SLY is able to activate Rho-family GTPases, which is associated with reorganisation of the actin cytoskeleton and formation of membrane ruffling. This might be a mechanism of *S. suis* invasion into epithelial cells. Ongoing studies of the mutated derivates of the recombinant wild type SLY will help to clarify the relevance of pore-formation in the SLY-mediated invasion process.

MPP65
Heterologous expression of pneumococcal surface proteins in *Lactococcus lactis*

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The surface proteins of *S. pneumoniae* are divided in three different classes: lipoproteins, cholin binding proteins and sortase dependent anchored proteins. The sortase A recognizes a signal peptide (LPXTG) in the C-terminus of these surface proteins and links covalently to the cell wall peptidoglycan. The gene product of *pavB* and *pspC*-like *hic* belong to the LPXTG proteins of *S. pneumoniae*.
For further functional analyses we cloned *pavB* and *hic* under the nisin inducible promoter in shuttle vectors pMSP3535 and pMSP3545. These recombinant plasmids were transferred by electroporation into the non pathogenic *L. lactis* MG1363. Heterologous expression of PavB was observed by immunoblot analysis using polyclonal anti-PavB antibodies. Localization of the heterologous expressed PavB was further visualized by immune fluorescence microscopy and quantified by flow cytometric analysis. The results demonstrated that PavB can be detected on the lactococcal surface indicating that PavB is secreted and anchored to the peptidoglycan. Adherence of recombinant *Lactococcus* to proteins of the extracellular matrix was studied with FITC labeled *L. lactis*. A significant binding to plasminogen was observed when PavB is expressed in *L. lactis*.
Hic contains a specific binding site for Factor H in the N-terminal part. Expression of Hic in the heterologous host was studied by immunoblot overlays using Factor H and Factor H antiserum.
The results demonstrate that *L. lactis* is suitable for the heterologous expression of pneumococcal surface proteins.

MPP66
Impact of the extracellular adherence protein (Eap) of *Staphylococcus aureus* on the internalisation into human keratinocytes

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Staphylococcus aureus (SA) is an opportunistic pathogen with the ability to cause acute-fulminant as well as chronic-persistent diseases. The Gram-positive bacterium is the leading cause of superficial and deep skin infections, and is frequently associated with non-healing wounds. SA has classically been considered an extracellular pathogen; however recent evidence indicates that SA invades and persists in non-professional phagocytes. The initial attachment of SA to the eukaryotic cells is mediated by a number of wall-bound adhesins as well as secreted proteins of the "secretable expanded repertoire adhesive molecules" (SERAM) family including the "extracellular adherence protein" (Eap). Eap was shown to exhibit a particularly broad spectrum of activities including immunomodulating and antiangiogenic functions.

We observed that extracellular Eap is effectively internalized by HaCaT keratinocytes. Cell fractionations and proteinase protection assays indicate that upon release from endosomal compartments the protein is localized freely within the cytosol. Moreover preincubation of HaCaT cells with Eap was found to clearly increase the internalisation of different SA strains into keratinocytes after 2 hours of co-cultivation. Although the interaction between SA and the host cells has been proposed to occur primarily through a bridging model where Fibronectin is bound by SA Fibronectin-binding proteins (FnBPs) as well as host cell integrins, preincubation with Eap enhances internalisation of strains with and without FnBPs. While the dual affinity of Eap to plasma proteins on the eukaryotic cell surface and to the bacterium itself is well documented only little is known about the bacterial cell surface components that are involved in the rebinding of Eap. We demonstrate that mutations affecting the teichoic acid composition of SA strain SA113 clearly decreased the Eap-stimulated internalisation of the pathogen into HaCaT cells. Furthermore incubation with Eap affects the ability of the bacterium to persist within these eukaryotic cells.
In conclusion, these findings of Eap uptake by keratinocytes in conjunction with its effect on internalisation of SA provides new aspects on the pathomechanisms involved and on the role of Eap in SA pathogenicity in skin infections.

MPP67
Investigation of protein glycosylation pathways in uropathogenic *Escherichia coli* strain 536

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For a long time, protein glycosylation has been regarded to be limited to eukaryotes. But increasing advances in analytical methods and genome sequencing provide constantly more evidences for N- and O-glycosylated bacterial proteins (Szymanski & Wren, 2005). Today, it is widely accepted that at least some eubacteria express glycoproteins, although detailed information about the glycosylation process and structure-function correlations is available for only a few eubacterial proteins. However, protein glycosylation in bacteria has been shown to play a part in different aspects of host-pathogen interaction, e.g., adhesion, antigenic variation, protection against proteolytic cleavage and protective immunity.
In *Escherichia coli*, a couple of autotransporter proteins, i.e. AIDA-I, TibA, and antigen 43 (Ag43), turns out to be O-glycosylated surface proteins (Sherlock et al., 2006). Ag43 represents the most abundant phase-variable outer membrane protein of *E. coli*. It belongs to the AIDA autotransporter family (Henderson et al., 2004) and has been characterized to impart aggregation and fluffing of cells, to promote biofilm formation, uptake and survival in macrophages as well as long-term persistence of uropathogenic *E. coli* (UPEC) in the murine urinary tract.
AIDA-I and TibA require O-glycosylation for their functionality and the associated heptosyl transferases have been determined. Anyhow, the enzyme responsible for O-glycosylation of Ag43 is still unidentified.
Therefore, we investigate protein glycosylation in uropathogenic *E. coli* strain 536 for the purpose of elucidation of glycoprotein biosynthetic pathways with a particular focus on Ag43. The studies also imply the comparative genomic analysis and functional characterization of putative glycosyl transferase-encoding open reading frames in *E. coli* strain 536.

MPP68
The contribution of the methionine metabolism to the virulence of *Pseudomonas aeruginosa*

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Patients with the genetic disorder cystic fibrosis (CF) suffer from increased mucus production in the upper airways. This mucus is rich in nutrients as e.g. amino acids and is colonized by a heterogeneous microflora, which causes persistent infection. Infections with the opportunistic pathogen *P. aeruginosa* are associated with a poor prognosis due to the failure of antibiotic treatment. *P. aeruginosa* colonizes CF mucus and adapts to the CF lung environment by mutation. Auxotrophic *P. aeruginosa* strains are frequently isolated but their contribution to persistent infection is poorly understood.
Most auxotrophic strains require the amino acid methionine for growth. Interestingly, the methionine metabolism of *P. aeruginosa* is closely connected with the formation of quorum sensing molecules the N-acyl-homoserine lactones (AHLs).
Here, we investigated and characterized methionine auxotrophic *P. aeruginosa* isolates to elucidate the underlying adaptation strategies. We identified that methionine auxotrophy was caused in clinical *P. aeruginosa* isolates by a point mutation in the *metF* gene. To elucidate additional phenotypes of a *metF* mutant, we constructed and characterized a knockout mutant in *P. aeruginosa* PAO1. Growth experiments in an artificial sputum medium containing all amino acids revealed no difference between the *P. aeruginosa* PAO1 wild type and the *metF* mutant. Unexpectedly, the media still contained high amounts of methionine after 48 h of growth. While we did not observe any growth differences between both strains, we noticed strongly reduced production of the virulence factors pyocyanin and the siderophore pyoverdin. In addition, we investigated the metabolic profiles of wildtype and knock out mutant using GC-MS analysis. We detected 237 metabolites of which 66 were identified.
Therefore, we propose that mutations in the methionine metabolism lead to a reduced virulence of the auxotrophic strains. We assume that this could be achieved by reduced production of the AHLs the quorum sensing molecules in the methionine auxotrophic strains.

MPP69
Inhibition of phosphoinositide-3 kinase/Akt signaling is critical for *Clostridium sordellii* Lethal Toxin-induced apoptotic cell death

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Virulent strains of *Clostridium sordellii* cause myonecrosis in obstetric patients and necrotizing fasciitis in injection drug users, in severe cases accompanied by a toxic shock syndrome. The released lethal toxin (TcsL) and hemorrhagic toxin are thereby regarded as the major virulence factors. TcsL mono-glucosylates low molecular weight GTP-binding proteins of the Rho/Ras families. In cultured cell lines, glucosylation, i.e. functional inactivation of Rho/Ras proteins, results in actin reorganization ("cytopathic effect") and apoptotic cell death ("cytotoxic effect"). Apoptotic cell death induced by TcsL is hypothesized to be based on inhibition of the phosphoinositide 3-kinase (PI3K)/Akt-survival pathway. In this study, the critical role of PI3K/Akt signaling in TcsL-induced apoptosis is investigated using the protective bile acid tauroursodeoxycholic acid (TUDCA) as pharmacological tool. TUDCA preserved the TcsL-induced dephosphorylation of Akt, suggesting that TUDCA activated PI3K/Akt signaling downstream of inhibited Ras signaling. Preservation of PI3K/Akt signaling by TUDCA prevented TcsL-induced apoptosis. This antiapoptotic effect of TUDCA was abolished by either LY294002, an inhibitor of PI3K, or the Akt inhibitor. These observations confirm that the antiapoptotic effect depends on PI3K/Akt signaling. Inhibition of Ras/Rho signaling by TcsL resulted in activation of p38 MAP kinase. Inhibition of p38 MAP kinase by SB203580 protected cells from TcsL-induced apoptosis. TUDCA induced activation of p38 MAP kinase, an aspect of the TUDCA effects that most likely did not contribute to its antiapoptotic activity. Due to its antiapoptotic activity, TUDCA is under investigation for its potential application as a therapeutic modulator of several apoptosis-related diseases, including biliary cirrhosis and neurodegenerative diseases. This study is the first example showing that TUDCA treatment may be also useful for the treatment of infectious diseases that includes bacterial toxin-induced apoptosis.

MPP70
Phagosomal integrity is generally maintained after *S. aureus* invasion of nonprofessional phagocytes, but is modulated in a SigB-dependent manner by strain 6850

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Background: *Staphylococcus aureus* is a major cause of a variety of both local and systemic infections. It can invade human host cells, a process that may account for disseminated and recurrent infections. *S. aureus* post-invasion events in non-professional phagocytes are only partially understood. While accumulating morphological data suggest a phagosomal escape, there is a lack of supporting functional data.

Methods: Using a combination of pH determination and morphologic techniques, we have tested the integrity of Staphylococcus-containing phagosomes in 293, HeLa and EA.hy926 cells over time for a number of *S. aureus* strains.
Results: Rapid acidification of *S. aureus*-containing phagosomes occurred and was sustained in all three non-leukocytic cell lines up to 24 h. All *S. aureus* strains tested displayed equally sustained intraphagosomal pH without exhibiting any correlation to pH level and hemolytic activity. Membrane morphology of the phagosomal compartment was heterogeneous, even at conditions where acidic pH was fully maintained, an observation incompatible with phagosomal membrane destruction. As an exception, *S. aureus* strain 6850 showed reduced fluorescence quenching 6 h after invasion. In LAMP-1-YFP-expressing HeLa cells, only strain 6850 failed to localize to LAMP-1-positive vesicles. This was, however, a rare event. Phagosomal modulation by strain 6850 was abolished in a mutant of the alternative σ factor SigB despite an increased hemolytic activity (2.7-fold and 1.9-fold for α-toxin and β-toxin, respectively, as determined by 2D gel electrophoresis). This argues against a role for *S. aureus* hemolytic toxins in this process.
Conclusion: Taken together, our data suggest that the *S. aureus*-containing phagosomal compartment in non professional phagocytes generally remains functionally intact. The exact mechanism of SigB-dependent modulation of intraphagosomal pH by strain 6850 needs further investigation.

MPP71
Persistent *Escherichia coli* in airway specimens of cystic fibrosis patients

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Background: Cystic fibrosis (CF) patients suffer from chronic recurrent bacterial airway infection which eventually lead to preterm death. *Escherichia coli* is not known to be a typical pathogen isolated from the airways of cystic fibrosis patients.
Aims: In this study, the prevalence and persistence of *E. coli* in the airways of CF patients from two different German centers were determined from 1994 until today. *E. coli* isolates collected since 2001 were available for further analysis.
Methods: *E. coli* isolates were phenotypically analyzed. Isolates from different patients and sequential isolates from individual CF patients were genotyped by ERIC PCR and sequencing of the *fimH* gene to assess clonality of the strains.
Results: 28 of 172 CF patients (16%) from two CF centers in Muenster were persistently colonized by *E. coli* with a mean persistence of 46.6 months. During the observation period a mean number of 19.6 isolates were cultured from the airway specimens of the patients. In some patients, phenotypically different isolates with differences in hemolysis on Columbia blood agar, in size including small colony variants and in mucoidity were observed. The mean age of patients with persistent *E. coli* colonization at first culture of *E. coli* was 12.4 years. Sequence results of Multi-Locus Sequence Typing (MLST) and the *fimH* genes of isolates of 6 patients revealed that sequential isolates of each patient belonged to a single clone, but all strains belonged to the major phylogenetic group B2 of *E. coli*. Also, ERIC PCR of isolates of 2 patients with persistent *E. coli* infection revealed that almost all isolates of these patients belonged to the same clone.
Conclusions: *E. coli* was isolated from the airways of a considerable number of patients. Molecular typing of sequential isolates revealed persistent colonization with single *E. coli* clones. Phenotypic variation of isolates indicate bacterial adaptation to the hostile environment. Further studies are required to determine the clinical significance of *E. coli* in the airways of CF patients.

MPP72
Improvement of molecular epidemiological strain typing of pathogenic and non-pathogenic *Escherichia coli*

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The ability to differentiate bacterial pathogens is a prerequisite for the identification and description of disease outbreaks as well as for the improvement of our knowledge on population genetics, epidemiology and ecology of bacterial pathogens. DNA-based bacterial typing by "multi locus sequence typing" (MLST) allows the robust and reproducible discrimination between individual bacterial clones. However, due to horizontal gene transfer and genome plasticity, the currently available *Escherichia coli* (*E.coli*) MLST schemes do not always result in a correct differentiation of phenotypes, especially when it comes to closely related pathogenic and non-pathogenic variants. To improve accuracy of strain typing, we aim at the expansion of the MLST scheme developed by M. Achtman and co-workers.
Additional marker genes were chosen by *in silico* analysis of publicly available complete *E. coli* genome sequences. A sample group of *E. coli* isolates was subjected to MLST based on the Achtman MLST scheme which was extended by additional marker gene fragments. The impact of additional genomic marker fragments on improved strain typing by MLST will be discussed.

MPP73
Characterization of a clinical isolate of *Yersinia enterocolitica* biotype 4; O:9: Variant plasmid architecture and a lack of YopO and YopP secretion

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Among the currently known 15 members of the genus *Yersinia*, three (*Yersinia pestis*, *Yersinia pseudotuberculosis* and *Yersinia enterocolitica*) are able to develop severe diseases in humans.
These human pathogenic *Yersinia* species utilise a plasmid-born "syringe-like" translocon apparatus (Type-III-Secretion-System, T3SS) to inject a cocktail of proteins (*Yersinia* outer proteins, Yops) into host cells. This is relevant for the pathogen to escape from the hosts' immune response.
The necessity of each Yop is not completely proven and in an attempt to reveal non essential Yops we analysed Yop secretion pattern of more than 400 clinical isolates of *Y. enterocolitica* in comparison to the *Y. enterocolitica* reference strain WA-314 (WAP).
We were able to identify a *Y. enterocolitica* Biotype 4; serotype O:9 isolate with a enlarged virulence plasmid which does not secrete the two Yops YopO and YopP in the media.
YopO is an 81kDa serine/threonine kinase which is involved in cytoskeleton rearrangements of host cells and inhibition of phagocytosis. YopP is a 33kDa acetyltransferase inhibiting signal cascades (MAPK and NFκB) in the innate immune response of the host.
PCR and DNA sequencing confirmed the presence of both genes for *yopO* and *yopP* as well as for the chaperone *sycO*. Also the transcripts for all genes were detected.
Currently we investigate the consequences of the variations in the context of host cell infections.

MPP74
Cytoskeletal elements of *Helicobacter pylori*

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Pathogenicity of the human pathogen *Helicobacter pylori* relies upon its capacity to adapt to a hostile environment and to escape the host response. Therefore, the shape, motility, and pH homeostasis of these bacteria are specifically adapted to the gastric mucus. Recently, we have published that the helical shape of *H. pylori* depends on coiled coil rich proteins (Ccrp), which form extended filamentous structures *in vitro* and *in vivo*, and are required for the maintenance of cell morphology in different extend. Next to these Ccrp59 and Ccrp1143 proteins, we have found that H. pylori possesses two additional Ccrps proteins building up likewise filamentous structures suggesting a complex network of Ccrps.
The tubulin ortholog FtsZ initiates cell division. By visualizing FtsZ of *H. pylori* we observed that *H. pylori* divides asymmetrically, yielding a considerably larger and smaller daughter cell, revealing that its cell cycle has several unusual features.
In most bacteria analysed so far, the actin-like protein MreB affects cell morphology. Contrarily, *H. pylori* MreB is not involved in the maintenance of cell shape, but affects the progression of the cell cycle. Mutant cells were highly elongated, characteristic for a delay in cell division, and contained non-segregated chromosomes. The persistence of *Helicobacter pylori* in the hostile environment of the human stomach depends on the activity of urease. Interestingly, mreB mutant cells displayed significantly reduced urease activity, revealing a novel connection between the cytoskeletal element and an enzyme, and thus with pathogenicity.

MPP75
Characterization of virulence-associated traits of *Escherichia coli* bovine mastitis isolates

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Bovine mastitis is the inflammation of the mammary gland in cows accompanying by high somatic cell counts. It is a major challenge for the dairy industry worldwide as it lowers milk yield, reduces quality and increases overall production costs. Bacterial mastitis is caused by invasion of the udder, bacterial multiplication and induction of inflammatory responses in the mammary gland. *E. coli* is one of the main causes of acute mastitis in dairy animal herds. Disease severity and the cause of disease are influenced by environmental factors and the cow's immune response. It has also been discussed that bacterial virulence factors may contribute to the disease. *E. coli* mastitis can be a good model system to transfer results to human extraintestinal infections and the characterization of factors involved increase our knowledge on pathogenesis of extraintestinal pathogenic *E. coli*.
This project focuses on the molecular characterization of virulence-associated traits of *E. coli* mastitis isolates. The distribution of selected determinants, e.g. autotransporter-encoding genes has been screened in a collection of bovine *E. coli* mastitis as well as in bovine fecal isolates. Our data indicate that bovine mastitis and fecal isolates cannot be clearly distinguished based on the distribution of virulence-associated genes. To find out whether differences in gene expression may contribute to the ability of *E. coli* variants to cause mastitis, we analyzed the fitness of *E. coli* mastitis and fecal isolates in milk. The *in vitro* transcriptome of mastitis isolate 1303 was investigated upon cocultivation with MAC-T immortalized bovine mammary gland epithelial cells to identify candidate genes involved in bacterium-host interaction. The results obtained will be discussed in the light of specific bacterial traits that may contribute to establishment of mastitis.

MPP76
En route towards a proteomic signature library of *Staphylococcus aureus*

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Since decades gel-based proteomics has been used to get a deeper insight into physiological processes of living cells. Although it is not possible to apply this technique to all subclasses of proteins (e.g. hydrophobic or very alkaline proteins), most of the cytoplasmic proteins covering important cellular processes as energy metabolism, biosynthesis and degradation of aminoacids, protein turnover, nucleotide metabolism and many others can be separated by 2D gel electrophoresis. Furthermore, using appropriate staining or labeling techniques, gel-based proteomics is able to visualize accumulation, modifications (e.g. phosphorylation), stability and synthesis of proteins. We investigated the response of *Staphylococcus aureus* COL to several stressors and nutrient limitations as well as antibiotic agents on proteomic level. A collection of nine proteomic signatures was used for an inter-experimental comparison revealing common and specific features of individual synthesis patterns. This might not only contribute to a more detailed understanding of interactive adaptational networks in *S. aureus*, but also to the assignment of completely new protein patterns observed for instance under infectious conditions to already existing patterns under well defined conditions. Therefore, a well-studied collection of proteomic patterns under defined stimuli is an essential basis to understand complex adaptational processes in *S. aureus* which are induced by an multi-stimulating environment as it is the case in *in-vivo* experiments and finally in the host.

MPP77
Role of the small GTPases RhoA, Rac1 and Cdc42 on the *Helicobacter pylori* induced actin cytoskeletal rearrangements

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Helicobacter pylori is a major etiological agent in the development of chronic gastritis, duodenal ulcer and gastric adenocarcinoma in humans. Virulent *H. pylori* strains harbor a type IV secretion system (T4SS) encoded by the cag pathogenicity island. This T4SS injects the CagA protein into gastric epithelial cells leading to actin-cytoskeletal rearrangements followed by cell elongation and scattering. However, the signalling leading to the actin-cytoskeletal rearrangements is not fully understood. To characterize the *H. pylori*-induced phenotype in more detail; we investigated the role of the small Rho GTPases RhoA, Rac1 and Cdc42, which are well known regulators of the actin cytoskeleton, on the *H. pylori*-induced phenotype. We used siRNAs, a set of specific GTPase inhibitors or GTPase-modifying toxins for RhoA, Rac1 and Cdc42 and analyzed the phenotypical outcome. Inactivation of the small Rho GTPases using clostridial toxin B or the Rac1 inhibitor NSC23766 significantly reduced the *H. pylori* induced elongation phenotype. The cytotoxic necrotizing factor CNF-1, which is known to activate Rho, Rac and Cdc42, did not significantly influence the *H. pylori*-induced phenotype. In contrast, treatment of cells with CNF-Y, known to activate RhoA, reduced the *H. pylori*-induced phenotype. Interestingly, treatment of cells with C3 toxin (known to inactivate RhoA) leads to a slightly enhancement of cell elongation. Furthermore the expression of RhoA siRNA and dominant-negative forms of RhoA had no inhibitory influence on the elongation phenotype. Conversely, the expression of constitutively active RhoA inhibited the *H. pylori*-induced actin cytoskeletal rearrangements. Taken together, we conclude that Rac1 is activated and RhoA is inactivated by *H. pylori*.

MPP78
PKA-mediated phosphorylation of EPEC-Tir at serine residues 434 and 463: a novel pathway in regulating Rac1 GTPase function

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Type-III or type-IV secretion systems of many Gram-negative bacterial pathogens are used to inject effector proteins into host cells which modulate cellular functions in their favour. A preferred target of these effectors is the actin-cytoskeleton as shown by studies using the gastric pathogens *Helicobacter pylori* (*H. pylori*) and enteropathogenic *Escherichia coli* (EPEC). We recently developed a co-infection approach to study effector protein function and molecular mechanisms by which they highjack cellular signalling cascades. This is exemplified by our observation that EPEC profoundly blocks *H. pylori*-induced epithelial cell scattering and elongation, a disease-related event requiring the activity of small Rho GTPase Rac1. While this suppressive effect is dependent on the effector protein Tir and the outer-membrane protein Intimin, it unexpectedly revealed evidence for Tir-signalling independent of phosphorylation of Tir at tyrosine residues 454 and 474. Instead, our studies discovered a long awaited function for protein kinase A (PKA)-mediated phosphorylation of Tir at serine residues 434 and 463. We demonstrated that EPEC infection activates PKA for Tir phosphorylation. Activated PKA then phosphorylates Rac1 at its serine residue 71 associated with reduced GTP-load and inhibited cell elongation. Phosphorylation of Rho GTPases such as Rac1 might be an interesting novel strategy in microbial pathogenesis.

MPP79

Proteomic comparison of a low and highly virulent *Mycoplasma hyopneumoniae* isolate

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Mycoplasma hyopneumoniae is the causative agent of porcine enzootic pneumonia (EP) which significantly impacts animal welfare and economical benefits in the pig industry. Field isolates of this micro-organism may significantly differ in virulence, but the knowledge on virulence factors is still limited and virulence markers are not yet available.
To elucidate what mechanisms define the level of pathogenicity of *M. hyopneumoniae*, a low and highly virulent *M. hyopneumoniae* isolate were compared by 2-dimentional difference gel electrophoresis (2D-DIGE) and comparative fluorescent 2D-Western blotting in combination with MALDI-ToF MS analysis. Many positional shifts and differentially present or absent spots were observed. In the low virulent isolate, cleavage fragments of the adhesin protein paralog families P97/P102 appeared to be present in higher abundance, both in number and intensity of spots. This is in agreement with the higher capacity of the isolate to adhere to cilia. Also, immunogenic proteins of both strains were identified. Other potentially interesting proteomic differences were observed between the highly and low virulent isolate that have not yet been identified and may lead to the identification of novel virulence determinants.

MPP80

Adaptation of *Pseudomonas aeruginosa* to changing environments using tRNA-dependent formation of alanyl-phosphatidylglycerol

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When exposed to acidic growth conditions the opportunistic bacterium *Pseudomonas aeruginosa* synthesizes significant amounts of the zwitter ionic 2' alanyl-phosphatidylglycerol (A-PG). Thereby, A-PG contributes up to 6% to the overall lipid content of the bacterium at pH 5.3.
Sequence analysis of *P. aeruginosa* revealed open reading frame (ORF) PA0920 showing 34% sequence identity to a protein from *Staphylococcus aureus* involved in tRNA-dependent formation of lysyl-phosphatidylglycerol. The gene product of ORF PA0920 from *P. aeruginosa* was predicted in bioinformatic approaches to be a transmembrane protein with 13 to 15 helices. The *P. aeruginosa* deletion mutant ΔPA0920 failed to synthesize A-PG. Consequently, the protein encoded by ORF PA0920 was named A-PG synthase. Transcriptional analysis of the corresponding gene in *P. aeruginosa* using a *lacZ* reporter gene fusion under various pH conditions indicated a 4.4-fold acid-activated transcription.
In order to get further insight into the biological role of A-PG synthase catalysis, a phenotype microarray analysis was performed. These experiments revealed that intact A-PG synthase is required to render *P. aeruginosa* resistant to the toxic effect of Cr^{3+} and the cationic antimicrobial peptide protamine sulphate. Furthermore, A-PG-dependent resistance to the β-lactam cefsulodin and the osmolyte sodium lactate were observed.
Heterologous overproduction of A-PG synthase in *Escherichia coli* resulted in the formation of significant amounts of A-PG, otherwise not synthesized by *E. coli*. The protein was identified as an integral component of the inner membrane and partially purified by detergent solubilization. By using an *in vitro* activity assay $tRNA^{Ala}$-dependent catalysis was demonstrated. While aminoacyl-PG synthase from *Enterococcus faecium* showed a relaxed specificity for lysine, arginine and alanine, for the A-PG synthase from *P. aeruginosa* a single, strict specificity for alanine was demonstrated.

MPP81

The role of phage-shock proteins and other envelope stress response systems in *Salmonella typhimurium* virulence

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Salmonella typhimurium (*S. typhimurium*) is one of the world's leading food-borne pathogens. It causes a wide range of diseases like diarrhoea, enteritis and typhoid fever.
Different envelope stress response systems enable Salmonella to cope with demanding environmental conditions that would attack the cell membrane during the infection route. The phage shock protein (*psp*) operon, *pspABCDE* is belonging to this category of systems. The *psp* genes are induced by stress factors like filamentous pIV phage proteins, extreme osmolarity, heat shock and ethanol treatment. Induction of the system results in maintenance of the proton motor force. Another phage shock protein gene, *pspG*, is co-expressed but is located distantly from the psp operon. PspG is thought to control iron usage.
Recent studies have indicated the importance of the *psp* response in Salmonella virulence as *pspABCDE* is highly up-regulated during infection of macrophages and epithelial cells.
In the current study, we have constructed single and double knock out mutants of both the *pspABCDE* and *pspG* loci. Mutants were characterized with respect to motility and susceptibility to different cell membrane stresses. Further, we showed that all mutants were dispensable for *S. typhimurium* invasion and survival in epithelial INT-407 and HeLa cells.
The study indicates that *psp* genes, although significantly induced during infection, are not essential for epithelial invasion. One possible explanation is that other envelope stress systems perform equal or compensatory functions in *Salmonella*.

MPP82

Genome sequence of a *Staphylococcus aureus* mutant showing high-level resistance to the glycopeptide antibiotic vancomycin

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S. aureus is an opportunistic human pathogen causing a broad range of human diseases, such as wound infections, pneumonia or endocarditis. In times of increased antibiotic treatment failure, there is an obvious need to understand how bacteria respond to the presence of antibacterial compounds and develop resistance. The glycopeptide vancomycin remains the main therapeutic agent for the treatment of serious infections caused by methicillin-resistant *S. aureus* (MRSA) that are refractory to other clinically used antibiotics. However, MRSA strains with reduced susceptibility to vancomycin have emerged during the last decade.
Characterization of clinical and laboratory vancomycin-intermediate resistant *S. aureus* strains (VISA) identified multiple, resistance-associated changes most probably due to stepwise mutations [1, 2]. In order to test the influence of an elevated mutation frequency on the acquisition of antimicrobial resistance, the mutator strain *S. aureus* RN4220Δ*mutS*, a *mutS* gene deletion mutant of the parent strain RN4220, was subjected to a stepwise vancomycin selection procedure [3]. Here, multiple passaging in the presence of increasing concentrations of vancomycin resulted in the generation of the mutant strain RN4220Δ*mutS*-VC40 that exhibits a significantly higher resistance level to vancomycin compared to the non-resistant parent, indicating full resistance of this strain (MIC: 64 µg/ml versus 2 µg/ml, respectively).
In this study, we performed full genome sequencing of strain VC40 that revealed multiple genetic changes of genes related to cell wall metabolism, transport and gene regulation. Further in-depth analysis of strain VC40 by transcriptomic and proteomic studies as well as mutational analyses will complete the genomic data in order to gain a better understanding of the mode of antimicrobial resistance development in *S. aureus*.

[1] McAleese *et al.* 2006, JBac 188:1120-1133
[2] Ohta *et al.* 2004, DNA Res 11:51-56
[3] Schaaff *et al.* 2002, AAC 46:3540-3548

MPP83
Characterization of a wall teichoic-less *Staphylococcus carnosus*

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Staphylococcus carnosus is a food grade staphylococcal species that is used as a host for heterologous expression of proteins. Here, a markerless Δ*tagO* mutant lacking covalently attached wall teichoic acids (WTA) was generated. In contrast to *S. aureus*, the *S. carnosus* mutant showed a higher lysozyme susceptibility than the parent. The reason for the different behavior of the two species is that *S. carnosus* genome does not contain an *oatA* gene that encodes a peptidoglycan (PG) specific O-acetyl transferase. The higher sensitivity against lysozyme facilitates the isolation of anchor proteins that still contain at their C-terminal end covalently bound PG. This was verified by expressing a fusion protein in *S. carnosus* composed of lipase (SHL) and the anchor sequence of fibronectin binding protein B (FnbpB) of *S. aureus*. This fusion protein was covalently anchored to the cell wall of *S. carnosus* clone and could be released by partial digestion with lysozyme, yielding lipase with different large portions of PG. By comparing the innate immunity activity of lipase with and without bound PG we could show that only the PG-bound lipase (SHL-PG) co-localizes with the pattern recognition receptors (PRRs) NOD2 and TLR2 in mouse keratinocytes.

MPP84
Role of serine/threonine phosphorylation for virulence and physiology of *Staphylococcus aureus<i>*

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In prokaryotes and eukaryotes phosphotransfer is a common theme in regulation of cellular functions. Obviously, modulation of cellular processes by eukaryote-like serine/threonine kinases (ESTKs) and phosphatases (ESTPs) are widespread also in bacteria including pathogenic species. Recently, first evidence on the role of Ser/Thr phosphorylation/dephosphorylation in <i>Staphylococcus aureus has emerged leading to the identification of a functional ESTPK and corresponding ESTP. Due to homology to known ESTPKs/ESTPs in other bacterial species the kinase was designated PknB or alternatively Stk/Stk1, and the phosphatase Stp. The functional role of these enzymes in *S. aureus* has been investigated by use of knock-out mutants and a kinase overexpressing strain. These studies uncovered PknB/Stk and Stp as modulators of cell wall structure and susceptibility to cell wall-acting antibiotics such as certain β-lactams and tunicamycin. Phosphoproteome analysis and transcriptional profile analysis revealed a strong regulatory impact of PknB/Stk on the expression of genes encoding proteins which are involved in purine and pyrimidine biosynthesis, cell wall metabolism, autolysis, and glutamine synthesis. Furthermore, PknB/Stk is able to phosphorylate a regulator of efflux pumps. Importantly, functional PknB/Stk is important for full virulence of *S. aureus*. These findings suggest a broad regulatory impact of Ser/Thr phosphorylation/dephosphorylation in regulation of central cellular functions as well as virulence in the major human pathogen *S. aureus*.

MPV01
FbaB Protein of group A streptococci mediates invasion into vasculature

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Streptococcus pyogenes (*S. pyogenes*, GAS) is related to severe invasive disease like toxic shock like syndrome (TSLS) or necrotizing fasciitis. Proteins SfbI and M1 are well-known major invasins of group A streptococci. Despite its high invasion potential M3 GAS lack these major invasins. One bacterial factor of M3 *S. pyogenes* associated with TSLS is FbaB, which was published first 2002 by Terao *et al.* as a fibronectin-binding protein and adhesion factor for epithelial cells.
As group A streptococci are related to TSLS and invasive disease they have to overcome the endothelial cell barrier to reach deeper tissues. Thus, its relation to invasive diseases makes FbaB an interesting candidate as invasin on endothelial cells.
The aim of this study was therefore to uncover the adhesion and invasion potential of FbaB on endothelial cells *in vitro*. By using a primary human endothelial cell infection assay we were able to show that FbaB functions as adhesin and invasin on endothelial cells. Purified recombinant FbaB protein was coated on latex beads and a *Lactococcus lactis* strain, expressing full length FbaB on its surface was designed. Both, FbaB-coated latex beads as well as the FbaB expressing *L. lactis* strain were effectively internalized into endothelial cells. Further analyses of the trafficking route revealed a membrane protrusion-mediated uptake which involves F-actin accumulation and Rac-1 activation. Furthermore it could be shown that once inside the cell FbaB mediates uptake into endosomes which subsequently fuse with lysosomes. This is in sharp contrast to the well studied SfbI protein-mediated and caveaolae-associated uptake of S. pyogenes and clearly discriminates the biological function of both proteins despite their structural similarities.
In conclusion FbaB may play an important role in endothelial cell invasion by *Streptococcus pyogenes*, a process that is essential for encounter with the vascular system and spreading into deeper tissues.

MPV02
Subversion of mast cell antimicrobial activities by *Staphylococcus aureus*

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Staphylococcus aureus is a significant human pathogen that causes several diseases, including skin infections, endocarditis, septic arthritis, and osteomyelitis. *S. aureus* has evolved clever ways to counteract host defense mechanisms and prevent the host from clearing the infection. In this study we have investigated the interactions of *S. aureus* with mast cells (MCs), which are multifunctional highly effective sentinel cells that line the surfaces of the body, including the skin, the respiratory tract, and the gastrointestinal tract. MCs are ideally positioned to be the first responder cells during microbial attack and to trigger the innate immune defenses against the invaders. MCs actively contribute to kill pathogens by either phagocytosis or by capturing them in extracellular structures consisting of antimicrobial granule proteins and DNA called extracellular traps (MCETs). Here, we show that, in serum-deficient conditions, such as the skin environment, MCs release MCETs where *S. aureus* in entrapped but, unlike other pathogens such as *Streptococcus pyogenes*, not killed. In the presence of DNA, *S. aureus* was found fully resistant to the antimicrobial peptide LL37, a major component of the MCETs. In the presence of serum or when MCs encounter *S. aureus* in blood, MCs are prevented for releasing MCETs and *S. aureus* gain access into the MCs without the loss of bacterial viability by a route distinct from the classical endosome-lysosome pathway. Viable *S. aureus* can be isolated from MCs exposed from long periods (5 days) to gentamicin indicating that intracellular *S. aureus* is refractory to this antibiotic therapy. As MCs are long-live cells, they might serve as reservoir of viable *S. aureus* in the host and contribute to recurrent infection. The virulence factors employed by *S. aureus* to survive either the extracellular antimicrobial activity of the MCETs or intracellularly after been ingested by the MCs are currently under investigation.

MPV03

Yersinia virulence factor YopM interacts with members of the PKN and RSK family of kinases and induces sustained RSK activation by interfering with dephosphorylation

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Yersiniae inject several effector proteins (Yops) into cells of the host immune system which subvert immune functions and enable the bacteria to survive within the host organism. While the biochemical activities of most Yops have been defined, the activity of YopM and its effect on host cell physiology has remained elusive, though deletion of YopM in pathogenic Yersinia results in a dramatic loss of virulence. In order to identify eukaryotic binding partners we used a near physiological approach with double-affinity-tagged YopM being translocated via the natural type three secretion system of yersinae into the macrophage cell line J774.1. Interacting proteins were purified by tandem-affinity-purification. We identified RSK2 and PKN1 as novel interaction partners of YopM in addition to the previously described interacting proteins PKN2 and RSK1. Moreover, GST pull-down experiments demonstrated direct binding of RSK3, RSK4 and PKN3 to YopM, indicating that YopM interacts with all members of the PKN- and RSK family of kinases. In analyzing the effect of YopM on RSK, we found that YopM induces sustained activation of RSK despite the absence of signalling from its activating upstream kinase ERK1/2. YopM protected specific residues from dephosphorylation which results in prolonged RSK activation. *In vitro* phosphatase assays showed that this effect was specific for phosphorylation sites downstream of the primary ERK1/2 target site of RSK. Thus, our study provides a mechanism how YopM induces over-activation of RSK.

MPV04

Small interfering RNA screening identifies novel signalling pathways that regulate *Chlamydia pneumoniae* and host cell interactions under normoxic and hypoxic conditions

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The interaction of C*hlamydia pneumoniae* with its host cell activates signalling cascades that turn on the anti-microbial defence mechanism and regulate immune responses of the host. Other pathways are used for the own benefit of the pathogen to govern the successful outcome of the infection. *Chlamydia pneumoniae* has well adapted to hypoxic conditions but the molecular mechanisms of pathways that regulate their increased growth have not been elucidated. The aim of the small interfering RNA (siRNA) screening was to reveal novel key host cell genes and molecular pathways that significantly affect C*hlamydia pneumoniae* infection of human lung epithelial cells under normoxic and hypoxic conditions. We have used systematic siRNA screening to selectively silence 100 genes of pathways possibly involved in Chlamydia infection and differentially regulated under hypoxic conditions. The effects of siRNAs on the infection of A549 cells by *Chlamydia pneumoniae* were monitored by automatic high-throughput microscopy using immunofluorescence-based assay. Customized image analysis algorithms were used to automatically quantify infection rates and host cell apoptosis. 27 siRNAs increased while eight siRNAs decreased chlamydial infection under both normoxic and hypoxic conditions. 26 siRNAs affected chlamydial infection only under hypoxic condition. The hit siRNAs target genes of pathways involved in internalization and intracellular trafficking, metabolism, signalling connected to cyclic-AMP, calcium and lipid signalling and reactive oxygen species generation. While some of them confirm the function of already known pathways, several of them indicates the role of so far unidentified pathways in chlamydial infection. The results of the siRNA screen not only contribute toward the better understanding of the molecular mechanism of the host-pathogen interactions, but they can also help to design prophylactic, therapeutic and diagnostic strategies to control chlamydial infection.

MPV05

Bacterial modulation of Bcl-x splicing prevents host cell apoptosis

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Apoptosis is an important innate immune response used by mammalian cells to control infection by intracellular pathogens. Many intracellular pathogens have evolved mechanisms to prevent apoptosis, which is critical for persistence of the organism inside of host cells. *Coxiella burnetii*, the causative agent of human Q fever, has been shown to prevent host cell apoptosis. The anti-apoptotic activity is critical for intracellular persistence of this obligate intracellular pathogen. How *Coxiella burnetii* prevents apoptosis of its host cell is not understood. Here, we demonstrate that AnkG, a *Coxiella burnetii* typeIV secretion system effector protein, has anti-apoptotic activity. AnkG binds specifically the mammalian protein p32 (gC1qR) and interfered with the pro-apoptotic functions of p32. AnkG neutralized the ability of p32 to modulate RNA splicing of Bcl-x. Iinhibition of p32 by AnkG resulted in reduced production of an alternatively-spliced transcript encoding the pro-apoptotic Bcl-xs protein. A similar defect in Bcl-x splicing occurred in cells infected with *Coxiella burnetii*. Thus, modulation of Bcl-x splicing by *Coxiella burnetii* represents a new paradigm that enables intracellular pathogens to disrupt a cell autonomous pathway that limits infection by promoting apoptosis.

MPV06

Evidence for the involvement of inflammatory *Propionibacterium acnes* in prostate carcinogenesis

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Prostate cancer is the second leading cause of male cancer deaths in the Western world. Current research implicates an inflammatory mechanism as a cause of prostate carcinogenesis. Here we present results from *in situ* immunofluorescence analyses, showing that the bacterium *Propionibacterium acnes* is prevalent in diseased, but absent from healthy prostates. Live *P. acnes* bacteria were isolated from cancerous prostates and co-cultured with prostate epithelial cells *in vitro*, resulting in a strong multifaceted inflammatory response. We found an intriguing correspondence between the *P. acnes*-induced host cell response and molecular features of the prostate cancer signature, including activation of the COX2-prostaglandin and the plasminogen-matrix metalloproteinase pathways. Long-term exposure to *P. acnes* initiated the process of malignant transformation of non-tumorigenic prostate cells. Our work adds to the growing body of work highlighting the presence of microorganisms in cancerous prostates and suggests that *P. acnes* could play an important role in prostate carcinogenesis.

MPV07

M1 protein mediates survival of group A streptococcus in phagocyte extracellular traps

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Group A Streptococcus (GAS) is a major bacterial pathogen responsible for a wide range of human disease. In the last 30 years, a striking resurgence in reports of severe, invasive GAS infections such as necrotizing fasciitis (NF) and toxic shock syndrome (TSS) has arisen to persist in many parts of the world. Serotype M1 strains have been the most common serotype isolated from invasive GAS infections associated with NF and TSS.

Here we identify a novel role of GAS M1 protein in the stimulation of neutrophil and mast cell extracellular trap formation, yet also subsequent survival of the pathogen within these DNA-based innate defense structures. Targeted mutagenesis and heterologous expression studies demonstrate a role of M1 protein in resistance to the human cathelicidin antimicrobial peptide LL-37, an important effector of bacterial killing within such phagocyte extracellular traps. Studies with purified recombinant protein fragments mapped the inhibition of cathelicidin killing to the M1 hypervariable N terminal domain. A survey of GAS clinical isolates found that strains from patients with NF or TSS were significantly more likely to be resistant to cathelicidin than GAS M types not associated with invasive disease; M1 isolates were uniformly resistant. We conclude increased resistance to host cathelicidin and killing within phagocyte extracellular traps contributes to the propensity of M1 GAS strains to produce invasive infections.
In summary, we have combined molecular microbiologic techniques and an epidemiologic survey to ascribe a novel virulence function of the N-terminal domain of M1 protein in survival avoidance of host extracellular traps and antimicrobial peptide defense. Our studies suggest that investigations of strain variations in cathelidicin and/or extracellular trap resistance/sensitivity may prove fruitful in understanding the epidemiology and pathogenesis of other bacterial infections important in human medicine.

MPV08
The Influence of Plasminogen Binding on the Adherence to Eucaryotic Cells by *Streptococcus pyogenes* Serotype M49

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Streptococcus pyogenes (group A streptococcus, GAS) is an important human pathogen, which causes diseases ranging from mild superficial infections of the skin and mucous membranes of the naso-pharynxs to severe toxic and invasive diseases. GAS is equipped with many virulence factors, which allow this human pathogen to infect and survive within the host. The regulation of virulence factor expression is fine tuned by two-component systems and stand alone regulators. Transcriptome analysis of a serotype M49 GAS strain and its isogenic Nra knock-out mutant revealed the transcriptional control of a new RALP family regulator gene, *ralp3* (Spy0735; RofA-like protein regulator type 3). Ralp3 is linked to a gene encoding Epf (extracellular protein factor from *Streptococcus suis*), a plasminogen-binding protein in GAS. To characterize the functional and virulence associated role of Epf encoded in the ERES region of GAS M49 , a *epf* insertion mutant was studied in several plasminogen-binding, adherence and internalization assays. The inactivation of this gene impaired plasminogen binding in GAS. Furthermore, the Epf mutant showed a decreased adherence and internalization. However, all tested M49 GAS wild type and mutant strains showed an increased adherence and internalization rate following preincubation with plasminogen. To be able to study the influence of plasminogen binding on the adhesion rate regardless of the genetic background, latex beads were coated with human plasminogen. Two different cell lines were incubated with uncoated and coated particles. Scanning electron microscopy revealed exclusive binding of plasminogen-coated beads to HEp-2 and HaCaT cell monolayers. In summary, plasminogen-binding triggers host cell adherence and internalization and could represent an independent mechanism apart from fibronectin-mediated attachment. In addition, Epf seems to be an important plasminogen-binding protein.

MPV09
Role of plasminogen during colonization of *Haemophilus influenzae*

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Plasminogen is a 90kDa protein that circulates in plasma. It is expressed in liver and is one of the key enzyme in coagulation. The typical structure of plasminogen is formed by five homologous triple loop structures designated "kringles" which are linked by 24 disulfide bridges. Moreover, a serine protease domain is attached at the C-terminus. The conversion of the zymogen, plasminogen to plasmin by proteolytic cleavage is mediated by the physiological activators urokinase (uPA) and tissue like plasminogen (tPA) activators and also by bacterial-derived activators, streptokinase, and staphylokinase. Plasmin binds and cleaves the extracellular matrix components fibrinogen, fibrin and laminin. Several bacteria like *H. influenzae, P. aeroguinosa, B. burgdorferi, H. pylori*, and streptococci and human pathogenic fungi express plasminogen binding proteins to invade tissue barriers by expressing proteins, which interact with plasminogen and also activate it.
Haemophilus influenzae is an important respiratory tract pathogen responsible for a variety of infections in humans. In 1996, aspartase of *H. influenzae* was described as a plasminogen binding protein. We identify two additional plasminogen proteins of *H. infuenzae*: haemophilus surface fibrils (Hsf) and protein E (PE). Each of these proteins is expressed on the surface and bound plasminogen can be activated by the urokinase plasminogen activator and is functionally active. These results suggest that *H. influenzae* can recruits plasminogen to its surface and use this host protein for colonization.
In order to locate the domains of plasminogen, which mediate binding to the bacterial proteins, recombinant plasminogen and deletion mutants were expressed in *Pichia pastoris.* Deletion constructs with single kringle-domains deleted (K3-5-P, K4-5-P and K5-P) in addition to a truncated variant with the kringle-domains K1-5 lacking the protease domain and a construct, which contains specifically the protease domain, were generated. When activated by urokinase recombinant plasminogen as well its truncated constructs are functionally active. Recombinant plasminogen and several dilution mutants bind to Haemophilus proteins Hsf and PE, and for PE the interaction site we localized to kringel 3th and 5th of plasminogen. This study will define whether the two plasminogen binding proteins use a similar, related or distinct binding patters.

MPV10
The essential YycFG (WalRK/VicRK) TCS of *Staphylococcus aureus*: Employing the full-length recombinant proteins in a model system

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The YycFG two-component regulatory system (TCS) of *Staphylococcus aureus* is almost ubiquitously distributed in the phylum of Firmicutes and represents the only essential TCS inside the group of Gram-positive bacteria with low G+C-content. YycG (WalK/VicK) is a sensor histidine-kinase and YycF (WalR/VicR) is the correspondent response regulator. Both proteins play a leading role in the development of the cell envelope [1]. Therefore, this TCS is also important for cell growth and viability and is regarded as a target for new or modified antibiotics [2]. However, the exact function of this system remains unclear. Especially the signal received by this TCS is still unknown.
To study the full function of the Yyc system we produced, by recombinant protein expression in *E. coli*, the full-length (including the transmembrane regions!) YycFGHI proteins of *S. aureus* SA137/93A. Additionally, we purified a mutant YycG kinase protein, that is characterized by an amino acid exchange in the putative cytoplasmic sensor PAS domain and therefore was named YycG(Y308N).
A detergent-micelle-model was used to show the ability of the full-length YycG and YycG(Y308N) kinases to autophosphorylate and subsequently transfer the phosphoryl group to the response regulator YycF. Furthermore we established a phospholipid-liposome-model providing an artificial membrane that mimics the *in vivo* situation much more closely. After the reconstitution of both kinases into unilamellar liposomes, only the mutated YycG(Y308N) kinase showed a high phosphorylation activity. This indicates that the amino acid exchange might have a triggering effect on the activity.
With regard to the auxiliary membrane proteins YycH and YycI this new model system provides an excellent experimental basis for studies on protein-protein interactions and for the elucidation of the signal(s) that activate the YycFGHI system.

[1] Dubrac *et al.* (2008); *Mol Microbiol, 70(6)*.
[2] Fabret and Hoch (1998); *J Bac, 180(23)*.

MPV11
Role of staphylococcal wall teichoic acid in targeting the major autolysin Atl

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Cell separation in staphylococci depends largely on the bifunctional autolysin Atl that is processed to amidase-$R_{1,2}$ and R_3-glucosaminidase. It has been shown that these murein hydrolases are targeted via the repeat domains (R) to the septal region of the cell surface to initiate cell separation, thereby allowing localized peptidoglycan hydrolysis and separation of the dividing cells while preventing random cell lysis. Here we show that the targeting of the amidase repeats is based on an exclusion strategy in which wall teichoic acid (WTA) plays a crucial role. In Δ*tagO* mutants that lack WTA, the specific targeting of either externally applied repeats ($R_{1,2}$) or of endogenously expressed amidase was abrogated; instead, binding was observed evenly on the cell surface. As a consequence the mutant became more fragile and more susceptible to introduced autolysis. Using Concanavalin-FITC conjugate we could localize WTA and show that the glycopolymer is essentially devoid in the septum. Our results indicate that WTA prevents binding of Atl to the staphylococcal side walls thereby providing an explanation for the long-known role of WTA in governing autolysin activity.

MPV12
The impact extracellular DNA driven biofilm formation for host colonization and between host transmission of *Neisseria meningitidis*

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Neisseria meningitidis is a major cause of septicemia and meningitis in children. During asymptomatic carriage meningococci reside within tonsillar tissue as microcolonies (Sim et al., 2000, Lancet), which are the basic unit of microbial biofilms (Costerton et al., 1995, Annu. Rev. Microbiol.). We recently established an *in vitro* biofilm model for*Neisseria meningitidis*, revealing that meningococcal biofilm formation is a general trait of un-encapsulated meningococcal variants, rendering the bacteria tolerant to penicillin (Lappann et al., 2006). We now show that genetic lineages of meningococci use two different strategies of biofilm formation. Biofilm biofilm formation of frequently carried pathogenic cc (e.g. sequence type ST-41/44 cc and ST-32 cc) depended on extracellular DNA (eDNA) to initiate biofilm formation (settlers), whereas biofilm formation of cc with low point prevalence (ST-8 cc and ST-11 cc) was independent from eDNA (spreaders). Extracellular DNA in settlers was released by lytic transglycosylase and cytoplasmic N-acetylmuramyl-L-alanine amidase activity. In mature biofilms outer membrane phospholipase A (OMPLA)-mediated autolysis was indispensable for shear force resistance of microcolonies only in settlers, whereas spreaders lacked a functional copy of the responsible autolysin. Taken together, *Neisseria meningitidis* evolved two distinct biofilm formation strategies, an eDNA-dependent one connected with shear force resistant biofilms, and an eDNA-independent one. Based on the experimental findings and previous epidemiological observations, we conclude that most meningococcal cc exhibit a settler phenotype, which is eDNA-dependent and results in a robust and long-lasting colonization of the host. In contrast, spreaders (ST-11 and ST-8 cc) are unable to use eDNA for biofilm formation and therefore might compensate for poor colonization properties by high transmission rates.

MPV13
The influence of CodY on virulence gene expression in *Staphylococcus aureus*: A proteomics approach

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CodY is a global regulator that mainly acts as a transcriptional repressor in exponentially growing cells. In *Bacillus subtilis*, CodY is activated by GTP and branched chain amino acids and thus couples gene expression to changes in the pool of these metabolites. A homologue of CodY has been found to be encoded in the genome of *Staphylococcus aureus*. To analyse the role of CodY in virulence gene expression, *codY* deletion mutants were constructed in three different *S. aureus* strains, RN1HG, UAMS-1, and Newman. Wild type and mutant strains were cultivated in complex medium and the extracellular protein pattern of exponentially growing and stationary phase cells was analysed by using 2D gel analyses combined with mass spectrometry for protein identification. A loss of CodY resulted in the overexpression of several virulence factors like lipases, proteases, nucleases and toxins, indicating that CodY represses, either directly or indirectly, the synthesis of extracellular proteins necessary for exploiting various nutrient resources. Comparison of the different strains uncovers an extreme heterogeneity of CodY dependent virulence gene regulation. Interestingly this phenotype was associated with differently derepressed levels of the accessory gene regulator RNAIII. Moreover, the level of other virulence associated regulators such as SarA, SigB, and SaeRS has been shown to be differently affected by a deletion of *codY*. As in *B. subtilis* the binding activity of CodY to its target sequence is increased in the presence of GTP and isoleucine under *in vitro* conditions. These studies allow a first glance into the connection of virulence gene expression and the metabolic state of *S. aureus*.

MPV14
Effect of CcpA on infectivity of *Staphylococcus aureus*

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The catabolite control protein A (CcpA) is a member of the LacI/GalR family of transcriptional regulators controlling carbon-metabolism pathways in low-GC Gram-positive bacteria. In *Staphylococcus aureus*, CcpA is also known to affect the expression of important virulence determinants such as α-hemolysin (*hla*), the *cap* operon (capsule production), the *ica* operon (biofim formation), protein A (*spa*), *RNAIII* of the *agr* locus, and toxic shock syndrome toxin 1 (*tst*). However, its impact on infectivity of *S. aureus* under *in vivo* conditions still remains to be evaluated.
Here we show that CcpA positively affects pathogenicity of *S. aureus* in nonobese diabetic (NOD) mice with elevated blood glucose levels (> 20 mM). In a murine catheter infection model, mice infected with *S. aureus* strains UAMS-1 and Newman with inactivated *ccpA*, respectively, showed significantly lower bacterial loads in tissue surrounding the catheter; in catheter lumina bacterial numbers of the Δ*ccpA* mutants were also slightly, but not significantly lower than those of mice infected with the wt strains. In contrast, no clear differences in infectivity were found between wt strains and Δ*ccpA* mutants in mice with normal blood glucose levels (< 10 mM), suggesting that CcpA affects virulence of *S. aureus* only in presence of elevated levels of glucose and that it helps to extend a local infection.

MPV15

Role of Glutamine Transporters on Expression of Virulence Factors and Pathogenesis of *Streptococcus pneumoniae*

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Streptococcus pneumoniae are obligate commensals of human respiratory tract, but are also human pathogens causing diseases like pneumonia, meningitis and septicemia. Pneumococci produce a large fraction of ABC transporters systems for carbohydrates and/or amino acids, which cannot be synthezised *de novo* autonomously, such as glutamate or glutamine. To maintain bacterial fitness these transporter systems are also required for assimilating a set of nutrients from host milieus, including bloodstream, nasophaynx or lungs. Genomic analysis revealed six operons encoding glutamine ABC transporter systems. Each glutamine transporter gene cluster consists of three genes, *glnQ*, a cytoplasmatic ATP-binding protein, *glnH*, representing a soluble glutamine binding protein and *glnP* the membrane-spanning permease. Glutamine and glutamate are also important components for the pneumococcal nitrogen metabolism. After insertion deletion mutagenesis of two of the six putative gene clusters encoding ABC transporter systems for glutamine/glutamate (*glnQPH*) in *S. pneumoniae* strain D39 we analyzed the role of *SPD_1098/1099* and *SPD_0411/0412* on growth kinetics, virulence gene expression and infection by using different *in vivo* infection models. The protein profiles of virulence factors including capsule, lipoproteins SlrA and PpmA and choline-binding proteins PspA and PspC were not affected in D39Δ*gln0411/0412* or D39Δ*gln1098/1099*. In contrast, CD1 mice infected intranasally with D39Δ*gln0411/0412* showed a delay in the development of pneumococcal pneumonia as measured in real-time by determination of the luminescence intensity with the IVIS Lumina system. Moreover, the knockout strain D39Δ*gln1098/1099* was massively attenuated and a four log higher doses of this mutant was required to show identical signs of disease in a septicaemia mice infection model as the wild-type strain D39. In the experimental mouse meningitis model, D39Δ*gln1098/1099* showed decreased levels of white blood cells in cerebrospinal fluids and also decreased numbers of bacteria in bloodstream compared to D39 and D39Δ*gln0411/0412*. In conclusion, our virulence studies and growth experiments demonstrated that the *glnQPH1098/1099* operon is essential for the glutamine metabolism while *glnQPH0411/0412* is probably less important but seems to be required under *in vivo* conditions.

MPV16

Adaptation of *Pseudomonas aeruginosa* to urinary tract infections

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Pseudomonas aeruginosa is an opportunistic human pathogen, which can cause severe acute and chronic infections, especially in immunocompromised patients. Due to its high intrinsic antibiotic resistance and the ability to form biofilms, infections with *P. aeruginosa* are difficult to eradicate. It has been described in 35 per cent of the cases as causative agent of catheter-associated urinary tract infections (UTIs). The enormous adaptability of *P. aeruginosa* and the production of several virulence factors often led to a progressive course of these infections.
We developed an *in vitro* biofilm model system which simulates the conditions during acute UTIs. *P. aeruginosa* PAO1 was grown under aerobic and anaerobic conditions in colony biofilms using an artificial urine medium (AUM) as nutrient source. Comprehensive proteome and transcriptome analyses were used to study the specific physiology of *P. aeruginosa* under the chosen conditions. We observed increased levels of virulence factors, biosynthesis of quorum sensing and pseudomonas quinolone signal (PQS) molecules, as well as a highly induced phenazine biosynthesis. Moreover, we identified several global regulators, indicating a complex adaptation response to the conditions in the urinary tract. Analyzing the data using the databases PRODORIC and SYSTOMONAS, we identified adaptation strategies of *P. aeruginosa*, important for infections of the urinary tract.

MPV17

Impact of a small heat shock protein of *Pseudomonas aeruginosa* during stressful and chronic infection in the cystic fibrosis lung

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To survive and adapt to permanently changing conditions during cystic fibrosis (CF) lung disease, *Pseudomonas aeruginosa* (PA) has to combat chronic inflammation and recurrent antibiotic treatment. Thus, long-term survival of PA results in remarkable changes in pathogenicity and physiology of this pathogen. In previous studies we showed that isolates from end-stage CF lung disease are characterized especially by adaptations in metabolism and pathogenicity, probably selected by the prevailing hypoxic and stressful conditions in the CF lung.
In end-stage likely best lung-adapted mutator strains of PA we found that small heat shock protein (sHSP) PA3126, with amino acid similarity of 67% to sHSP IbpA of *E. coli*, was significantly hyperexpressed especially under hypoxic growth conditions. Heat shock proteins (HSP) protect intracellular proteins by refolding or proteolytic degradation of damaged or misfolded proteins, whereas sHSP help to prevent protein aggregation by substrate-binding and create a reservoir of non-native proteins for subsequent refolding by ATP-dependent major HSP. This prompted us to investigate the function of IbpA in PA for CF lung adaptation and first focussed on the regulation and function of PA3126 under stress conditions expected to prevail in the chronically infected CF lung.
In CF mutator isolates of PA we found reduced *ibpA* promoter activity under low oxygen conditions in artificial sputum medium (ASM) that we used to mimic composition of CF mucus. Furthermore, we identified a striking effect of macrophages and heat with oxidative stress on the relative gene expression of *ibpA*. Interestingly, under the combination of stress factors an *ibpA* transposon mutant shows worse survival and by proteome analysis of the *ibpA* mutant under low oxygen, heat and oxidative stress we found a lack of proteins as well. These results suggest that IbpA of PA exhibits an important function during persistent infection of the chronically inflamed CF lung.

MPV18

Intrinsic thermal sensing controls proteolysis of the *Yersinia pseudotuberculosis* virulence regulator RovA

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Yersinia pseudotuberculosis is a food-borne human pathogen that causes a variety of intestinal and extraintestinal symptomes. During infection of the intestinal tract the bacteria invade through the M-cells of the epithelial cell layer and colonize the Peyer´s patches. This step is primarily mediated through the outer membrane protein invasin.
Invasin expression is regulated by the transcriptional activator RovA. Expression of the *rovA*-gene itself is regulated via a complex network, including autoregulation, the LysR-type regulator RovM, the Csr-System and the nucleoid-associated proteins H-NS and YmoA. The post-transcriptional regulation of RovA is strongly dependent on temperature and growth phase. We could show, that RovA acts as a protein thermometer. Thermal shifts from 25°C to 37°C upon host entry lead to a reversible change of conformation, which reduces the RovA DNA-binding capacity and makes it more susceptible to proteolysis by the ATP-dependent proteases Lon and ClpP. This process is also subject to growth phase control. RovA degradation could be observed at 37°C in exponential but not in stationary growth phase. Therefore we assume the presence of an accessory factor that either stabilizes the RovA protein or affects the protease in stationary growth phase. Current work is directed to identify this factor in stationary phase grown *Y. pseudotuberculosis*.

MPV19
Regulation of Type III secretion system activation in *Shigella flexneri*

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Type III secretion systems (T3SSs) are essential virulence determinants of many gram-negative bacteria including animal and plant pathogens. They are protein transport devices used to inject bacterial proteins into host cells to manipulate them during infection. In general, T3SSs consists of a cytoplasmic bulb, a transmembrane region, a hollow needle protruding from the bacterial surface which serves as a secretion conduit for effector proteins and a distal needle tip complex which inserts a pore into the host cell membrane through which effectors can be translocated.
<*Shigella flexneri* is the etiological agent of bloody dysentery in humans, a disease characterised by invasion of, massive inflammation in, and destruction of the colonic mucosa. *Shigella* uses a T3SS to invade and disseminate through the epithelium. T3S is a tightly regulated process: When the environmental conditions are appropriate for *Shigella* invasion, the virulence genes are expressed and the T3SS is built. However, secretion of early effectors and transcription of later ones is blocked until contact with a host cell generates an activation signal. One key question is how this signal is transmitted to the cytoplasmic side of the T3SS to allow orderly effector translocation.
There is a cytoplasmic mechanism linked to the T3SS that actively blocks secretion and premature late effector transcription in the absence of host cell mediated activation. In *Shigella*, MxiC, for which homologues are found in many T3SS, plays a key role in the regulation of secretion responding directly or indirectly to external signals transmitted through the T3SS.
We have analysed the possible association of MxiC with needle complexes and the role of the needle in transmitting an activation signal to MxiC. Additionally, we have generated several MxiC mutants to examine its function. We propose a model of secretion activation that goes from the needle tip complex to the cytoplasmic MxiC via the needle.

MPV20
An antimicrobial peptide induces adhesion of *Salmonella typhimurium*

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Antimicrobial peptides are important components of the innate immune response. These molecules can integrate within the bacterial membrane, thus forming pores which leads ultimately to bacterial killing.
Salmonella enterica is a food-borne pathogen able to cause a wide range of diseases ranging from mild gastroenteritis to systemic infections. During almost all stages of the infection process *Salmonella* is likely to be exposed to a wide variety of host-derived antimicrobial peptides. However, the mode of action of the peptides and how *Salmonella* responds is not fully understood.
We observed that addition of Bactericidal/Permeability-increasing Protein (BPI) to *Salmonella enterica sv. typhimurium* (*S. typhimurium*) cultures has neither bacteriostatic nor bactericidal effects in concentrations up to 10 µg/ml. Surprisingly, we found that this peptide can promote biofilm formation and triggers adhesion of *S.* typhimurium to different surfaces at a concentration of 1 µg/ml. Inhibition of H^+ ATPases or proton motive force strongly inhibited the effect, suggesting an energy-depending process at the inner membrane. Addition of chloramphenicol as an inhibitor of translation had nearly no effect on the capability to induce adhesion. Scanning electron microscopy revealed the appearance of proteinaceous appendages upon addition of BPI. By screening a set of *Salmonella* mutants we could identify these appendages to be type I pili (fimbriae).
Here we present a new mechanism where *Salmonella typhimurium* responds to the presence of an antimicrobial peptide with the activation of virulence functions. Surface-binding of BPI induces functional expression of type I pili. These fimbriae can mediate adhesion to a wide variety of surfaces and enhance biofilm formation, both of which are key functions to promote colonization of and persistence within the host.

MPV21
The primary transcriptome of the major human pathogen, *Helicobacter pylori*

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Genome sequencing of *Helicobacter pylori* has revealed the potential proteins and genetic diversity of this prevalent human pathogen, yet little is known about its transcriptional organization and noncoding RNA output. Massively parallel cDNA sequencing (RNA-seq) has been revolutionizing the analysis of transcriptomes. Here, using a novel differential approach (dRNA-seq) selective for the 5' end of primary transcripts, we present a global map of *H. pylori* transcriptional start sites (TSS) and its operon structure. We discovered hundreds of TSS within operons, and opposite to annotated genes, suggesting that the complexity of gene expression from the small *H. pylori* genome is increased by uncoupling of polycistrons and by genome-wide antisense transcription. We also discovered an unexpected number of ≈60 small RNAs including the ε-subdivision counterpart of the regulatory 6S RNA and associated pRNAs, and potential regulators of cis- and trans-encoded target mRNAs. Our approach establishes a paradigm for mapping and annotating the primary transcriptomes of many living species.

MSP01
Comparison of different genotyping methods for phylogenetic classification of genetically monomorphic bacteria using *Mycobacterium tuberculosis* as a model

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Because genetically monomorphic bacterial pathogens harbour little DNA sequence diversity, most current genotyping techniques used to study the epidemiology of these organisms are based on mobile or repetitive genetic elements. Molecular markers commonly used in these bacteria include Clustered Regulatory Short Palindromic Repeats (CRISPR) and Variable Number Tandem Repeats (VNTR). These methods are also increasingly being applied to phylogenetic and population genetic studies.
Using the *M. tuberculosis* complex (MTBC) as a model, we evaluated the phylogenetic performance of CRISPR- and VNTR-based genotyping, which in MTBC are known as spoligotyping and Mycobacterial Interspersed Repetitive Units (MIRU)-VNTR-typing, respectively. We used as a gold standard the complete DNA sequences of 89 coding genes from a global strain collection.
Our results showed that phylogenetic trees derived from these multilocus sequence data were highly congruent and statistically robust, irrespective of the phylogenetic methods used. By contrast, corresponding phylogenies inferred from spoligotyping or 15-loci-MIRU-VNTR were incongruent with respect to the sequence-based trees. 24-loci-MIRU-VNTR performed significantly better, however, it was still unable to detect all strain lineages with high confidence. The sequence data showed virtually no homoplasy, but the opposite was true for spoligotyping and MIRU-VNTR, which was consistent with the higher rates of convergent evolution observed and the low statistical support obtained for phylogenetic groupings defined by these markers. Our results also revealed that the discriminatory power of the standard 24 MIRU-VNTR loci varied by strain lineage.
Taken together, our findings suggest that SNPs are ideal for defining deep phylogenetic groupings. While initial screening of larger strain collections might be done by VNTR-based markers, final strain assignment to specific strain lineages should be based on more robust markers such as SNPs.

MSP02
Factor H binding of capsule null locus meningococci

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Capsule null locus (cnl) meningococci are frequently carried commensals of the human nasopharynx, which due to a lack of a capsular polysaccharide only rarely cause invasive disease. As such, cnl meningococci might be positively involved in the development of protective natural immunity against pathogenic meningococcal variants. The current development of vaccines utilizing the meningococcal factor H binding protein (fHbp) as antigen raises the question about fHbp expression and variability in cnl meningococci. In a total of 17 cnl strains derived from 8 sequence types, of which 4 belonged to two different clonal complexes, we demonstrate that all cnl strains harbored fhbp alleles belonging to the variants 2/3 or synonymously, variant A. Accordingly, the strains did not bind variant 1 specific antibodies to FHBP. The analysis of variant 2/3 FHBP expression is ongoing. None of the strains bound purified fH in contrast to control strains as evidenced by FACS analysis. This finding suggests that fH binding might be irrelevant for survival on the mucosal surface and that at least under the conditions employed there are no alternative fH binding sites. To elucidate other complement regulator binding activity of cnl meningococci, data will be presented on C4bp binding. Since FHBP is an important vaccine antigen, the expression of additional proteins utilized in investigational vaccines, such as NadA, will be demonstrated.

MSP03

Will not be presented and published

MSP04
Taxonomic position of the morphospecies *Angiococcus disciformis*

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A set of 14 strains presumely assigned to the species *Angiococcus disciformis* on the basis of their morphological appearance was studied taxonomically. The 16S rRNA gene sequence available for the type strain ATCC 33716^T clusters with those of *Cystobacter violaceus* and *Archangium gephyra*. However, the whole cell fatty acid pattern of the studied strains was highly similar to those of the *Myxococcus-Corallococcus* branch (M-C group). Five of the isolates grouped with the type strains of *M. virescens* and *C. coralloides* while the other strains were more similar to the *M. fulvus* and *A. disciformis* type strains. The latter group also included strains assigned to *Pyxidicoccus fallax*, and these were indistinguishable from the *Angiococcus* strains. The fatty acids contributing most to the difference between these two groups are iso-$C_{15:0}$ present in higher percentages in the M-C group and $C_{16:1}$ ω5c with significantly higher percentages in the *Angiococcus-Pyxidicoccus* group, as compared to the other group, respectively.
This chemotaxonomic bisection is reflected by the hydrolytic abilities of the isolates. The strains of the M-C group strongly hydrolyse starch and xylan and cleave esculin whereas the second group of strains did not react in this manner. Thus the *Angioccoccus* strains of the M-Cgroup displayed properties characteristic for the genus *Corallococcus*.
The disaccord between the phylogenetic position of the type strain of *A. disciformis*, as determined by 16S sequencing, and its high similarity to species of the M-C cluster in respect to the fatty acid pattern deserves further clarification.
The close phylogenetic neighbourhood shown earlier and the chemotaxonomic similarities of the genera *Myxococcus, Corallococcus, Pyxidicoccus* and *Angiococcus* suggest an more detailed evaluation of the pooling of (some of) these genera.

MSP05
Genotyping *Streptococcus agalactiae* isolates with DNA-microarrays

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Streptococcus agalactiae or group B streptococcus (GBS) is frequently carried in the normal vaginal flora, but it is also a common cause of neonatal sepsis and meningitis. This pathogen has several different virulence-associated markers which might be used to assess its ability to cause invasive disease. In order to obtain information on all these markers within a single experiment, a microarray was developed and tested on sequenced strains and clinical isolates. The capsular gene cluster, the alleles of the gene encoding the alpha-like protein *alp*, the variants of the quorum sensing gene cluster *rgf*, the presence or absence of the gene encoding the beta antigenic cell wall protein as well as absence or presence and variation of two gene clusters encoding pili can be simultaneously assayed. These markers provide a framework to split *Streptococcus agalactiae* in a number of strains or clusters since they vary independently from each other. Thus, the combination of these genes might be used as genetic fingerprint in order to facilitate tracing of transmissions. 110 isolates and reference strains tested so far have been assigned to 21 of these strains or clusters. The correlation to MLST (multilocus sequence typing) is currently under investigation. Comparative screening of clinical and surveillance isolates is planned in order to identify predictors for increased virulence among gynecological *S. agalactiae* isolates and to assess the risk of invasive infection in newborn infants.

MSP06
Microevolution of *Pseudomonas aeruginosa* isolates of patients with cystic fibrosis

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Cystic fibrosis patients typically harbour the initially acquired *Pseudomonas aeruginosa* clone for ten years or more in their airways. The conversion of phenotype and the evolution of genome organisation in P. aeruginosa isolates from CF-airways have only been studied in single, independent isolates. At Hanover Medical School there is a strain collection of sequential *P. aeruginosa* isolates from the onset of colonisation over a period of about 20 years. The phenotypic and genomic evolution of *P. aeruginosa* in CF airways was analysed in six clones each represented by sequential non-hypermutable isolates from patients with variable clinical course. First and late isolates were tested for virulence and persistence in a murine lung infection model. Sequential isolates from one patient displayed a broad spectrum of phenotypes and colony morphologies. Each clone had evolved differently in its unique CF habitat. According to the mouse experiments *P. aeruginosa* evolved in CF airways towards a persistor pathotype attenuated in acute infection. Single growth experiments in rich and sputum media showed a higher growth rate for late than first isolates, whereas in minimal medium the first isolates were growing faster, i.e. nutrient depletion favoured the non adapted and environmentally related first isolates. In contrast late isolates were well adapted to high nutrient levels. Late CF isolates were not compromised in their metabolic versatility. The divergent evolution of the six clones in separate CF habitats points to an instrumental role of the human host in shaping the *P. aeruginosa* microevolution. Ongoing next-generation sequencing of sequential isolates belonging to the worldwide dominant clonal complexes C and PA14, respectively, indicate an on-going microevolution that vary in the individual patient between fifteen and more than a thousand de novo mutations per 10-year colonization period.

MSP07

The evolutionary basis of *Alphaproteobacterial* diversity

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In order to elucidate the role of recombination, adaptation and selection in shaping bacterial diversity, the family *Sphingomonadaceae* (*Alphaproteobacteria*) was used as the model group. First a multilocus sequence analysis (MLSA) approach which targets a set of 9 housekeeping genes (atpD, dnaK, EF-G, EF-Tu, gap, groEL, gyrB, recA, rpoB) in *Sphingomonadaceae* was established. Then they were used to elucidate the population structure and the significance of recombination events.
The new primers for these housekeeping genes were designed based on all available genome sequences of 5 strains of *Sphingomonadaceae* and 2 strains of the genus *Erythrobacter* (closest phylogenetic relative to the *Sphingomonadaceae*). Subsequently, 96 strains of *Sphingomonadaceae* were isolated from Starnbergersee and Walchensee, and subjected to MLSA analyses. Based on their rRNA gene sequences, these strains fall into 16 different groups. While 16S rRNA gene sequences were identical for certain members of one 16S rRNA group, the concatenated tree of all 9 housekeeping genes revealed the presence of a significantly larger divergence between the different strains. Most significantly, MLSA revealed the presence of distinct subclusters among individual 16S rRNA groups, suggesting different selection pressure between subclusters and the existence of distinct evolutionary units despite the identical or very similar 16S rRNA gen sequences.

MSP08

Phylogenetic and demographic analysis of methicillin-resistant *S.aureus* ST22

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Objectives: Over the past several decades, infections with methicillin-resistant *Staphylococcus aureus* (MRSA) among hospitalized patients have become common.
We aimed to investigate MRSA with the sequence type 22 (ST22) which was first identified in England in the early 1990s and causes an ongoing epidemic of hospital infections in many parts of the world.
To elucidate the mechanisms of emergence and epidemic spread we investigated the microevolution by means of single nucleotide polymorphism (SNP) and reconstructed the demography of ST22.
Methods: SNP analysis was performed by dHPLC for 88 metabolic loci (35,955 bp) within an international strain collection of 111 S.aureus isolates.
Results: We identified 66 bi-allelic polymorphisms (BiP), corresponding to 1 BiP per 0.5 kilobases.
The BiPs included 21 synonymous base substitutions and 45 non-synonymous substitutions.
The 66 bi-allelic polymorphisms were used to construct a minimum spanning tree which was associated in 50 haplotypes among the 111 strains and estimated population size through time.
Conclusion: Results are consistent with an "epidemic population structure", where the epidemic strain within few years has spread into hospitals in multiple countries.
Furthermore we observed that community-acquired MRSA emerged independently from hospital-acquired MRSA.

MSP09

Staphylococcus aureus colonizing anterior nares of Central African Babongo Pygmies

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About 20% of the world´s population is permanently colonized by *S. aureus*. While *S. aureus* colonization is well studied for the developed world, data are missing for developing countries, especially for indigenous people who have often been described to be colonized and/or infected by community-associated (CA) methicillin-resistant *S. aureus* (MRSA) isolates in recent investigations. In this study, we collected and characterized *S. aureus* isolates colonizing the anterior nares of Central African Babongo Pygmies.
Within the German-African network on staphylococci and staphylococcal diseases, nasal swabs were taken from semi-nomadic Babongo Pygmies (n=100) living in the area of Ikobé, Central Gabon. Demographic data and data regarding hospitalisation, travel habits and the use of antibiotics were recorded. *S. aureus* isolates were confirmed by *nuc* gene detection and further characterized pheno- and genotypically.
Approximately 25% of Babongo Pygmies living in the study area were enrolled. 35% of the participants were *S. aureus* carriers, 60% of them were shown to carry penicillin-sensitive *S. aureus* isolates; no MRSA isolates were found. Of note, Panton-Valentine leukocidin (PVL)-encoding genes were detected in more than a half of the isolates tested. The distribution of *spa* types was heterogeneous with *spa* types t1848, t186 and t084 being the most prevalent. Furthermore, a so far unknown *spa* type, t5941, was detected.
While beta-lactam resistance is still rare among Babongo Pygmies including the absence of CA-MRSA, a high prevalence of PVL gene was found. Besides unknown *spa* types, mostly those were identified that have been already described for Northern Hemisphere isolates. *Spa* typing indicated a high heterogeneity of *S. aureus* within this indigenous population.

MSP10

Redesign and Validation of an Existing Scheme for Multiple Locus VNTR Analysis (MLVA) of *Neisseria meningitidis*

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A typing scheme for Multiple Locus Variable-Number-Of-Tandem-Repeats Analysis (MLVA) of *Neisseria meningitidis* was developed in 2006 by Schouls and colleagues based on the then available genome-sequenced strains Z2491, MC58, and FAM18. It was devised as a uniplex PCR method using carboxyfluorescein labeled primers targeting 8 loci and amplicon length determination using a capillary sequencer (e.g. ABI genetic analyzer). We have changed above scheme to a multiplex method targeting 4 loci at a time, thus facilitating full typing with 2 PCR rounds/ strain. This required redesign of primer sequences to curb unwanted interactions, e.g. competing amplification, using a tool named muPlex by Rachlin and colleagues (2005). Also, primers were adjusted to 5 newly available genomic sequences. In total, 8 meningococcal genomes informed primer design, including the 4 carrier isolates α14, α153, α275, α710, and the 4 invasive isolates FAM18, MC58, NMCC053442, Z2491. Furthermore, PCR volume was reduced and primer concentrations adapted to optimize product yield. Redesign was validated using a set of 30 meningococcal strains, analyzed with MLVA in Bilthoven, NL in a previous study (Elias et. al, EID, in revision), and 7 of the 8 above genome-sequenced strains. Results were consistent with known differences between previously analyzed strains and reproduced repeat lengths predicted by genomic sequences. In addition to refinement of laboratory technique, we are working on a method to automatically read out results from sequencer generated files with a script based on the R package seqinR (http://r-forge.r-project.org/projects/seqinr/). This new tool, which will be available for free, may greatly facilitate MLVA type assignment and generation of data for online repositories (e.g. http://www.miru-vntrplus.org). In summary, the enhanced MLVA scheme represents a quick, discriminatory, and reliable typing method for both invasive and carrier isolates of *N. meningitidis*.

MSP11
Classification of bacterial commensals isolated from veterinary specimens

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During the period September 2008 to July 2009 unusual bacterial strains considered to be commensals were isolated from primary cultures of veterinary specimens from patients with different diseases. These isolates were subjected to partial 16S rRNA gene sequencing and the sequences were compared to entries in gene banks. Highest sequence similarities identified the isolates as close relatives of *Acinetobacter spp., Bacillus cereus, Brachybacterium paraconglomeratum, Clostridium colicanis, Comamonas terrigena, Corynebacterium diphtheriae, Kurthia gibsonii, Myroides spp*., '*Phytobacter diazotrophicus*', *Pseudomonas putida, Staphylococcus xylosus*, and *Solibacillus silvestris*, respectively.
If 16S rRNA gene sequence similarities suggested relationships at the species level PCR-based genomic fingerprints of the isolate and the corresponding species were employed to substantiate the indicated relationship. Strains showing only moderate 16S rRNA gene similarity with a recognized species were subjected to a set of phenotypic and genetic examinations including analyses of polar lipids, quinones, polyamines, physiological reactions and genomic fingerprintings and compared to data of related species. Taxonomic conclusions are drawn based on these examinations.<

MSP12
The diversity of methicillin-susceptible and –resistant *Staphylococcus aureus* strains in the Sub-Saharan African Countries

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Background Genetic characterization of *Staphylococcus aureus* is important for better understanding of the global and dynamic epidemiology of this organism as we witness the emergence and spread of virulent and antibiotic-resistant clones. The epidemiologic characteristics of *S. aureus* bacteremia in many African countries are unknown. Methods We genotyped *S. aureus* isolates from West, Southwest and East Africa (n=98 methicillin resistant, MRSA and 507 methicillin susceptible, MSSA) using a combination of multilocus sequence typing, spa typing, toxin gene analysis including Panton-Valentine leucocidin (luk-PV), and SCCmec typing for MRSA. Results MRSA was detected in approx. 20% of the Nigerian isolates and in 10.9% of the Namibian *S. aureus* isolates. In the Eritrean population no MRSA was detected, only two MSSA isolates (ST25). The Nigerian MSSA strains (ST1, ST5, ST15, ST7, ST8, ST25, ST30, ST72, ST80, ST121, and ST508) were heterogeneous as compared to the MRSA clones, ST250 (spa t194, t292) and ST241 (t037) as HA-MRSA and CA-MRSA ST88 (t186). The ST88 strain was resistant to trimethoprim-sulfamethoxazole besides to penicillin and oxacillin. The MSSA strains in Nigeria were mainly resistant towards penicillin, cotrimoxazole and tetracycline. The majority of Namibian MRSA strains belonged to the clonal complex CC8 (ST8, spa-types t104 and t064) and were spread throughout the country. Other less frequent MRSA STs were ST5 (t311), ST26 (t078), ST30 (t318), and ST101 (t056). The resistance profile "penicillin-oxacillin-cotrimoxazole-rifampicin-tobramycin" for ST8 in Namibia was remarkable. The Namibian MSSA strains (ST1, ST5, ST8, ST12, ST15, ST22, ST45, ST30, ST72, ST97, ST121, ST188, ST484 and ST1441) were heterogeneous and were penicillin-resistant and nearly 5% additionally towards cotrimoxazole (SXT) and tetracycline. Conclusion The Namibian MRSA lineage is rather heterogeneous and expressed more toxins as compared to major Nigerian lineage. The spread of MRSA in Eritrea was not confirmed. Our results underscore the importance of routine testing for resistance to SXT in sub-Saharan Africa. Most of the Namibian and Nigerian MRSA and MSSA isolates were luk-PV positive. Future molecular study in East African region is mandatory to understand the entire *S. aureus* population in Sub-Saharan Africa.

MSV01
Microevolution and phylogeography of *Clostridium difficile* ribotype 027

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Objectives: *Clostridium difficile* is the most frequent cause of antibiotic-associated gastrointestinal disease. Incidence rates of *C. difficile* infections (CDI) have recently increased worldwide. Currently, it is unclear to what extend this increase can be attributed to the emergence and spread of more virulent strains designated ribotype 027. To examine this question, we have investigated the phylogenetic relationships among international 027 isolates and monitored the spatial distribution of ribotype 027 within Germany.
Methods: 670 *Clostridium difficile* isolates collected from patients with severe infections in 84 German hospitals 2007 to 2008 were characterised to generate the first nationwide surveillance data. To investigate the microevolution and phylogeography of ribotype 027, 60 representative isolates from an international strain collection were compared by mutation discovery at 283 metabolic loci and MLVA.
Results & Conclusions: Our nationwide surveillance data on *C. difficile* indicated an increased prevalence of ribotype 027 in the South West of Germany and gradual spread into other regions of the country. Metabolic loci revealed extremely low diversity reflecting the recent emergence of *C. difficile* 027. MLVA data defined geographically associated clades of recent epidemic 027 outbreak isolates but dispersed geographic patterns of randomly sampled or historic strains. Whole genome comparisons of historic and epidemic strains will provide additional insights into evolutionary history of ribotype 027 in Germany.

MSV02
Evaluation of Sequence Data for Detection of Phylogenetic Lineages of the *Mycobacterium tuberculosis* Complex

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Pathogens of the *Mycobacterium tuberculosis* complex (MTBC) can be classified into several genotypes/phylogenetic lineages based on genetic polymorphisms like deletions or genotyping data e.g. MIRU-VNTR (Mycobacterial Interspersed Repetitive Units – Variable Number of Tandem Repeats) typing. Recent studies show that sequence-based tools like single nucleotide polymorphisms (SNP) can also discriminate MTBC strains. However, an evaluation in a larger set of clinical isolates has not been done so far.
A population based set of 105 MTBC strains obtained in year 2007 from tuberculosis patients living in Hamburg, Germany, was analyzed to detect and verify genotype specific SNPs in ten genes to establish an SNP-assay for fast genotyping of MTBC strains. All strains were pre-classified based on MIRU-VNTR typing and spoligotyping data. Maximum parsimony trees were calculated based on the generated SNP data and in combination with available data from MIRU-VNTR analysis.
Sequence analysis showed at least one lineage specific SNP for every genotype of the MTBC enclosed in this study. Assignment to genotypes based on SNPs was in full concordance with previous classification based on MIRU-VNTR typing. In addition to known variants, our results reveal further SNPs that can be used for identification of previously not described lineages. The maximum parsimony tree based on the SNPs data reflects exactly the proposed population structure of the MTBC. A combination of SNP and MIRU typing data allows a higher discriminatory power by maintaining phylogenetic information.

Our data demonstrate that sequence variations in a limited number of genes can be used to identify the majority of phylogenetic lineages of the MTBC with a very high specificity. This opens the door for establishing high throughput SNP based assays that complement routine genotyping e.g. based on repetitive elements.

MSV03

Footprints of selection and the gain and loss of genetic information in genomes from methicillin-resistant *Staphylococcus aureus* clonal complex 5

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Objectives: The application of next-generation sequencing technologies enables the study of bacterial population genetic processes and microevolution at the genomic level. We show potential signals of selection and its recent impact using whole genome sequences.
Methods: We compared whole-genome sequences from altogether 25 isolates of MRSA affiliated to a single clonal complex (CC5). 21 of these isolates were sequenced using next-generation sequencing technologies (454 & Solexa). On the basis of genome-wide SNPs discovered from the genome sequences, we were able to reconstruct the phylogeny within CC5.
Results: The presented phylogeny of CC5 has the greatest resolution currently possible. By mapping mobile genetic elements, genomic rearrangements, and accumulated pseudogenes onto this phylogeny, we revealed high dynamics of gene aquisation, inactivation, and loss. In particular prophages show great variability and their speed moving through the staphylococcal population must be very high. Furthermore we could observe atypical patterns of variation disclosing molecular footprints of natural selection.

MSV04

Using a Predictive Model for MRSA-Carriage As a Guide For Rapid Molecular Testing

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In collaboration with a study group consisting of medical and nursing staff from the University Hospital of Würzburg we performed a prevalence study to assess MRSA carriage using molecular testing (BD-GeneOhm™). We screened 3952 (67%) admissions to a defined study area between July 2007 and March 2008. Rate of culturally confirmed carriers among admissions ("bringers") was 2.1%, while rate of initially negative patients recognized as MRSA carriers ("acquirers") during stay was 0.5%. Infection was more common among acquirers (51% vs. 3%); furthermore, most infections in our setting (11 of 16, 69%) were nosocomial. Using logistic regression, we identified risk factors associated with bringer and acquirer status: these were age, referral from nursing home, and emergency admission for bringers, and length of inpatient stay for acquirers. Receiver-operator-curve analysis revealed good conservative performance of the predictive model for bringers ("PMB"); nevertheless, no useful threshold for use in risk-adapted screening could be identified due to high share of misses. E.g. screening above the 90th and 70th percentile of predicted probabilities (i.e. 10% and 30% of admissions) only include 30% and 50% of all bringers, respectively. Overall positive predictive value (PPV) of BD-GeneOhm™ was 0.62 due to culturally unconfirmed results and *S. aureus* harbouring *mecA* deletions. Employing non-linear regression, however, we found an exponential relationship between PBM's predicted probabilities and PPV of the molecular test. Thus, PPV of molecular testing can be raised from 0.62 to 0.89 for patients above 95th percentile of predicted probabilities. In summary, we propose that comprehensive and cost-efficient MRSA screening entails: a) restriction of molecular testing to high-risk groups to increase PPV and acceptability while saving costs, b) testing of lower-risk groups with cultural methods.

MSV05

A localised outbreak of Hemolytic Uremic Syndrome caused by sorbitol-fermenting enterohemorrhagic *Escherichia coli* O157:H- in Germany, 2009

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Hemolytic uremic syndrome (HUS) is the major cause of acute renal failure in childhood [1], and infection by enterohemorrhagic *E.coli* (EHEC) its principal cause. The most frequently isolated EHEC serotype from HUS patients is O157:H7/H-. The inability to ferment sorbitol is a criterion to differentiate this serotype from other *E. coli*. In 1988, Karch et al. [2] first described sorbitol-fermenting (sf) EHEC O157:H-. Thereafter, these sf O157:H- have caused several outbreaks of HUS spread out over large areas in Germany [3].
In 2009, a localised outbreak of HUS was noticed in Lurup, a suburb of Hamburg, Germany. An investigation was initiated in collaboration with state and local health authorities to identify the source and stop the outbreak. A case-patient was defined as person residing in Lurup with clinically diagnosed HUS or infection with sf EHEC O157:H- from July 23rd to August 25 th, 5 children with HUS aged 3 to 9 years, all boys, were ascertained, one child died. In four cases, sf EHEC O157:H- identical with respect to the virulence factors and PFGE-RFLP could be isolated.
By screening of more than 200 contact persons for sf O157:H-, it was possible to identify four more cases with and without symptoms carrying the same clone compared to this from the HUS-patients. In a case-control study, an association between visiting a playground and HUS was found (odds ratio: 12.9, 95% confidence interval: 1.5 + infinity, p-value < 0.02). Environmental samples taken from sand, toys and paddling pool on this playground tested negative, rendering the reservoir of sf EHEC O157 still elusive. The role of playgrounds requires further investigation, particularly because playing in a sandbox was the most important risk factor for sporadic EHEC infection in children in a nationwide case-control study in Germany.

MSV06

Lyme borreliosis in Germany: Preliminary results from a nationwide serological survey in children and adolescents

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Lyme borreliosis (LB), the most frequent tick-borne disease in the northern hemisphere, is a multi-systemic disease caused by Borrelia burgdorferi sensu lato (Bb). Central Europe including Germany is regarded as a highly endemic area; however, data on the extent of endemicity are scanty. The most frequent clinical manifestation is erythema migrans. If untreated other organs can be affected. Sera from the nationwide German Health Interview and Examination Survey for Children and Adolescents (KiGGS) of the Robert Koch Institute (2003 to 2006), were analysed to assess the prevalence of anti Bb antibodies in children and adolescents. Data collected by interview (study participants and/or parents) were used to evaluate possible risk factors.
Methods: Sera were analysed for the presence of anti-Bb IgG by ELISA and reactive sera were subjected to Western Blot analysis. The following variables were used to identify possible risk factors: age, sex, type of residence (geographic area, rural versus urban), pets and recreational activities.

Results: Preliminary analysis of data from 3,759 individuals (1,899 males and 1,860 females; age range: 1 to 17 years) revealed 155 (4.1%) seropositive for anti-Bb IgG antibodies. Interestingly, only two cases are linked to previously diagnosed LB. The likelihood to be seropositive increased by 1.08 per life year. Seropositivity was linked to residence in rural areas or small towns (OR=1.80; 95%CI: 1.29–2.50). Participants with pets had a higher risk to be seropositive (OR=1.43; 95%CI: 1.02–1.99). No geographical differences could be detected between the eastern and western part of Germany.
Conclusions: We present the results from the first nationwide serological study on the prevalence of anti Bb IgG antibodies in children and adolescents indicating that LB is evenly distributed in all parts of Germany and present even in very young children. The results of this study indicate the need to promote health messages to prevent infections with Bb.

MTP01
Physiological adaptation of the membrane proteome to alternative carbon sources in actinomycetes

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Actinomycetes such as *Corynebacterium glutamicum* and *Rhodococcus jostii* RHA1 are catabolically versatile, degrading a broad range of natural and man-made compounds. This versatility is a key advantage during the constant competition for nutrients in natural environments. *C. glutamicum*, an amino acid producer, grows on a variety of monocyclic aromatic components that occur in natural environments either as degradation intermediates of natural products or as pollutants. We quantitatively compared the membrane proteome of *C. glutamicum* during growth on benzoate or glucose using MudPIT technology [1] and metabolic labeling. Adaptation of the membrane exceeded the regulation of substrate uptake systems, including changes in energy and sulfur metabolism, as well as a starvation response [2], consistent with a global response. *R. jostii* RHA1 degrades a wider range of organic compounds including steroids. Recently, the cholesterol uptake system in this bacterium was identified, coded by the *mce4* gene cluster [3]. It has been shown that cholesterol degradation in the related *Mycobacterium tuberculosis* is crucial for the establishment of a chronic infection, and that a *mce4* deletion in *M. tuberculosis* leads to a phenotype with decreased virulence [4]. The membrane proteomes of *R. jostii* RHA1 grown on cholesterol, cholic acid, and pyruvate were compared using MudPIT analysis, with iTRAQ labeling [5] and label-free spectral counting for relative protein quantification. The subunits of the Mce4 complex were detected in cholesterol-grown cells only. Abundance differences were also detected for other proteins from transport and fatty acid metabolism.

[1] Washburn *et al.*(2001)NatBiotechnol 19(3):242-7
[2] Haußmann *et al.*(2009)Proteomics 9(14):3635-51
[3] Mohn *et al.*(2008)JBC 283(51):35368-74
[4] Pandey & Sassetti(2008)PNAS 105(11):4376-80
[5] Ross *et al.*(2004)MCP 3(12):1154-69

MTP02
Hexo- and glucokinase from *Aspergillus fumigatus*: More than redundant enzymes?

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Fungi need to phosphorylate sugars for metabolism by soluble sugar kinases, because sugar transport is independent of a phosphoenolpyruvate-dependent transport system. Thereby, glucose and fructose phosphorylation seems exclusively attributable to glucokinase and hexokinase. The impact of glucokinase was assumed redundant to that of hexokinase, since a deletion did not alter the phenotype of colonies. Here, we characterized the catalytic glucokinase and hexokinase from the opportunistic human pathogen *Aspergillus fumigatus* and showed that both enzymes display different biochemical properties and exhibit different impacts during growth and development. Although both enzymes displayed a similar substrate spectrum, glucokinase was highly specific for glucose activation and hardly phosphorylated fructose. Transcript analyses revealed a high abundance of glucokinase mRNA in resting conidia, whereas hexokinase transcripts were most abundant during vegetative growth. In agreement, a glucokinase mutant showed a delayed germination of conidia in the presence of low glucose concentrations, but morphology of colonies was indistinguishable from the wild type. In contrast, deletion of hexokinase only slightly affected germination but reduced colony growth rates especially on sugar containing media. Interestingly, both enzymes contributed to carbon catabolite repression as revealed from increased isocitrate lyase activity of deletion mutants on glucose/ethanol containing media. Most strikingly, a deletion of both genes was not possible, implying an essential contribution of sugar phosphorylation to cell viability.

MTP03
Evidence for a novel cytochrome *c* nitrite reductase (rHAO) in Epsilonproteobacteria

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The octahaem hydroxylamine oxidoreductase (Hao) catalyzes the oxidation of hydroxylamine to nitrite in the energy metabolism of aerobic ammonia oxidizers. A similar enzyme is predicted in the genomes of several anaerobic Epsilonproteobacteria like *Campylobacter* and *Nautilia* species and it was hypothesized that this multihaem cytochrome *c* might catalyze nitrite reduction in a nitrate-ammonifying pathway [1]. Interestingly such putative reverse Hao (rHao) enzymes lack the critical tyrosine residue that covalently binds the catalytic haem *c* group within a conventional Hao homotrimer.
A genetic strategy will be presented that aimed at the efficient overproduction of a strep-tagged rHao from *Campylobacter fetus* using the closely related non-pathogenic Epsilonproteobacterium *Wolinella succinogenes* as host organism. *W. succinogenes* has a high capacity to produce *c*-type cytochromes using the so-called biogenesis system II for covalent haem attachment. The organism is easy to grow and tools for genetic engineering are available. Using this approach, rHAO from *C. fetus* was produced and purified from the *W. succinogenes* soluble cell fraction by affinity chromatography. Data on the characterization of the enzyme will be presented. Most notably, rHAO was found to reduce nitrite with high specific activity.

[1] Campbell *et al.* (2009) *PLoS Genet* 5(2):e1000362

MTP04
Metabolic responses of novel cellulolytic and saccharolytic soil bacteria to oxygen

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Cellulose is the most abundant biopolymer in terrestrial ecosystems and is degraded by microbial soil communities. However, the diversity and function of prokaryotes that participate in cellulose degradation remains poorly resolved. 16S rRNA ^{13}C-based stable isotope probing was used to evaluate active cellulolytic and saccharolytic bacteria in an aerated agricultural soil. Cellulose, cellobiose, and glucose were converted under anoxic conditions to acetate, butyrate, carbon dioxide, hydrogen, and traces of propionate and iso-butyrate; the production of these fermentation end products was concomitant with the apparent reduction of iron(III). These substrates were mineralized to carbon dioxide under oxic conditions. [^{13}C]cellulose was mainly degraded under anoxic conditions by the *Kineosporiaceae (Actinobacteria)* and cluster III *Clostridiaceae*, and novel clusters within *Bacteroidetes*, whereas degradation under oxic conditions was facilitated by novel family-level taxa of the *Bacteroidetes* and *Chloroflexi*, and a family-level taxon of *Planctomycetes*. Active anaerobic sub-communities in anoxic [^{13}C]cellobiose and [^{13}C]glucose treatments were dominated by cluster I *Clostridiaceae (Firmicutes)*, whereas *Intrasporangiaceae* and *Micrococcaceae (Actinobacteria)* were prevalent in oxic treatments. 28 of the detected 48 taxa did not closely affiliate with known families. Active archaea were not detected in the treatments. These findings suggest that (a) a large uncultured diversity of *Bacteria* was involved in the utilization of cellulose, (b) the active saccharolytic community differed phylogenetically from the active cellulolytic community, (c) oxygen impacted differentially on the activity of taxa, and (d) different redox guilds competed or interacted during cellulose degradation.

MTP05
A novel mechanism of osmosensing: salt dependent protein-DNA interaction in the cyanobacterium *Synechocystis* sp. PCC 6803

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The *de novo* synthesis of compatible solutes in bacteria during an osmotic challenge is regulated by the immediate biochemical activation of preformed enzymes and by activation of gene expression. Whereas the transcriptional response was investigated intensively the mechanisms of enzyme activation in dependence on the osmotic stress situation is still elusive. Here we addressed this problem for the moderately halotolerant cyanobacterium *Synechocystis* sp. PCC 6803 which synthesizes glucosylglycerol (GG) as compatible solute. In an *in vitro* system using the recombinant key enzyme of GG synthesis (GgpS) reversible salt dependent inactivation by addition of *Synechocystis* cell extract was found. We excluded a proteinaceous inhibitory factor and confirmed that nucleic acids block GgpS activity. Inhibition of GgpS was shown to be due to salt dependent binding of nucleic acids proven by gel mobility assays. Upon DNA binding accessibility of the enzyme for proteolytic degradation was altered, substrate binding inhibited and enzyme activity was slowed down. We co-purified GgpS bound to DNA from *Synechocystis* cells after cross-linking indicating that the enzyme can interact with DNA under *in vivo* conditions as well. Constitutive expression of the *ggpS* gene revealed alterations in GG synthesis in *Synechocystis* cells that are in agreement with the proposed novel mechanism of GgpS activity regulation by the unspecific, salt dependent binding of the enzyme to nucleic acids.

MTP06
Global regulation of nitrogen metabolism in *Streptomyces coelicolor* M145

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Streptomyces coelicolor M145 is a Gram-positive bacterium and a model organism for antibiotic-producing streptomycetes. In its environment, the soil-dwelling organism has to cope with permanent nutrient limitation. Especially nitrogen is a limited bioelement in soil. Nitrogen is required as building block of almost all components in the cell, which on the other hand are also needed to synthesise secondary metabolites. Streptomycetes possess a complex sensing and signalling system which adapts the whole bacterial metabolism to the different requirements caused by variations in nitrogen supply. Nitrogen assimilation and metabolism is regulated on transcriptional as well as on posttranslational level.
Glutamine synthetases (GS) are key enzymes in nitrogen assimilation, mediating the incorporation of ammonium into glutamate resulting in synthesis of glutamine. *S. coelicolor* contains two functional glutamine synthetases GSI and GSII. By analysing the nitrogen metabolism we could show that the GSI is expressed constitutively and functions as the major GS, whereas the GSII is expressed only under severe nitrogen limitation. On posttranslational level the activity of GSI is regulated via (de)adenylylation by the adenylyl transferase GlnE. Whilst the regulation of GlnE in *Enterobacteriaceae* is well characterised, the regulation in *S. coelicolor* was not known for a long time. Now, recent results of bacterial two-hybrid studies indicate that GlnE is regulated by the GS-like protein GlnA2.
Besides that, nitrogen metabolism is regulated on transcriptional level by the global transcriptional regulator GlnR. We could show that GlnR controls transcription of at least 15 genes involved in utilisation of different nitrogen sources as well as in ammonium assimilation and supply.

MTP07
Insights into the utilization of elemental sulfur by different phototrophic sulfur bacteria

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Phototrophic sulfur bacteria are generally able to use elemental sulfur as an electron donor for anoxygenic photosynthesis [1], but the process of mobilization and uptake of elemental sulfur is only poorly understood. In this study, we investigated elemental sulfur utilization in *Allochromatium vinosum* and *Thiocapsa roseopersicina*, two members of the *Chromatiaceae* that transform elemental sulfur into intracellular sulfur globules. Furthermore, *Halorhodospira halophila* was investigated. This organism belongs to the *Ectothiorhodospiraceae* and forms extracellular sulfur globules during elemental sulfur metabolism. Elemental sulfur is mainly a mixture of *cyclo*-octasulfur and polymeric sulfur [1]. Therefore, we investigated the influence of sulfur speciation on the availability of elemental sulfur using X-ray absorption near edge structure (XANES) spectroscopy. The organisms strongly preferred polymeric sulfur chains as a substrate and did not use *cyclo*-octasulfur. Furthermore, our results showed that tight cell-sulfur contact was essential for elemental sulfur utilization in *Alc. vinosum*, *Tca. roseopersicina* and *Hlr. halophila*. In case of *Alc. vinosum*, outer membrane proteins of photoorganoheterotrophically grown cells and cells grown photolithoautotrophically on sulfide and sulfur were isolated and the cell surface charge of the organism during growth with elemental sulfur was investigated. In addition, we searched for soluble intermediates of sulfur mobilization, again using XANES spectroscopy.

[1] Frigaard N-U & Dahl C (2009). In *Advances in Microbial Physiology*, Poole RK (eds.), London: Academic Press, pp. 105-200.

MTP08
Methanotrophy under extreme conditions: Biochemistry and physiology of *Methylacidiphilum fumariolicum* SolV

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Methane is a potent greenhouse gas, which is emitted to the atmosphere both from natural and anthropogenic sources. Aerobic methanotrophic bacteria utilizing methane are commonly found at the oxic/anoxic interfaces within aquatic sediments, landfills etc. In addition, natural geological sources such as mud volcanoes, may also act as sinks for methane. Until recently, all aerobic methanotrophs could be phylogenetically placed into the Alpha- and Gamma-proteobacteria. In 2007–2008 the isolation of thermoacidophilic methanotrophs from geothermal areas was described. These isolates represented a distinct lineage within the bacterial phylum Verrucomicrobia for which recently the new genus name *Methylacidiphilum* was proposed. It was the first time that the widely distributed Verrucomicrobia phylum, from which most members remain uncultivated, were coupled to a geochemical cycle.
Methylacidiphilum fumariolicum strain SolV isolated from the very acidic and hot soil of the Solfatara volcano (Naples, Italy) could grow under oxygen limitation on methane as the sole source of energy (yield 6.4 g DW per mol of methane). Strain SolV showed major differences with the classical methanotrophs, e.g. extreme acid tolerance, absence of typical membrane structures, distinct enzymes of the methane oxidation and carbon fixation pathways. Initial analysis of a draft genome (220 large contigs, 2.33 Mbp, coverage 35.5-fold) made clear that the diagnostic genes of the ribulose-monophosphate pathway seem to be absent. Many genes of the C1 metabolism were, indicating that *M. fumariolicum* SolV may use a new combination of the serine, tetrahydrofolate and ribulose-1,5 bisphoshosphate carboxylase pathways for carbon assimilation. Conversion of formaldehyde seems to be tetrahydrofolate-dependent pathway or by formaldehyde dehydrogenase. Methanol dehydrogenase activity could be demonstrated but the gene cluster encoding this activity seems to be rather different compared to classical methanotrophs. None of the subunits of sMMO were found. Three complete pmoCAB operons were identified. Phylogenetic analysis of the pmoA genes showed that pmoA1 and pmoA2 are highly similar but represent a new branch as is also the case for the third gene (pmoA3).

MTP09
The Role of the β-Barrel Protein MtrB in the Dissimilatory Metal Reduction of *Shewanella oneidensis* MR-1

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The outer membrane β-barrel protein MtrB is an essential element of the electron transport chain to insoluble iron species in *S. oneidensis*. One major hypothesis concerning the function of this protein is that MtrB is the core of an outer membrane (OM) spanning complex with the OM cytochrome OmcB and the periplasmic cytochrome MtrA. This complex might bridge the outer membrane in a way that electron transfer from the periplasm to the extracellular metal oxides can occur [1].
The insertion of β-barrel proteins into the OM depends usually on a machinery composed out of soluble and membrane-anchored subunits. The periplasm contains molecular chaperons, as well as folding catalysts [2]. For the assembly of β-barrel proteins into the OM, a protein designated as Omp85 is said to be essential [3]. We observed that the presence of the general machinery for β-barrel protein insertion is not sufficient for stability and correct localization of MtrB. The construction of several markerless deletion mutants and subsequent Western-blotting analysis revealed that there is a direct involvement of the periplasmic protein MtrA in the expression of MtrB. Since mRNA stability of *mtrB* is not affected by a *mtrA* deletion we postulate that a periplasmic interaction between MtrA and MtrB is necessary for the integrity of MtrB.
Interestingly, MtrA and MtrB single deletion mutants share almost the same phenotype and are not able to reduce ferric iron. These phenotypes are believed to rely on the function of these single proteins in electron transfer. Regarding our data, it is obvious that an *mtrA* mutation results concomitantly in a *mtrB* loss of function phenotype. Hence, past results and the general function of MtrA in dissimilatory iron reduction should be regarded carefully.

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MTP10
Pathway of anaerobic methane oxidation coupled to nitrite reduction

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Anaerobic oxidation of methane coupled to denitrification has been demonstrated in several freshwater enrichment cultures dominated by bacteria belonging to the NC10 phylum [1],[2],[3]. At the moment the role of archaea is unclear [3], and in some cultures they were shown to be dispensable[2], [4]. Our research has therefore focused on the elucidation of the genome and biochemical pathways of the NC10 bacteria.
From the metagenome of the mixed enrichment culture the complete 2.75 Mb genome sequence of the dominant NC10 bacterium could be assembled. Although grown under anaerobic, denitrifying conditions, it contained the full enzymatic pathway of aerobic methane oxidation. All genes and gene products were also represented in the transcriptome and proteome of the culture. The denitrification pathway, on the other hand, was found incomplete, with notably nitrous oxide reductase missing. Activity experiments using stable isotopes demonstrated that nitrous oxide was indeed not an intermediate in the production of dinitrogen from nitrite. Based on these paradoxical findings, a new biochemical mechanism was proposed and stable isotope experiments are currently conducted to experimentally verify this hypothesis.

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[3] Hu, *et al.* (2009) Enrichment of denitrifying anaerobic methane oxidizing miroorganisms. Environmental Microbiology Reports 1 (5): 377-384.

MTP11
The role of selenophosphate for selenoprotein synthesis in archaea

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Biosynthesis of selenocysteine, the 21st proteinogenic amino acid, starts by charging dedicated tRNA ($tRNA^{sec}$) with serine in all three domains of life, Bacteria, Eukarya and Archaea, but differences exist between the subsequent step(s) in bacteria and eukaryotes/archaea. While bacteria convert seryl-$tRNA^{sec}$ to selenocysteinyl-$tRNA^{sec}$ in one step employing selenocysteine synthase (SelA) which uses selenomonophosphate as the selenium donor, eukaryotes and archaea phosphorylate seryl-$tRNA^{sec}$ to *O*-phosphoseryl-$tRNA^{sec}$ employing a kinase before converting it to selenocysteinyl-$tRNA^{sec}$ using *O*-phosphoseryl-$tRNA^{sec}$:selenocysteine synthase (SepSecS). As phosphorylating the seryl-moiety could render the requirement to activate selenium obsolete, the role of selenophosphate and the enzyme providing it, selenophosphate synthetase, in archaeal selenoprotein synthesis was addressed by mutational analysis. The gene encoding a homolog of eukaryal selenophosphate synthetase was deleted in *M. maripaludis* resulting in a strain unable to synthesize selenoproteins. Complementing with *selD*, encoding selenophosphate synthetase of *E. coli*, restored selenoprotein synthesis, demonstrating that the deleted gene encodes selenophosphate synthetase and that selenophosphate is the *in vivo* selenium donor for selenoprotein synthesis of this organism. We also showed that in *M. maripaludis* selenophosphate synthetase itself is a selenoprotein, thus possibly functioning as an autoregulatory checkpoint for balancing the ratio of cellular selenocysteinyl-$tRNA^{sec}$ and selenoprotein mRNA in response to availability of selenium.

MTP12
The crystal structure of an [Fe]-hydrogenase-substrate complex reveals the framework for H_2 activation

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[Fe]-hydrogenase is one of the three different types of enzymes known to activate molecular hydrogen (H_2). It catalyzes the reversible transfer of a hydride ion from H_2 to the C14a carbon atom of methenyl-tetrahydromethanopterin (methenyl-H_4MPT^+), resulting in methylene-H_4MPT, which is an intermediary step of methanogenesis in many methanogenic archaea. The homodimeric protein is built up of one central and two peripheral globular domains, and harbors a unique iron-guanylylpyridinol (FeGP) cofactor in the active site located between each domain. The low-spin iron(II) ion of FeGP cofactor is octahedrally coordinated by two carbon monoxide ligands, one C(O)-CH_2-, one S-CH_2- (Cys176), a sp^2-hybridized nitrogen atom on the pyridinol ring, and a solvent molecule.
In order to gain insight into the H_2 activation mechanism, we determined the crystal structure of C176A mutated holoenzyme in complex with its substrate, methylene-H_4MPT, in this study. The overall structure of the binary complex is in an open form. Due to the mutation of the cysteine residue, one dithiothreitol molecule supplied to the crystallization solution was bound to the iron center of FeGP cofactor buried in the peripheral domain. The iron center is at a distance of 9 Å away from C14a of the substrate bound to the central domain, indicating that it is obviously too far for hydride transfer. Therefore, its closed form was modeled by superimposing the respective domains onto those of the apoenzyme structure in a closed form that was experimentally determined previously. In this model, the iron center lies in front of the *Re*-face of the pterin ring within 3 Å from C14a, which could be a reasonable distance for hydride transfer from H_2 captured at the iron site. These results suggest that such a large movement of the peripheral domain is required to form a catalytically productive conformation in the reaction.

MTP13
Initial steps in anoxic testosterone degradation by *Steroidobacter denitrificans*

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Steroid compounds have many important physiological activities in higher organisms. In addition, testosterone and the related steroids are important environmental contaminants that disrupt endocrine systems of animals and aquatic species. The degradation of steroids is challenging in term of its complex chemical structure, especially under anoxic conditions. Recently, we have proposed initial steps in anoxic cholesterol catabolic pathway using a β-proteobacterium, *Sterolibacterium denitrificans*, as the model organism (Chiang et al., 2007). In this study, a denitrifying γ-proteobacterium, *Steroidobacter denitrificans*, able to grown anaerobically on a variety of steroids as the sole carbon and energy source was used as the model organism. Herein, the optimal conditions, such as pH and artificial electron acceptors, for the production of testosterone-derived intermediates have been studied. In addition, we demonstrated that the enzymes participating in the initial steps of anoxic testosterone catabolism are cytoplasmic or periplasmic proteins. We then purified and identified the initial intermediates involved in anoxic testosterone degradation by Steroidobacter denitrificans. From these results, the initial steps in the anoxic pathway of testosterone degradation is proposed.

MTP14
The unprecedented supercomplex of oxoglutarate- and pyruvate-dehydrogenase in the central metabolism of *Corynebacterium glutamicum*

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Oxoglutarate dehydrogenase (ODH) and pyruvate dehydrogenase (PDH) complexes catalyze key reactions in central metabolism, and in *Corynebacterium glutamicum* an unusual supercomplex of AceE (E1), AceF (E2) and Lpd (E3) together with OdhA exists. OdhA is an apparent fusion protein of additional E1 and E2 domains and *odhA* orthologs are present in all *Corynebacterianeae* encluding *Mycobacterium tuberculosis*. We here show that deletion of any of the individual domains of OdhA results in loss of ODH activity, whereas PDH activity is not affected. By contrast, deletion of AceE resulted in absence of PDH activity, whereas surprisingly deletion of AceF disabled both PDH and ODH activity. We furthermore demonstrated that OdhA is non-lipoylated and therefore suggested that lipoylated AceF might perform both dihydrolipoyl transacetylase and dihydrolipoyl transsuccinylase activity within the complex. However, *in vitro* assays with isolated proteins and free dihydrolipoamide showed that AceF acts specifically on acetyl-CoA, and OdhA has acyltransferase activity too, acting on succinyl-CoA only. Further support for transsuccinylase activity of non-lipoylated OdhA came from muteins OdhA-T294A, OdhA-H352C, and OdhA-Q356D with mutations based on the structure of the active center of E2p of *Azotobacter vinelandii*. As a result the E2-activity was strongly influenced. Notably the T294A mutation fully prevented E2-activity (while maintaining E1-activity) thus strengthening the view derived from E2p of *A. vinelandii* that T294 of OdhA is required for transition state stabilization during acyl-group transfer. In summary, these data give rise to the view that *C. glutamicum* possesses a so far unprecedented supercomplex where OdhA catalyzes specifically the E1 and E2 reaction to convert 2-oxoglutarate to succinyl-CoA, and AceE and AceF catalyze specifically the E1 and E2 reaction to convert pyruvate to acetyl-CoA. The E2 reaction of OdhA fully relies on the lipoyl residues provided by AceF.

MTP15
Anaerobic O-Demethylation: Reductive Activation of Corrinoid Enzymes

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Corrinoid enzymes are mainly divided into three subfamilies – the adenosylcobalamin-dependent mutases and eliminases, the dehalogenases and the cobalamin-dependent methyltransferases. The latter enzyme family includes bacterial *O*-demethylases, methyltransferase systems of methylotrophic archaea, and B_{12}-dependent methionine synthases. In these methyltransferase systems, the corrinoid cofactor acts as a methyl group carrier. To bind a methyl group from a donor, e.g. phenyl methyl ethers, methanol, methyl amines or methyl tetrahydrofolate, the corrinoid cofactor has to be in its superreduced cob(I)alamin form. Inadvertent oxidation of the superreduced corrinoid cofactor leads to the formation of inactive cob(II)alamin. To "reactivate" (= reduce) the corrinoid cofactor different strategies were evolved.
We investigated the reductive activation of corrinoid enzymes in bacteria with the focus on anaerobic *O*-demethylation in *Acetobacterium dehalogenans* and *Desulfitobacterium* spec.. The key enzyme involved in corrinoid activation of different *O*-demethylase systems in *A. dehalogenans* is the Activating Enzyme, a 2Fe/2S protein and a member of the COG3894-protein family, which was identified for the first time as a Reductive Activator of Corrinoid Enzymes (RACE). RACE-proteins are found in Bacteria and Archaea. The corresponding genes are usually located within or close to operons encoding B_{12}-dependent methyltransferase systems. In most cases the function of these putative RACE-encoding genes has not been proven. The aim of our studies is to elucidate the function of different RACE-proteins as well as to gain insights in the activation mechanisms of corrinoid enzymes.

MTP16
Anaerobic *O*-Demethylation: Characterization and Mutagenesis of the Methyl Transferase I of *Acetobacterium dehalogenans*

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Anaerobic bacteria such as *Acetobacterium dehalogenans* are able to grow on a variety of phenyl methyl ethers. Key enzymes in the utilization of these substrates are the *O*-demethylases, which mediate the cleavage of the ether bond and the transfer of the methyl group to tetrahydrofolate. The first step of the ether cleavage is catalyzed by the zinc containing methyl transferase I (MT I) which transfers the methyl group to a super-reduced corrinoid protein. *A. dehalogenans* has at least three different methyl transferases I, MT $I_{veratrol}$, MT $I_{vanillate}$ and MT $I_{syringate}$, which differ with respect to their substrate spectra. By deletion or exchange of the N-termini of MT $I_{vanillate}$ and MT $I_{veratrol}$ the influence of this part of the peptide chain on the substrate specificity was studied. In addition, the zinc binding motifs were identified using site-directed mutagenesis.

MTP17
Anaerobic metabolism of toluene in denitrifying, Fe(III) and sulfate reducing bacteria

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Anaerobic degradation of toluene is initiated by an unusual addition reaction of the toluene methyl group to the double bond of a fumarate cosubstrate to form the first intermediate (*R*)-benzylsuccinate. This reaction is catalyzed by the glycyl radical enzyme benzylsuccinate synthase, encoded in the toluene-inducible *bss*-operon. The *bss*DCAB genes code for the subunits of benzylsuccinate synthase (BssA, B and C) and an additional activating enzyme (BssD). Genetic analysis of this operon show high similarity between sulfate reducing, Fe(III)-reducing and denitrifying bacteria.
(*R*)-benzylsuccinate is then further degraded via several steps to benzoyl-CoA and succinyl-CoA in a modified ß-oxidation pathway. It appears that the genes encoding the enzymes of this pathway are arranged in very similar operons in Fe(III)-reducing and denitrifying bacteria capable of degrading toluene. Genes and gene products of these *bbs*-operons are analyzed genetically and biochemically to establish the conservedness or differences in energy metabolism between different physiological groups of toluene degrading bacteria.

MTP18
The 3-Hydroxypropionate Bicycle of *Chloroflexus aurantiacus* and the Distribution of its Key Enzymes among other Bacteria

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The phototrophic bacterium *Chloroflexus aurantiacus* uses the 3-hydroxypropionate bicycle for autotrophic CO_2 fixation. The whole bi-cyclic pathway results in pyruvate formation from three molecules of bicarbonate and involves 13 enzymes, catalyzing 19 reaction steps [1]. A complete or even a rudimentary 3-hydroxypropionate bicycle allows co-assimilation of small organic compounds, which may be advantageous in oligotrophic aquatic habitats. Yet, the distribution of the characteristic enzymes/genes of the 3-hydroxypropionate bicycle is limited. The whole bicycle may also operate in *Chloroflexus aggregans*, *Roseiflexus castenholzii*, and *Roseiflexus sp.* RS-1 (Chloroflexaceae), which contain all postulated genes of the cycle. The involvement of key enzymes of the 3-hydroxypropionate bicycle in the carbon metabolism under heterotrophic growth conditions was demonstrated in *Chloroflexus aurantiacus*.
Erythrobacter sp. NAP1 and some phototrophic gamma-Proteobacteria (NOR5-3, NOR51-B) harbor the Chloroflexus-type genes required for acetyl-CoA conversion to succinyl-CoA, while lacking other genes of the 3-hydroxypropionate bicycle. Likewise, the heterotrophic *Congregibacter litoralis* and *Nitrococcus mobilis* and the photolithoautotrophic *Chloroherpeton thalassium* contain the propionyl-CoA synthase gene only. We studied the functioning of these enzymes in a marine gamma-proteobacterium (NOR5-3) and *Congregibacter litoralis*. These bacteria may use parts of the 3-hydroxypropionate bicycle for the mixotrophic assimilation of acetate, 3-hydroxypropionate and/or propionate, and succinate.

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MTP19
Modelling the glycolytic pathway in *Streptococcus pyogenes*

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Streptococcus pyogenes (group A streptococci, GAS) is an important human pathogen causing a wide variety of diseases ranging from mild superficial infections of skin and throat up to life threatening systemic or invasive diseases and sequelae. GAS is equipped with many virulence factors allowing the bacteria to infect and persist within the host cell. During infection GAS are exposed to different host environments with changing nutritional conditions potentially affecting the central metabolism of the bacteria. In order to understand the central metabolism of *S. pyogenes* and to be able to compare it to other lactic acid bacteria, we set up a detailed, quantitative model of glycolysis in this organism. The model was based on literature, data as found in the SABIO RK database, and on experimental data. Experimental data were measured in glucose limited continuous cultures as well as glucose pulse experiments. Moreover, individual glycolytic enzymes of *S. pyogenes* M49 were cloned and kinetic parameters (Km values, allosteric regulation) were measured for these *in vitro*. It turned out that the allosteric regulation of glycolysis differs in *S. pyogenes* in comparison to other lactic acid bacteria (*Lactococcus lactis, Enterococcus faecalis*). Especially the role of inorganic phosphate in allosteric regulation of lactate-dehydrogenase activity is contrary to the situation in *L. lactis*. In summary, kinetic models of *S. pyogenes* as human pathogen allow us to further define the similarities and differences between pathogenic and non-pathogenic lactic acid bacteria and may uncover existing links between central metabolism and virulence.

MTP20
Methanol assimilation in *Methylobacterium extorquens* AM1: Regulation and limiting steps

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Methylobacterium extorquens AM1 is an aerobic facultative methylotrophic α-Proteobacterium that uses reduced one-carbon compounds such as methanol as sole carbon and energy source, which makes this organism an interesting target for biotechnological applications.
In *M. extorquens* the assimilation of C1 compounds proceeds via the serine cycle producing acetyl-CoA[1]. This cycle requires continuous regeneration of glyoxylate. It was demonstrated that the glyoxylate regeneration proceeds via the recently established ethylmalonyl-CoA pathway,[2,3,4] which is used for the further assimilation of acetyl-CoA.
The connection of the ethylmalonyl-CoA with the serine cycle represents an elegant solution of methanol assimilation where methanol and carbon dioxide contribute nearly equally to cell carbon.
The regulation of enzymes involved in C1 assimilation was studied on the level of enzymatic activities in cell extracts of *M. extorquens* grown on methanol (C_1), acetate (C_2) and succinate (C_4). The enzymes were strictly upregulated on the transcriptional level being highest in the order methanol >> acetate > succinate grown cells. Moreover, we identified the limiting reaction steps of methanol assimilation, surprisingly all involved in the serine cycle, which could be helpful to improve strains for industrial applications.

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MTP21
A methylaspartate cycle in halobacteria: a third strategy of acetate assimilation

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Many organic substrates enter metabolism via acetyl-CoA. Their assimilation depends on the presence of a special pathway responsible for the synthesis of all cell constituents from acetyl-CoA. Up to now, two such pathways are known, namely the glyoxylate cycle and the ethylmalonyl-CoA pathway. In search of the function of a biotin dependent carboxylase in halobacteria, we discovered that *Haloarcula marismortui* possesses a third pathway, which starts with acetyl-CoA conversion to glutamate via reactions of the citric acid cycle. Glutamate is transformed to mesaconate via methylaspartate. Further metabolism of mesaconate via 3-methylmalyl-CoA leads to propionyl-CoA and glyoxylate. Biotin-dependent propionyl-CoA carboxylation finally yields succinyl-CoA, and glyoxylate condensation with another acetyl-CoA molecule gives rise to malate. In summary, an additional C_4 compound is made that can be used for biosynthesis. Tightly connected with nitrogen metabolism, this cycle represents an unusual strategy of acetate assimilation. Its emergence by combination of reactions belonging to different metabolic processes exemplifies the concept of evolutionary tinkering introduced by François Jacob more than 30 years ago.

MTP22
Overexpression of the monoterpene transforming enzyme linalool dehydratase-isomerase of *Castellaniella defragrans* 65Phen

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Monoterpenes, natural occurring hydrocarbons, are well-known as essential oils and fragrances. Microorganisms degrade these hydrocarbons under aerobic and anaerobic conditions. *C. defragrans* 65Phen, a monoterpene-mineralizing denitrifying Betaproteobacterium, initiates the degradation of myrcene by a novel enzyme, a linalool dehydratase-isomerase. Myrcene is converted by addition of a water molecule into the tertiary alcohol linalool and further isomerised into the primary alcohol geraniol. The enzyme was purified and the corresponding gene identified (Brodkorb et al., submitted). The coding sequence for the enzyme was amplified from genomic DNA of *C. defragrans* 65Phen by PCR and cloned into the overexpression vector pET42a-c. *E. coli* BL21(DE3)Star® containing the plasmid overexpressed the desired enzyme by induction via IPTG. Enzymatic activity of the enzyme was observed in crude extracts, indicating a functional protein as product of the overexpression. According to SDS-PAGE analysis, the quantity of the linalool dehydratase-isomerase was 40% of the total protein content of the cell. Although the gene codes for a precursor protein with a periplasmatic location. The actual molecular mass of the functional and mature enzyme was identified by SDS-PAGE. Therefore, the betaproteobacterial enzyme was exported and cleaved with the translocation machinery of *E. coli*.
The stability of the enzyme activity was characterized in the presence of denaturing reagents. Urea concentrations of up to 4 M impaired the enzyme activity, but did not inhibit it completely. This coincidence with an unfolding intermediate observed in denaturing gradient gel electrophoresis. The physiological relevance of a slow unfolding is discussed with respect to the natural hydrocarbons as substrate.

MTP23
Characterisation of putative D-serine-Transporters in *Staphylococcus saprophyticus*

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The amino acid D-serine occurs in relatively high concentrations in human urine and has a bacteriostatic or toxic effect on many non-uropathogenic bacteria. *Staphylococcus saprophyticus* is an important cause of urinary tract infections in young women. It possesses the cytoplasmatic enzyme D-serine-deaminase DsdA, which is found in many uropathogens. It enables *S. saprophyticus* to cleave D-serine into pyruvate and ammonia. This is probably a factor that makes it possible to colonize the urinary tract. In contrast to *E. coli* the dsdA-operon of *S. saprophyticus* does not encode a specific D-serine-transporter, but there are three genes in the genome of *S. saprophyticus* that encode for putative D-serine-transporters. It is therefore our approach to create knock-out mutants of these genes in order to characterise the D-serine-transport of *S. saprophyticus* via a radioactive labelled D-serine uptake assay. Alternatively the uptake could be measured by analysing the supernatant of the culture by NMR-spectroscopy. The results will presumably offer us more insights in to the physiology of virulence of *S. saprophyticus*.
Because selective markers for *S. saprophyticus* are limited, we searched for additional resistance determinants in clinical isolates. We found a tetracycline resistant *S. saprophyticus* strain and isolated and sequenced a plasmid, which belongs to the pT181-family that is usually found in *Staphylococcus aureus* and *S. epidermidis*. It contains the *tet*K gene encoding for a tetracycline efflux pump, which may be used as a selective marker for the generation of mutant-strains.
It must be noted that one of the most important cause of urinary tract infections in young women is frequently Tetracycline-resistant. 33 of our 323 clinical *S. saprophyticus* isolates apear to be resistant against Tetracycline. These stains possess the *tet*K-gene most likely on a pT181-family plasmid.

MTP24
Development of a chemically defined medium for *Staphylococcus saprophyticus* and other staphylococci

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A basic requirement for the analysis of metabolism of staphylococci is the development of a chemically defined medium. For *Staphyloccoccus aureus* such media already exist, but these media are not suitable for coagulase-negative staphylococci. Here we describe a chemically defined medium that can be used for the study of many staphylococci. As we are interested in the metabolism and the mechanism of virulence of *Staphylococcus saprophyticus*, the medium has been optimized for this species.
S. saprophyticus is the only species of the staphylococci that is typically uropathogenic. Several virulence factors have already been identified, but it has been suggested that also certain metabolic activities may contribute to virulence. The genome of *S. saprophyticus* is the only one of all sequenced staphylococci which posseses a D-serine deaminase, an enzyme which converts D-serine to pyruvate and ammonia. Interestingly, this enzyme is also present in other uropathogenic bacteria like *E. coli* (UPEC).The amino acid D-serine is present in relatively high concentrations in human urine and is toxic or bacteriostatic to several non-uropathogenic bacteria. Therefore the uncommon ability to degrade D-serine may play an important role for the virulence of uropathogens. By systematically adding or removing components from the defined medium we identified pantothenic acid as essentiell for growth and could show that *S. saprophyticus* is able to use D-serine as the sole carbon and energy source. It was shown for *E. coli* that the toxicity of D-serine is due to the inhibition of the biosynthesis of pantothenic acid. As we showed that *S. saprophyticus* requires pantothenic acid for growth regardless of the presence of D-serine, we conclude that the toxic action of D-serine has a different target in *S. saprophyticus*.

MTP25
Development of a random mutagenesis system for *Methanococcus maripaludis*

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Methanococcus maripaludis, a marine mesophilic archaeon, uses H_2+CO_2 or formate as sole carbon and energy source. Utilization of the latter proceeds by oxidation of formate to CO_2 by formate dehydrogenase and subsequent reduction of CO_2 to CH_4 in the methanogenesis pathway. To identify factors required for formate-dependent energy metabolism we developed an *in vitro* system for random mutagenesis based on the *Haematobia irritans Himar*1 transposon. The respective transposase was heterologously produced in *E. coli* and purified. Strikingly, by incubating the transposon (located on a plasmid) with the purified transposase in the absence of chromosomal *M. maripaludis* DNA, and transferring this mixture into *M. maripaludis*, transposon-induced mutants of *M. maripaludis* could be isolated. These mutants were shown to carry random and stable transposon insertions in their genome suggesting activation of the transposon in *trans*. Random mutants we screened for those unable to grow on formate and the position of transposon integration was subsequently determined. Since two isoforms of formate dehydrogenase are encoded in the genome, it was surprising to find that all mutants unable to grow on formate carried transposons either within or in the vicinity of MMP1297 and MMP1298, coding for the subunits of formate dehydrogenase 1. Vicinal genes disrupted by transposons were those for a putative formate transporter, a two-component system and two proteins of unknown function, strongly suggesting their critical involvement in formate metabolism. Interestingly, the genes for formate dehydrogenase 2 (MMP0138 and MMP0139) were not found affected, indicating that they are either not expressed or their products are not involved in formate metabolism.

MTP26
Crystal structures and biochemical characterization of the GacH receptor of *Streptomyces glaucescens* GLA.O involved in acarbose metabolism

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GacH is the substrate-binding component (receptor) of the putative oligosaccharide ATP-binding cassette (ABC) transporter GacFG, encoded in the acarbose biosynthetic gene cluster (*gac*) from *Streptomyces glaucescens* GLA.O. The characteristic core structure of the secondary metabolite acarbose, the pseudo disaccharide acarviose, consists of a C7 cyclitol bound via an imino bridge to 4-amino- 4,6-dideoxyglucose and is therefore responsible for the inhibitory effect of alpha-glucosidases of competitors in the natural habitat. Thus, acarbose is industrially used for the treatment of patients suffering from diabetes mellitus type II. In the context of the proposed function of acarbose as a "carbophor", the transporter, in complex with a yet to be identified ATPase subunit, is supposed to mediate uptake of longer acarbose homologs as well as of acarbose for recycling purposes [1].
Binding assays with radiolabeled maltose and isothermal titration calorimetry identified GacH as a maltose/maltodextrin binding protein with considerable binding activity for acarviosyl- 1, 4 -maltose- 1, 4 -glucose- 1, 1 -glucose (component 5C) but only low affinity for acarbose (acarviosyl- 1, 4 -maltose). In contrast, maltose-binding protein of *Salmonella typhimurium* (MalE) displayed high-affinity acarbose binding. Furthermore, we have determined the crystal structures of GacH in complex with acarbose, component 5C and maltotetraose as well as in the unliganded form. The structures revealed that GacH does not specifically bind the acarviosyl group but displays specificity towards binding of the maltose moiety in the inner part of its binding cleft. Our results provide first biochemical and structural evidence for the proposed role of GacHFG in acarbose metabolism.

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MTP27
Catalytic mechanism of the dissimilatory sulfite reductase from the hyperthermophilic archaeon *Archaeoglobus fulgidus*

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Dissimilatory sulfite reductase (dSiR) uses a siroheme-[4Fe-4S] cluster coupled cofactor to catalyze the reduction of sulfite to sulfide - one key process within the global biogeochemical sulfur cycle. We determined the structure of dSir from the strictly anaerobic hyperthermophilic archaeon *Archaeoglobus fulgidus* which revealed an oligomeric state and the presence of four siroheme-[4Fe-4S] sites, and four extra [4Fe-4S] clusters [1]. To obtain insight into the catalytic reaction of dSir, we solved the X-ray structures of the dSir-sulfite, -sulfide, -nitrite, -CO and -CN- complexes complemented by HPLC, kinetic studies and by *in vitro* product analysis [2]. In contrast to the assimilatory sulfite reductases (aSir) dSir from *A. fulgidus* metabolizes and produces thiosulfate and trithionate *in vitro*, which could be structurally rationalized by a wide entrance to the sulfite binding pocket. In particular, the 1.8 Å resolution structure of the dSir-sulfite complex provides an accurate picture of the siroheme-Fe bound sulfite conformation, the polypeptide-sulfite oxygen interactions and the binding sites of a large number of solvent molecules that extend from two sulfite oxygens to the entrance of the active site cleft. Some aspects of substrate and intermediate binding significantly deviate from those of aSir from *E. coli* [3].

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MTP28
SACOL0731, a putative catabolite control protein C (CcpC) homolog of *Staphylococcus aureus*

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Carbon catabolite repression (CCR) in bacteria is a widespread, global regulatory phenomenon that allows modulation of the expression of genes and operons involved in carbon utilization and metabolization in the presence of preferred carbon source(s). In CCR, the presence of a preferred carbon source represses the expression of genes and operons whose products are involved in the metabolism of alternative, less-preferred carbon sources. In low-GC gram-positive bacteria, CCR is achieved via transcriptional control, inducer exclusion, and induction prevention. In *Bacillus subtilis*, regulation of carbon catabolism involves a variety of different factors in addition to the catabolite control protein A (CcpA), the master regulator of CCR in this organism. We recently identified the CcpA homolog of *Staphylococcus aureus* and showed that the DNA-binding protein affects carbon metabolism, virulence determinant production, biofilm formation, and resistance to cell wall-directed antibiotics. Computational analyses of the *S. aureus* genomes, however, suggest this pathogenic organism to harbour additional homologs of *B. subtilis* genes that are known to affect the regulation of carbon catabolism. One of these homologs is SACOL0731. The predicted protein shares 61% similarity and 35% identity with the *B. subtilis* CcpC, a citrate-inhibited repressor of the tricarboxylic acid (TCA) cycle genes *citZ* (encoding citrate synthase) and *citB* (encoding aconitase). We report here the construction of markerless deletions of SACOL0731 in *S. aureus* strains Newman and SA113. Preliminary data suggest that inactivation of SACOL0731 in *S. aureus* affects biofilm formation and the *in vitro* growth capacities of the mutants, especially during the later stages of growth, indicating that the putative *ccpC* homolog of *S. aureus* might affect TCA cycle activity in this organism as well.

MTP29
Phenylacetaldehyde ferredoxin oxidoreductase: a molybdenum- or tungsten-containing enzyme involved in phenylalanine metabolism in "*Aromatoleum aromaticum*" strain EbN1

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Aldehyde ferredoxin oxidoreductases play an important role in peptide fermentation in hyperthermophilic archaea and catalyze the conversion of aldehydes generated from amino acids to carboxylates. These enzymes contain a tungsten cofactor and were long believed to occur only in thermophilic and hyperthermophilic anaerobic prokaryotes. When grown on phenylalanine as sole carbon source, the mesophilic denitrifying bacterium "*Aromatoleum aromaticum*" strain EbN1 produces an enzyme homologous to these thermophilic tungsten enzymes. Simultaneously, an induced phenylacetaldehyde ferredoxin oxidoreductase activity has been observed in the corresponding cell extracts. The enzyme is currently purified and characterized for its biochemical features and the presence of metals. "*A. aromaticum*" may be a model system for the coexistence of molybdenum- and tungsten-enzymes in the same cell, as indicated by detailed genome evaluation. This revealed hints for the existence of several isoenzymes for metal insertion in molybdenum-cofactor biosynthesis and for molybdate or tungstate transport.

MTP30
Characterisation of the Formate Channel Protein FocA of *Escherichia coli*

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Formate is one of the major products of mixed-acid fermentation in *Escherichia coli* and is an important electron donor for many anaerobic bacteria. The accumulation of formate in the *E. coli* cell can lead to acidification of the cytoplasm and therefore a mechanism to regulate its level must be available. We identified a predicted <u>fo</u>rmate <u>c</u>hannel (FocA) that belongs to the family of formate–nitrite transporters (FNT). FocA is encoded by the *focA* gene, which is co-transcribed with *pflB*, encoding pyruvate formate-lyase, the enzyme responsible for formate generation. A mutant unable to synthesise FocA accumulates formate in its cytoplasm and, although it still transports formate, it has a clearly reduced capacity to excrete and re-import the acid. Based on these findings, FocA has therefore been proposed to catalyse the bidirectional movement of formate across the cytoplasmic membrane. *N*- and *C*-terminally *Strep*-tagged variants of FocA were purified and the analysis of their oligomeric state by blue native-PAGE revealed FocA to have an unexpected pentameric quaternary structure. Previous topological analyses had revealed FocA to have six membrane-spanning alpha-helices and the predicted high alpha-helical content of the purified protein was confirmed by CD spectroscopy. Different focA mutants have been generated to identify the amino acids that are important for formate transport activity. The importance of these amino acids in bidirectional formate activity will be examined.

MTP31
FocA-Independent Formate Transport in *Escherichia coli*

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During anaerobic growth with glucose enterobacteria such as *E. coli* convert up to one third of the substrate to formate. Formate is generated through the cleavage of pyruvate by the enzyme pyruvate formate-lyase (PflB). Accumulation of high cytoplasmic levels of formate is prevented by excretion of the organic acid into the periplasmic space where it can function as a substrate for respiratory formate dehydrogenases (FDH), but only if a suitable electron acceptor such as nitrate is available. In the absence of exogenous electron acceptors formate is re-imported into the cell where it is sensed by the transcriptional activator FhlA, which induces synthesis of the formate hydrogenlyase (FHL) complex: FHL disproportionates formate into CO_2 and H_2 and thus dissipates the excess reducing equivalents. Mutants that are devoid of FocA excrete less formate into the medium and accumulate higher formate levels in the cytoplasm, suggesting that FocA is a formate transporter. Notably, *focA* null mutants still can excrete and import formate, indicating the existence of at least one further system involved in formate transport. We used a formate-dependent *fdhF*::*lacZ* transcriptional fusion as a reporter to monitor changes in the intracellular formate concentration, and demonstrate that *E. coli* has a cAMP-dependent formate transport system. Moreover, in a *focA* mutant, hypophosphite, a toxic formate analogue, is also transported into the cell but this transport activity is independent of cAMP. Together, these data suggest that, as well as FocA, two further transport system exists: one that is cAMP-dependent but which cannot transport hypophosphite; and a second that can transport hypophosphite and possibly also formate but which is cAMP-independent. Strategies towards the identification of these FocA-independent transport systems will be presented.

MTP32
Post-translational requirement for formate on the maturation pathway of [NiFe]-hydrogenase 3 of *Escherichia coli*?

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During anaerobic growth on glucose *E. coli* synthesizes three membrane-bound, [NiFe]-hydrogenases (Hyd). Functionally, Hyd-1 and Hyd-2 are proposed to be mainly hydrogen uptake hydrogenases, whereas the third enzyme is part of the hydrogen-evolving formate hydrogen lyase (FHL) complex, which disproportionates formate to CO_2 and H_2. The active site of each Hyd enzyme contains nickel and iron with the diatomic ligands CO and CN^- bound to the iron atom. A number of accessory, Hyp, proteins are involved in the complex assembly process of these metals and their ligands. While synthesis of Hyd-3 is strictly dependent upon formate, synthesis of the other two enzymes seems to be controlled in response to different signals. During maturation of the Hyd enzymes, a precursor form of the large subunit is processed to a mature species, which can also be used to monitor completion of the [NiFe]-cofactor insertion process; mutants blocked in cofactor synthesis or assembly show only the precursor form of the large subunit. In order to identify further factors with a potential role in hydrogenase enzyme synthesis, maturation or activity we monitored the influence of key metabolic intermediates on hydrogenase activity using defined knockout mutants with defects in key metabolic enzymes. Analysis of *adhE*, *ackA*, *pta* and *pflB* mutants revealed that they produce only the precursor of the Hyd-3 large subunit. Enhancing the copy number of the *hyp* maturation genes together with nickel ion supplementation had no effect on processing, indicating that these factors do not limit the processing event in these mutants. Rather, addition of formate to the growth medium restored processing, suggesting that formate acts post-translationally on the maturation pathway of Hyd-3.

MTP33
Key enzymes of anaerobic degradation of ethylbenzene in *Aromatoleum aromaticum* EbN1: Ethylbenzene dehydrogenase and acetophenone carboxylase

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The aromatic hydrocarbon ethylbenzene is degraded by *Aromatoleum aromaticum* under denitrifying conditions via a unique pathway. The initial step is the anaerobic oxidation of ethylbenzene to (*S*)-1-phenylethanol, catalysed by the periplasmatic, molybdenum/iron-sulfur/heme b enzyme ethylbenzene dehydrogenase (EbDH), which belongs to the DMSO reductase family of molybdenum enzymes. (*S*)-1-Phenylethanol is transported into the cytosol and further oxidised to acetophenone. Subsequently, acetophenone is carboxylated to benzoylacetate in a novel ATP-dependent, biotin-independent reaction, catalysed by acetophenone carboxylase (Apc). Ethylbenzene dehydrogenase and acetophenone carboxylase have been biochemically characterised and the catalytic properties of both enzymes were studied. The respective reaction mechanisms were investigated and new data on the properties of both enzymes will be shown.

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[2] Jobst, B., K. Schühle, U. Linne, and J. Heider. 2009. ATP-dependent carboxylation of acetophenone by a novel type of carboxylase. J. Bac., submitted.

MTP34
Biosynthesis of glutamate and benzoate in *Syntrophus aciditrophicus*

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S. aciditrophicus SB grows non-syntrophically on crotonate, which is oxidized to acetate with formation of ATP and reduced via glutaconyl-CoA to cyclohexane-carboxylate and benzoate [1]. Therefore, we assumed that glutaconyl-CoA decarboxylase must play an important role in this pathway catalyzing the carboxylation of crotonyl-CoA, which never has been observed *in vivo* before. Glutamate is usually synthesized from acetyl-CoA via citrate. However, no gene for *Si*-citrate synthase has been detected in the genome of *S. aciditrophicus*. We cloned the gene coding for *Re*-citrate synthase [2] and expressed it as active enzyme in *Escherichia coli*. Together with assays in *S. aciditrophicus* extracts we turned down the idea of glutaconyl-CoA via 2-hydroxyglutarate as precursor of glutamate.

Glutaric acid together with a diamine can polymerize to a polyamide related to Nylon®. We obtained glutaconate production in a recombinant *E. coli* strain expressing six genes from the 2-hydroxyglutarate pathway of glutamate fermentation in *Clostridiales*. For the reduction of glutaconyl-CoA we expressed the genes encoding the non-decarboxylating glutaryl-CoA dehydrogenase (Gdh) [3] and the electron-transferring flavoprotein (Etf) from *S. aciditrophicus* in *E. coli*. We suppose that Gdh together with Etf catalyze the reductions of glutaconyl-CoA and ferredoxin by 2 NAD(P)H [4].

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MTP35
Biotransformation of the NSAID fenoprofen in environmental samples as well as by the filamentous fungus *Epicoccum nigrum* DSM 838 and identification of a major intermediate

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Pharmaceutical residues can be detected in the ng/l range from nearly every surface water. Since concentrations of the individual ingredients fall below activity levels by several orders of magnitude the risk of acute effects on living members of the bioscenosis is generally low. However, little knowledge exists on chronic effects caused by long-term exposure, by synergestic effects of drug cocktails, as well as by metabolites. In contrast to human metabolism the mechanisms of microbial transformation of most pharmaceuticals are poorly understood and little is known about relevant metabolites and their ecotoxicological impact.
The present work deals with investigations concerning the biotransformation of the non-steroidal anti-inflammatory drug (NSAID) fenoprofen by a river sediment as well as by a pure culture of the filamentous fungus *Epicoccum nigrum* DSM 838.
It was shown by HPLC analysis that fenoprofen loads of up to 130 µM are eliminated within 3 days by a river sediment under aerobic conditions. Surprisingly no metabolites could be detected in such incubations.
A major problem of such environmental approaches in respect to elucidate the pathway of degradation is the lack of potential metabolites as reference compounds. Fungal cytochrome-P450 systems could be an appropriate tool for the preparation of environmentally relevant metabolites. In order to get access to oxygenated fenoprofen derivatives several filamentous fungi were screened for their ability to convert this drug. *E. nigrum* DSM 838 was shown to metabolize fenoprofen completely up to concentrations of 760 µM into one major product, 4´-hydroxyfenoprofen (3-(4-hydroxyphenoxy)-α-methyl-benzeneacetic acid), structurally identified by MS and NMR techniques. 4´-hydroxyfenoprofen has been described to be a major transformation product during human fenoprofen metabolism and *E. nigrum* DSM 838 is the first reported biological system with the ability to produce this metabolite in significant amounts.

MTP36
Distribution of the tungsten containing class of benzoyl-CoA reductases in obligately anaerobic bacteria

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Benzoyl-CoA reductases (BCR) catalyze the reductive dearomatization of benzoyl-CoA, the central intermediate in the anaerobic degradation of aromatic compounds. Obligately anaerobic bacteria use an ATP-independent benzoyl-CoA reductase complex encoded by the benzoate-induced *bamBCDEFGHI* genes [1]. Recently, BamBC the W-pterin and FeS cluster containing active site components of this complex were purified and characterized from the strict anaerobe *Geobacter metallireducens* [2]. The remaining BamDEFGHI components are proposed to be involved in a SeCys-/FeS-/Flavin-containing electron activating machinery. The distribution and regulation of the W-containing class of BCRs in aromatic compound degrading anaerobic bacteria were investigated by (i) a specific oligonucleotide DNA probe for *bamB*, (ii) proteome analysis coupled to mass spectrometry, (iii) Western blot analysis using antibodies against BamB from *G. metallireducens* and, (iv) by *in vitro* assays following the reverse reaction, the electron acceptor dependent rearomatization of the reduced dienoyl-CoA to benzoyl-CoA The results obtained clearly show that the tungsten containing class of BCR is present in all tested obligately anaerobic bacteria including Fe(III)-respiring, sulphate reducing and fermenting bacteria.

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[2] Kung *et al.* (2009), PNAS PNAS 106:17687-92

MTP37
Towards elucidating the biosynthetic pathway of unique FeGP-cofactor of [Fe]-hydrogenase

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Hydrogenases catalyse the reversible activation of molecular hydrogen. The third type of hydrogenase, [Fe]-hydrogenase, harbours a unique iron-guanylylpyridinol (FeGP) cofactor in the active site, in which a low-spin iron is ligated by two CO, one C(O)-CH_2-, one S-CH_2-, and a sp^2-hybridized nitrogen of the pyridinol ring. The structure of the guanylylpyridinol part has not been found before. In all three hydrogenases, the iron centre is ligated by three potential πacceptor ligands consisting of CO and CN^- or, in case of [Fe]-hydrogenases, an acyl-carbon. The intrinsic acyl-carbon ligand is only discovered in [Fe]-hydrogenase.
In this study, the first insight into the biosynthetic pathway of FeGP-cofactor was provided by stable isotope-labeling experiments. The acetate auxotroph *Methanobrevibacter smithii* was cultivated in the presence of [1-^{13}C]acetate and [2-^{13}C]acetate. FeGP-cofactor was extracted and the mass of the guanylylpyridinol part was analyzed by MALDI-TOF-mass spectrometry. The data indicated that three carbons of the pyridinol ring are derived from C-1 of acetate, two carbons are from C-2 of acetate, and thus four carbons are from CO_2. Additionally, the autotrophic *Methanothermobacter marburgensis* was cultivated in the presence of L-[methyl-$^2H_3$]methionine. The mass of guanylylpyridinol part of the cofactor was shifted to + 3 Da, indicating that one of the two methyl groups is derived from methionine. NMR spectroscopic analysis of the methyl-$^2H_3$ labeled guanylylpyridinol part revealed the position of the methyl-$^2H_3$ group on the pyridinol ring. [2,3-$^{13}C_2$]succinate, [1-^{13}C]propionate and [1-^{13}C]glycine could be excluded as possible precursors for the pyridinol ring biosynthesis because labeling by these compounds was not observed.

MTP38
Propanediol and ethanolamine metabolism of *Salmonella enterica serovar typhimurium*

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Propanediol and ethanolamine are abundant compounds in processed food and in the human gut. Their utilization as the main source of carbon, nitrogen and energy under aerobic and anaerobic conditions by bacteria requires cobalamin as a cofactor that is synthesized under anaerobic conditions only. The responsible gene cluster eut, pdu and cob covering about 1% of the genome are present in the food-borne pathogens *Salmonella enterica serovar typhimurium*, *Listeria monocytogenes* and *Clostridium perfringens* but absent from most other genera. So far, conditions under which the presence of the eut, pdu and cob provides a growth advantage are largely unknown.

In Salmonella, the propanediol utilization operon (pdu) has three and ethanolamine utilization operon (eut) has one promoter which controls the expression of the respective operons. We investigated the expression of the pdu and eut operons under different conditions using a promoter based luciferase assay. The Ppdu promoter showed high induction in minimal medium containing only propanediol as the carbon source. Fucose and rhamnose are widely presumed as the sources of propanediol; consequently, the pdu operon is also induced in minimal medium containing these sugars. On the other hand, the promoter of the ethanolamine operon, Peut, showed induction both in minimal medium containing ethanolamine and propanediol. It is assumed that expression of pdu and eut genes may help in the general survival of Salmonella in sugar scarce conditions. In the presence of milk, high induction of Peut was observed, while the Ppdu induction was minimal. We conclude that the fucose largely present in the glycoconjugates of the intestinal epithelial cells and the cell membranes rich in phospholipids serve as sugar source for Salmonella especially in an intestinal environment where other sources are absent. We are also currently studying the redundant role of different diffusion facilitators in transporting these compounds into the bacterium.

MTP39

A unique way of energy conservation by the glutamate fermenting *Clostridium tetanomorphum*

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Clostridium tetanomorphum produces ammonia, acetate, butyrate, CO_2 and hydrogen from glutamate [1]. In the glutamate fermentation, five pyruvate derived from 5 glutamate are oxidized by 5 ferredoxin (Fd) to 5 CO_2 and 5 acetyl-CoA. The 5 reduced ferredoxins (Fd^{2-}) are redoxized by 2 H^+ and 4 NAD^+ to yield 1 H_2 and 4 NADH, respectively and 4 acetyl-CoA are reduced by 4 NADH to 2 butyryl-CoA. Three ATP are generated from 2 butyryl-CoA and the remaining 1 acetyl-CoA. Thereby additional energy is conserved via butyryl-CoA-dehydrogenase/electron transferring flavoprotein (Bcd/Etf) and Rnf complex [2]. The Rnf complex purified from *C. tetanomorphum* catalyzes the ferredoxin ($E^{\circ\prime} \leq$-420 mV) dependent reduction of NAD^+ ($E^{\circ\prime}$ = -320 mV). The 6 genes comprising the Rnf operon has been sequenced and aligned with the sequences of *C. tetani* [3]. The complex contains both non-covalently bound flavin as well as covalently bound flavin. The non-covalently bound flavin was identified as FMN and riboflavin in 1:1 stochiometric ratio, each 0.3 mol/mol Rnf complex (180 kDa). The 3 genes comprising the Bcd/Etf operon has been sequenced and aligned with the sequences of *C. tetani*. The Bcd/Etf complex ($\alpha 2\beta\gamma$) has been purified from *C. tetanomorphum*. It was shown to catalyze the endergonic reduction of ferredoxin coupled to the exergonic reduction of crotonyl-CoA to butyryl-CoA ($E^{\circ\prime}$ = -10 mV) with 2 NADH. In this work, we have combined Bcd/Etf with Rnf and catalytic amounts of ferredoxin according to the equation: Crotonyl-CoA + NADH + H^+ = Butyryl-CoA + NAD^+. The concomitant formation of $\Delta\mu H^+/Na^+$ remains to be established.

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MTP40

2-Hydroxyglutaryl-CoA dehydratase - a radical enzyme, alternative to coenzyme B12-dependent glutamate mutase

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Clostridium symbiosum, Acidaminococcus fermentans and *Fusobacterium nucleatum*, ferment glutamate via (R)-2-hydroxyglutarate to ammonoia, CO_2, acetate, butyrate and H2. The key enzyme of this pathway is 2-hydroxyglutaryl-CoA dehydratase, catalysing the mechanistically difficult reversible syn-elimination of water from (R)-2-hydroxyglutaryl-CoA to (E)-glutaconyl-CoA. The enzyme contains iron-sulfur clusters and uses a single electron as cofactor that is supplied by an activator protein concomitant with hydrolysis of ATP. The dehydratase recycles this electron for about 10,000 turnovers until it requires another 'shot' by the activator. This work determined the substrate specificity of this enzyme that catalysed the hydration of several unsaturated adipyl-CoA derivatives and the mono CoA-thioester of acetylenedicarboxylic acid that were prepared with glutaconate CoA-transferase from A. fermentans. Hydration of acetylenedicarboxyl-CoA and subsequent spontaneous hydrolysis yielded oxaloacetate. Different substrates induced different EPR-spectra, indicating the involvement of radicals in the reaction. One of the proposed radicals has been recently observed in the related (R)-2-hydroxyisocaproyl-CoA dehydratase from Clostridium difficile [1]. In addition, the equilibrium constants of the dehydrations of lactyl-CoA ($K' = 0.02$), (R)-2-hydroxyglutaryl-CoA ($K' = 8$), and (R)-2-hydroxyisocaproyl-CoA ($K' = 1600$) were calculated. This agreed well with the experimentally determined data: lactyl-CoA ($K' = 0.017$) and (R)-2-hydroxyglutaryl-CoA ($K' = 50$). The dehydration of (R)-2-hydroxyisocaproyl-CoA was irreversible under the applied conditions. Glutaryl-CoA and a mixture of 2,2- and 4,4-difluoroglutaryl-CoA inhibited the dehydration.

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MTP41

Export of carboxylic acids in *Corynebacterium glutamicum*

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Corynebacterium glutamicum is an apathogenic soil bacterium, which is one of the major model organisms in industrial biotechnology. In particular it is used for industrial production of amino and carboxylic acids like L-glutamate and L-lysine.
The uptake of precursors and the export of substrates through the membrane is crucial for the efficiency of biotechnical production processes. For most substrates the presence of a carrier was postulated but the majority of transport systems is still unknown. We have identified and characterized uptake systems for carboxylic acids in *C. glutamicum* recently, including the uptake system for pyruvate (MctC) [1] and the carriers for succinate, fumarate and malate DctA and DccT [2]. Beside lactate pyruvate and succinate are excreted as unwanted by-products during biotechnological production processes under conditions of transient low oxygen tension or because of a deregulated metabolic flux. Although the export of these substrates under anaerobic conditions is long known also in eukaryotic cells, export systems are unknown in eukaryotes as well as in prokaryotes [3].
We aimed to set up screening strategies for identification of export systems for selected carbohydrates. On the one hand, we performed bioinformatics searches for putative candidates. On the other hand, we established high throughput screening systems using transposon mutant or site directed mutant libraries. The results will be discussed and compared. Identified candidates were further characterized in terms of biochemical methods in order to proof their participation in carboxylic acid transport.

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MTP42

A metabolomic view of *Staphylococcus aureus* and its eukaryotic-like serine/threonine kinase and phosphatase deletion mutants: Involvements in cell wall biosynthesis

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Staphylococcus aureus is an emerging pathogen causing severe nosocomial and community acquired infections. Little is known about such pathogens in terms of intracellular metabolite pools. Therefore, we gave insights into the metabolome using an improved method based on HPLC-mass spectrometry analysis. The glimpse onto the alteration of the metabolism of *S. aureus* should provide treasures information on how the pathogen regulates the central biochemical pathways in order to adapt to changing environmental conditions.
One key regulatory system in eukaryotic and prokaryotic cells is the regulation of enzymatic functions via phosporylation and dephosphorylation by kinases and phosphatases. The eukaryotic-like protein serine/threonine kinase PknB and phosphatase STP enzymes were very recently investigated for several adaptive reactions in staphylococci.

In this report we present quantitative metabolite levels of *S. aureus* wild-type grown in different media to compare the basic physiology. More in detail we analyzed the wild-type *S. aureus* strain 8325 and the isogenic eukaryotic-like protein serine/threonine kinase (Δ*pknB*) and phosphatase (Δ*stp*) deletion mutants. Detection of several remarkable differences e.g. in nucleotide metabolism and especially within cell wall precursor metabolites emphasize a new impact of serine/threonine kinase/phosphatase on peptidoglycan and wall teichoic acid biosynthesis. These findings possibly lead to new insights into the regulation of staphylococcal cell wall metabolism and could open a door to new targets of antimicrobial therapy. Further, metabolomics can provide essential informations about the impact of protein phosphorylations on bacterial physiology.

MTP43

Enzymes involved in the degradation of halogenated benzoic acids in *Thauera chlorobenzoica*: a novel mode of reductive dehalogenation

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Halogenated aromatic compounds are produced in large amounts by human activities and are harmful to men and the environment. In anaerobic bacteria dehalogenation is usually accomplished by dehalorespiration in which chlorinated organic compounds serve as terminal electron acceptors in anaerobic respiratory chains [1]. However the denitrifying *Thauera chlorobenzoica* completely oxidizes halogenated benzoic acids to CO_2 and HCl by a completely different, so far unknown pathway [2]. We investigated the catabolism of 3-chloro- and 3-bromo-benzoate in *T. chlorobenzoica* by (i) *in vitro* HPLC assays, (ii) CoA-ester metabolome analysis, and (iii) enzyme purification and characterization. The results obtained revealed that during growth on meta-halogenated benzoic acids a specific halobenzoate CoA ligase is expressed. The corresponding enzyme was purified and characterized from cells grown on 3-chloro-benzoate. In the next step, the major part of the halogenated benzoyl-CoA analogue is then reductively dehalogenated forming benzoyl-CoA and HCl/HBr. This reaction is catalyzed by ATP-dependent benzoyl-CoA reductases (BCR) [3] and represents a previously non-described mode of reductive dehalogenation. Surprisingly, also isolated BCR from *T. aromatica*, which cannot grow on halogenated aromatic compounds, catalyzed the reductive dehalogenation.

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MTP44

Characterization of the putative operon *glnX-glnH-pknG* in *Corynebacterium glutamicum*

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We previously identified a novel regulatory mechanism for the TCA cycle enzyme 2-oxoglutarate dehydrogenase (ODH) in *Corynebacterium glutamicum*, a Gram-positive soil bacterium used industrially for the production of L-glutamate and L-lysine. In this mechanism serine/threonine protein kinase G (PknG) plays an important role due to phosphorylation of OdhI. In its unphosphorylated state OdhI inhibits the activity of the ODH complex by binding to the OdhA subunit [1]. This inhibition is essential for efficient glutamate production [2].
In corynebacteria and mycobacteria *pknG* is located in a putative *glnX-glnH-pknG* operon. The first gene of this cluster codes for a hypothetical membrane protein with four potential transmembrane helices, whereas the second gene, *glnH*, codes for a putative glutamine-binding lipoprotein. Similar to a *pknG* deletion mutant of *C. glutamicum*, mutants lacking *glnX* or *glnH* showed a growth defect on agar plates containing glutamine as sole carbon and nitrogen source. This indicates a functional association of these proteins [1].
We now analysed the transcriptional organization of the *glnX-glnH-pknG* cluster, the localisation of the corresponding proteins and potential interactions of these proteins. The results of these studies will be presented.

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[2] Schultz *et al.* (2007) Appl. Microbiol. Biotechnol. 76: 691-700

MTP45

Cytoplasmic sulfurtransferases in the phototrophic sulfur oxidizer *Allochromatium vinosum*

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The cytoplasmic protein DsrEFH is encoded in the *dsr* operon, which plays an essential role during sulfur oxidation in the purple sulfur bacterium *Allochromatium vinosum*. Evidence is accumulating that DsrEFH and DsrC are involved in sulfur-trafficking between persulfidic sulfur imported into the cytoplasm and sulfite reductase (DsrA). In *E. coli*, the DsrEFH- and DsrC-homologous proteins TusBCD and TusE are part of a sulfur relay system during thiouridine biosynthesis. This system also involves the protein TusA. A TusA-encoding gene is also present in *A. vinosum* (AlvinDRAFT_1105). It is preceded by a gene (1106) encoding a potential single domain thiosulfate:sulfurtransferase (rhodanese). It was shown that DsrC and AvTusA interact with DsrEFH *in vitro* and conserved cysteine residues in each of the proteins play an important role for this interaction. We collected data on the function of the 1106-encoded protein, assessed whether it is able to transfer sulfur to AvTusA and studied the specificity of persulfuration of DsrEFH and DsrC. We found that the 1106-encoded protein indeed exhibits thiosulfate:cyanide-sulfurtransferase acitivity. MALDI-TOF mass spectrometry proved persulfuration of the protein after incubation with thiosulfate. While AvTusA itself is not able to mobilize sulfur from thiosulfate, it is persulfurated in the presence of thiosulfate and the 1106-encoded protein, proving specific sulfur transfer between these proteins. DsrEFH and DsrC are persulfurated in the presence of sulfide and in the presence of cysteine and the cysteine desulfurase IscS. Sulfur transfer occurs specifically to active site residue Cys_{78} of DsrE but not to the conserved Cys_{20} of DsrH. Cys_{111} appears to be the sulfur-accepting site of DsrC. In summary, we provide new information on sulfur transfer reactions in purple sulfur bacteria and propose that sulfur transfer mechanisms may be of general importance for oxidative sulfur metabolism.

MTP46

Benzoyl-CoA oxygenase BoxB of Azoarcus evansii: Structure and proposed mechanism of benzoyl-CoA dearomatization via an epoxide

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Benzoyl-CoA oxygenase BoxB catalyzes the dearomatization step of benzoyl-CoA degradation found in a new but widely distributed pathway of aerobic benzoate metabolism [1]. According to recent data benzoyl-CoA is oxidized to an epoxide that is in equilibrium with its tautomeric oxepin form [2], both compounds being non-aromatic. Monomeric BoxB (M_r 55 kDa) is part of a two protein component system and harbours a diiron center detected by EPR and Mößbauer spectroscopic analysis. The crystal structure of BoxB established at 1.9 Å resolution revealed an architectural relationship to soluble methane monooxygenase and ribonucleotide reductase R2 [3], in particular, in the diiron active site region. The μ-hydroxo-bridged irons are ligated to three glutamates, one aspartate and two histidines. The latter residues lie distal to the putative benzoyl-CoA binding site which is embedded in a 25 Å long tunnel. Including data of other diiron proteins we propose a mechanism how the aromatic ring of benzoyl-CoA is activated and the epoxide is produced. Benzoyl-CoA oxygenase BoxB has a counterpart in phenylacetyl-CoA oxygenase, which also forms an epoxide of an aromatic CoA thioester. Both are the first representatives of a new aerobic route to cleave aromatic compounds.

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MTP47

New insights into the Sox-multienzyme complex and putative periplasmic sulfur transferases of the purple sulfur bacterium *Allochromatium vinosum*

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In many sulfur-oxidizing bacteria, the periplasmic Sox multienzyme complex is involved in the oxidation of thiosulfate to sulfate. Those organisms which do not form sulfur globules during thiosulfate oxidation (e.g. *Paracoccus pantotrophus*) contain three core enzymes, SoxXA, SoxB and SoxYZ, as well as Sox$(CD)_2$. The latter is not present in organisms forming sulfur globules (e.g. *A. vinosum*). Central to both processes is SoxYZ that carries the pathway intermediates on a conserved cysteine residue near the C-terminus of SoxY. We gained evidence for the crucial function of the conserved cysteine by studying *A. vinosum* $SoxY_{152}Z$ in which conserved Cys_{152} was replaced by serine. The mutated protein was neither active *in vitro* nor was it able to functionally complement a Δ*soxY* mutant.
The proposed mode of action for the *A. vinosum* Sox system involves the transfer of SoxY-bound sulfane sulfur to growing sulfur globules by sulfur-transferase-catalyzed steps. A possible sulfur transferase, SoxL, was found to enhance the oxidation rate of thiosulfate *in vitro* without increasing the electron yield [1]. These findings argue for a faster recycling of SoxYZ by transfer of one or more sulfur atoms away from its substrate-binding cysteine residue in the presence of SoxL. This conclusion is substantiated by our finding that replacement of single potential active site cysteines ($Cys_{179,184}$) by serine rendered SoxL inactive *in vitro*. Previously, we suggested that other sulfur transferases may act as back-up system for SoxL *in vivo* [2]. Analysis of the complete *A. vinosum* genome sequence revealed two further genes encoding potential periplasmic sulfur transferases. We assess the role of these proteins by phenotypic characterization of deletion mutants and by studying their activity *in vitro*.

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MTP48

A Coupling Domain in the T Unit of Energy-Coupling Factor (ECF) Transporters

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Energy-coupling factor (ECF) transporters are a recently described class of micronutrient importers, which are composed of a substrate-specific transmembrane protein (S unit) and a conserved dedicated (subclass I) or shared (subclass II) energy-coupling module, consisting of a transmembrane protein (T unit) and pairs of ABC-ATPases (A units) [1]. The role of two short motifs in the C-terminal half of T units, each with a strongly conserved Arg residue, was studied recently. Arg-to-Glu substitutions were generated in BioN, the T unit of the subclass I biotin transporter (RcBioMNY) of a purple bacterium, and in EcfT, the T unit of a set of promiscuous subclass II vitamin transporters of a lactobacterium. Double replacements destroyed the transporter complexes in each case and abolished activity. Individual replacements affected the transporters differentially and had a stronger impact on the function of the subclass II systems [2]. These results point to a role of the Arg-containing signatures in T units similar to that of the "EAA loop" in the transmembrane domains of canonical ABC transporters. In the latter, the EAA loop is in close contact with the "Q loop" of the ABC ATPases and is involved in intersubunit signaling. Cysteine-scanning mutagenesis was applied to analyse the role of the Arg-containing signatures in BioN as a potential contact site. A Cys-less BioMNY complex lacking the three and one Cys residues in BioM and BioN, respectively, was not affected in stability and retained one third of wild-type ATPase activity. Twentyone variants with single Cys residues in the Q loop of BioM or the Arg signature in BioN have been constructed. Site-specific cross linking is underway and expected to provide clues to the contact sites.

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[2] Neubauer O. *et al.* (2009) J. Bacteriol. 191: 6482–6488

MTP49

„Archaeal chocolate“ - stress protection by trehalose?

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Trehalose, a widespread non reducing disaccharide, consisting of two a-1,1 linked glycosyl-glucose molecules, is shown to function as compatible solute in Eucarya and Bacteria, protecting the cell against a wide range of different stress conditions [1]. Still its function in Archaea is unknown.
From comparative genomic and biochemical analysis four pathways of trehalose synthesis were identified in Archaea: (i) the two step trehalose-6-phosphate synthase (TPS) / trehalose-6-phosphatase (TPP) pathway (OtsA/OtsB) [2], (ii) the two step maltooligosyl-trehalose synthase / trehalohydrolase pathway (TreY/TreZ) [3], (iii) the trehalose glycosyltransferring synthase pathway (TreT) [4] and (iv) the trehalose synthase pathway (TreS) [5].
In order to unravel the function of trehalose in Archaea, two crenarchaea which differ in pathway composition, the hyperthermophilic *Thermoproteus tenax* harbouring the TreT [6] and the TPSP pathway and the thermoacidophilic *Sulfolobus acidocaldarius* containing the TreT and the TreY/TreZ pathway, were selected.
To determine the role of trehalose metabolism and its regulation in these two organisms, growth curves were performed under different stress conditions (i.e. osmotic, temperature, desiccation, nutrition stress), and the effect is analyzed at protein (enzymatic activities in crude extracts) and transcript (Northern Blot analysis) level. In addition, recombinant enzymes were analyzed and knock out mutants in *S. acidocaldarius* are under investigation. This study gives first inside into the regulation of trehalose metabolism and its role in Archaea.

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MTP50

Enzymatic volatilisation of heavy metal ions: Characterisation of the unexpected biomethylation ability of the methanogenic enzyme Methylcobalamin-CoM-Methyltransferase (MtbA) of *Methanosarcina mazei*

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It is well known that a plenitude of biologically produced methylated metals and metalloids can be detected in the environment. These biogenic derivatives are mostly volatile and – with only few exceptions – more toxic than their inorganic educts due to their greater hydrophobicity which results in higher solubility in and permeability through cell membranes. In recent studies we could prove that members of methanoarchaea play a predominant role in their synthesis. However, knowledge regarding the mechanism of organometal(loid) biosynthesis is still scarce.
The methanogen *Methanosarcina mazei*, which uses various methyltransferases for methane formation depending on the carbon source (CO_2, methylamines, methanol, and methylsulfide), is used as a model organism in this study. We could show that Methylcobalamin-CoM-Methyltransferase (MtbA), which catalyzes the methyltransfer from trimethylamine to Coenzyme M via a corrinoid cofactor, is also able to transform heavy metal ions into methylated volatile derivatives. Macromolecular and kinetic investigations with the recombinant enzyme were performed to define the molecular prerequisites for that unexpected activity and to get insight into the mechanism of methyltransfer to metal ions.

MTP51
Comparison of ADP- forming acetyl-CoA synthetase and succinyl-CoA synthetase in hyperthermophilic archaea

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ADP-forming acetyl-CoA synthetase (ACD) and the well known succinyl-CoA synthetases (SCSs) of the citric acid cycle belong to the protein superfamily of NDP-forming acyl-CoA synthetases (acyl-CoA + NDP + Pi <--> acid + NTP + CoA) [1]. ACD from *Pyrococcus furiosus* and the first archaeal, hyperthermophilic SCS to be characterised from *Pyrobaculum aerophilum* were comparatively analysed. The results indicate certain differences between these two proteins: (i) ACD and SCS consist of five homologous domains which are differently organised in both enzymes. (ii) This domain shuffling results in a novel spatial domain arrangement in ACD compared to SCS, however maintaining a similar catalytic site. (iii) In contrast to the three step mechanism of SCS, ACD reaction follows a novel four step mechanism: The first two partial reactions, the formation of acylphosphate and the phosphoryltransfer to a conserved histidine in the α subunit (H257α), proceed in analogy to SCS. But in contrast to SCS, the phosphorylgroup is not directly transferred to ADP. Instead, it is transferred in a novel third partial reaction from H257α to a conserved histidine in the β subunit (H71β). The fourth step is then the phosphoryltransfer from H71β to ADP [2]. (iv) In ACD a conserved lysine is directly adjacent to H71β. In SCS this lysine is substituted for arginine. Both residues play a crucial role in the final partial reaction, the phosphoryltransfer to ADP, in ACD and SCS, particularly in nucleotide binding. However, both arginine and lysine cannot be substituted for each other in SCS and ACD, respectively. (v) ACD and SCS differ in substrate specificity: ACD exhibit a broad substrate spectrum converting several organic acids. In contrast, SCS is highly specific for succinate.

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[2] C. Bräsen *et al.*, (2008) J. Biol. Chem. 283(22), 15409-15418

MTP52
YggB of *Corynebacterium glutamicum* - Dual function in osmotic stress response and glutamate production

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Corynebacterium glutamicum is a gram-positive, apathogenic soil bacterium with exceptional importance for the industrial production of various amino acids, especially L-glutamate. Recently, evidence was provided that the small mechanosensitive channel protein YggB (MscS) of *C. glutamicum* is linked to glutamate excretion under glutamate production conditions in biotechnological applications (1). YggB was identified as mechanosensitive channel which is required for solute efflux in response to hypoosmotic conditions (2, 3). It is homologous to MscS from *E. coli* (286 AA) concerning its N-terminal part, but, in addition, carries a long C-terminal domain of approximately 250 amino acids including a fourth transmembrane segment.
Comprehensive analysis of *C. glutamicum* YggB with respect to its function as mechanosensitive channel on the one hand and concerning a possible new role in export of glutamate, on the other, revealed a significant contribution of the YggB protein to both functions. Deletion and complementation studies in *C. glutamicum* showed not only significant contribution to betaine efflux in response to hypoosmotic conditions, but also an important role in fine-tuning the steady state concentration of internal betaine accumulated in response to hyperosmotic stress (4). Additionally, homologous expression of different C-terminal truncations of *C. glutamicum yggB* as well as heterologous expression of *E. coli mscS* variants provided strong evidence for an involvement of YggB in glutamate production by *C. glutamicum*, mediating the excretion of this industrial important amino acid.

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MTP53
Clostridium ljungdahlii – insight in the metabolism of a homoacetogenic Clostridium

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Clostridium ljungdahlii is a mesophilic acetogenic bacterium that was isolated for its ability to produce ethanol autotrophically from synthesis gas. It is of industrial interest because syngas is an inexpensive substrate that can be easily generated by gasification of coal, biomass, or municipal waste. Recently, the genome of this organism was sequenced and a DNA microarray was established.
During autotrophic growth no net-gain of ATP seems to be possible by substrate level phosphorylation as the ATP generated by acetate kinase has to be used for formate activation. In contrast to *Moorella thermoacetica*, we could not identify genes for cytochromes. Instead, genes encoding a Rnf-complex were identified in the genome, as has been described for the Na^+-dependent homoacetogen *Acetobacterium woodii*. As *C. ljungdahlii* grows autotrophically in the absence of Na^+, we propose that the Rnf-complex is involved in the generation of a proton gradient that could be used for ATP synthesis by electron transport phosphorylation.
C. ljungdahlii can not only produce ethanol but can also use it as an electron donor with betaine as electron acceptor thereby producing trimethylamine and acetate. This mechanism could be seen as a new variation of a stickland reaction with an alcohol as electron donor and a glycine-derivative as electron acceptor. Using the DNA microarrays we compared the levels of mRNA in H_2/CO_2 grown cells with cells grown on fructose or ethanol and betaine and try to reconstruct the carbon and energy metabolism during growth on these substrates. *C. ljungdahlii* possesses two genes for betaine reductases of which only one is highly up-regulated when betaine is used as an electron acceptor.

MTP54
Regulation of a *Pseudomonas stutzeri* aspartokinase with possible implications for hydroxyectoine biosynthesis

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Synthesis of the compatible solutes ectoine and hydroxyectoine is limited by the availability of aspartyl phosphate. This precursor is generated from aspartate by aspartokinases which are known to be strictly feedback regulated by amino acids of the aspartic acid family: lysine, threonine and methionine [e.g. Curien 2008]. In heterologous production of ectoine this feedback regulation leads to a metabolic bottle neck which can be overcome by making use of a deregulated aspartokinase [Bestvater 2008].
In the recently published *P. stutzeri* genome [Yan 2008] two genes for aspartokinases have been annotated. One of them, *pst_0177*, is located next to a cluster of genes encoding the ectoine and hydroxyectoine biosynthesis pathway *ectABCD* [Seip 2009]. This proximity on the genome indicates a possible evolutionary and functional correlation. Here we present kinetic and regulation studies of *P. stutzeri* Pst_0177 in comparison with other aspartokinases.

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[3] Yan Y, Yang J *et al.* (2008) PNAS 105:7564
[4] Seip B, Stein M, Galinski EA, Kurz M (2009) VAAM Annual Meeting PX23

MTP55
"Hot Spots" of thermoadaptation in the central carbohydrate metabolism of *Sulfolobus solfataricus*

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Recent studies indicate that the thermoacidophilic archaeon *Sulfolobus solfataricus*
uses the Embden-Meyerhof-Parnas (EMP) pathway for gluconeogenesis and the branched Entner-Doudoroff (ED) pathway for glycolysis [1, 2]. This study is part of the *Sulfolobus* Systems Biology ("SulfoSYS") project, which focuses on the effect of temperature change on a biological network, i.e. the central carbohydrate metabolism (CCM) of *S. solfataricus* [3]. In general, life at high temperature requires special adaptation strategies and besides the stability of proteins also the stability of intermediates seems to be crucial [4]. Beside 1,3 DPG and GAP, PEP was identified as instable intermediate in the CCM of *S. solfataricus*. In order to elucidate the effect of temperature change on the network at PEP level, PEP converting enzymes were analyzed. Enolase catalyses the reversible conversion of 2-phosphoglycerate (2-PG) to phosphoenolpyruvate (PEP) and pyruvate kinase the ATP generating conversion of PEP to pyruvate, the final step of glycolysis. The *S. solfataricus* genes encoding enolase (SSO0913) and the pyruvate kinase (SSO0981) were cloned, the encoded proteins heterologously expressed in *E. coli*, purified and analysed with respect to their catalytic properties and physiological functions. In addition, the half-lifes of 2-PG and PEP at different temperatures have been determined. The results reveal first insights into one of the hot spots of the CCM of *S. solfataricus*: PEP conversion.

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MTP56
Metabolic Pathways in *Streptococcus pneumoniae* as analyzed by ^{13}C-Isotopologue Profiling

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Streptococcus pneumoniae are human pathogens, which are able to transmigrate the host nasopharyngeal barriers and cause severe diseases such as pneumonia, meningitis and septicemia. As pneumococci are members of the clade of lactic acid bacteria, the major metabolic end-product of carbohydrate metabolism under microaerophilic is lactic acid. Pneumococci produce a large fraction of ABC transporter systems involved in uptake and metabolism of sugars, soluble compounds and amino acids. Here we have started to analyze by ^{13}C isotopologue perturbation the carbon metabolism after growth of pneumococci in chemically defined media (CDM) in the presence of U-$^{13}C_6$ glucose as the sole C-source. GC/MS analysis showed a pattern of labeled and non labeled amino acids. The results suggest that pneumococci have to take up specific amino acids, while others are synthesized by the classical or non-classical biosynthesis pathways. There was no labeling of His, Glu, Ile, Leu, Val, Pro and Gly, which is a hint for the lack of *de novo* synthesis pathways. Strikingly, the essential branched amino acids (Ile, Leu and Val) data are unexpected, as genes encoding enzymes required for the synthesis were annotated. Moreover, Asp is not synthesized via the general pathway including pyruvate and acetyl-CoA but through conversion of phosphoenolpyruvate (PEP) and CO_2 to oxaloacetate by the PEP carboxylase. Oxaloacetate is converted into 2-oxoglutarate with the lateral conversion reaction glutamate to aspartate via the aspartate transaminase. GC/MS analysis also showed complete labeled aromatic amino acids synthesized from erythrose-4-phosphate and two molecules of PEP via the intermediate chorismate. Finally, unlabeled Gly is not synthesized out of one labeled Ser, but *vice versa*. In pneumococci, Ser is labeled by hydroxymethylation from
5,10-methylenetetrahydrofolate as part of the One Carbon Pool. These data show for the first time the variety of amino acid synthesis pathway in pneumococci and the essential role of pneumococcal amino acid transporter systems for bacterial fitness und host milieu adaptation.

MTP57
Pseudomonas aeruginosa NirE: a SAM-dependent uroporphyrinogen III methyltransferase for heme d_1 biosynthesis

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Pseudomonas aeruginosa uses the anaerobic respiration process of denitrification as a powerful strategy for energy generation under anaerobic growth conditions. The second step of this process is performed by a nitrite reductase which posesses two different tetrapyrroles as prosthetic groups, heme *c* and heme d_1. The biosynthesis of the isobacteriochlorin heme d_1 is currently unknown. Mutant studies in *P. aeruginosa* revealed the involvement of proteins encoded by the *nir*-operon (*nirSMCFDLGHJEN*) in heme d_1-biosynthesis. NirE, encoded by the *nirE* gene, was predicted to be a SAM-dependent uroporphyrinogen III mehtyltransferase (SUMT) based on its aminoacid sequence.
Recombinant *P. aeruginosa* NirE was produced in *E. coli* and purified chromatographically to apparent homogenity. *In vitro* activity assays provided evidence for the predicted function of NirE as a SUMT which converts uroporphyrinogen III to precorrin-2. Like known SUMTs NirE was inhibited by its product uroporphyrinogen III and a reaction by-product *S*-adenosyl-homocysteine. Gelfiltration experiments and bacterial two hybrid screens showed that NirE is a dimeric protein. NirE was crystallized and the structure was solved by molecular replacement using CobA as a model.

MTP58
Fermentation of *Clostridium acetobutylicum* at different pH values: product spectrum, proteome and transcriptome

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Clostridium acetobutylicum is characterized by its acetone-butanol (AB) fermentation (1, 2). An important interest of the COSMIC project concerns the detailed analysis of the AB-fermentation for the production of reliable data for modeling. It is well known that in a chemostat *C. acetobutylicum* is able to switch its metabolism between 'acidogenesis' and 'solventogenesis' in reaction to the change of the external pH. At pH 5.7, cells mainly produce acids (acetate, butyrate) whereas at pH 4.5 the dominating fermentation products are solvents (acetone, butanol) (3, 4). Knowledge about the regulation is still limited.
Thus, we established a continuous chemostate culture under phosphate limitation at pH 5.7 (acid production) and switched to pH 4.5 (solvent production) and investigated differences in the steady state proteomes and transcriptomes, respectively. Moreover, a systematic approach, analyzing cells growing at further five different pH values between pH 5.7 and pH 4.5 in steps of 0.2 (pH 5.5, 5.3, 5.1, 4.9 and 4.7) was started. Here we present the results, primarily focusing on the alterations of the product spectrum.

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MTP59
Crh regulates a glycolytic bypass in *Bacillus subtilis*

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For efficient proceeding of life processes, all cellular activities must be balanced. It is already known that HPr plays a very important role in the regulation of central carbon metabolism (Singh *et al.*, 2008). The physiological role of HPr has been thoroughly investigated, whereas almost nothing is known about the function of Crh, which is a paralog of the HPr protein from the phosphotransferase system (PTS). Like HPr, Crh can be phosphorylated by the metabolite-dependent protein kinase HPrK on the seryl residue at position 46, but it cannot be phosphorylated by enzyme I and is therefore not involved in PTS-catalyzed sugar transport (Galinier *et al.* 1997). The fact that Crh is not organized in an operon with the genes that encode PTS proteins strongly suggests that it has a different function. Here, we demonstrate that Crh interacts with the methylglyoxal synthase MgsA. This enzyme catalyzes a glycolytic bypass, which probably occurs when the phosphate supply is insufficient. In contrast to the conventional C3-part of glycolysis, phosphate is not consumed in this pathway, but is released. Our results indicate that phosphorylated Crh activates MgsA. Crh is probably phosphorylated if a good carbon source like glucose is available. In the presence of glucose the flow in glycolysis is high and a substantial quantity of phosphate is consumed. One hypothesis is that activation of the bypass allows C-metabolism to take place in case of limited inorganic phosphate.

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MTP60
Life in the hot lane: How (hyper-)thermophiles cope with the instability of metabolites

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An outcome of the SulfoSYS project (*Sulfolobus* Systems Biology; [5, 6]) is that metabolite instability is one important challenge for organisms growing at high temperature. The project investigates how changes in temperature influence the central carbon metabolism (CCM) of *Sulfolobus solfataricus*. Thermal instability of metabolites means for the cell to deal with loss of free energy and carbon, or with accumulation of dead-end compounds. Analyses of heat stability of CCM-intermediates revealed, that glyceraldehyde 3-phosphate (GAP; half-life 80°C: 1.6 min) and 1,3 diphosphoglycerate (1,3 DPG; half-life 60°C: 1.6 min; [1]) of the semi-phosphorylative Entner-Doudoroff branch are unstable at high temperature.
S. solfataricus P2 is a thermoacidophilic Crenarchaeon that optimally grows at 80°C and pH 2-4 [2]. In *S. solfataricus* two different GAP converting enzymes were characterized, the classical phosphorylating GAP dehydrogenase(GAPDH; [3]) and the non-phosphorylating GAPDH (GAPN; [4]). GAPDH is present in all Archaea, whereas GAPN is almost exclusively found in (hyper-)thermophiles [4]. By using the GAPN, instead of the classical enzyme couple GAPDH and phosphoglycerate kinase, GAP is directly converted into 3-phosphoglycerate omitting the instable 1,3 DPG.
This study focuses on the temperature-dependent biochemical, kinetic and regulatory properties of the two GAP converting enzymes and gives first insights into strategies of how (hyper-)thermophiles deal with thermal instability of metabolites.

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MTP61
Transcriptional response of *cbrA* to trichlorobenzenes in *Dehalococcoides* sp. strain DCMB5

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The genome of *Dehalococcoides* sp. strain DCMB5, which dehalogenates chlorinated benzenes and dioxins, harbours at least 22 reductive dehalogenase-homologous (*rdh*) genes, 19 of which possess orthologues in *Dehalococcoides* sp. strain CBDB1 and three are orthologous to *rdh* genes in strains BAV1 or 195. The *rdh* gene *cbrA* in strain CBDB1 encodes a dehalogenase for which reductive dechlorination of 1,2,3-trichlorobenzene (TrCB) was demonstrated [1]. The *rdh* gene *cbrA* was transcribed at high levels when cells were grown in the presence of 1,2,3- or 1,2,4-TrCB, leading to the hypothesis that CbrA is also involved in reductive dechlorination of 1,2,4-TrCB [2]. In accord with the presence of a *cbrA* orthologous gene, strain DCMB5 also reductively dechlorinates 1,2,3-TrCB. However, in contrast to strain CBDB1 it is unable to dechlorinate 1,2,4-TrCB. Here we demonstrate that 1,2,3-TrCB, but not 1,2,4-TrCB, induced *cbrA* gene expression in strain DCMB5. This result suggests that a reductive dehalogenase different from CbrA, and which is absent from strain DCMB5 is actually responsible for reductive dechlorination of 1,2,4-trichlorobenzene in strain CBDB1. In attempts to elucidate the function of further reductive dehalogenases in *Dehalococcoides* strains we observed that the CBDB1-specific *rdhA* gene *cbdbA1624* showed a relatively high expression in the presence of 1,2,4-TrCB, suggesting that it might be responsible for 1,2,4-TrCB dehalogenation in strain CBDB1 [2].

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MTP62
Function of outer membrane cytochromes in *Shewanella oneidensis*

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Dissimilatory metal reduction is a respiratory process in which the reduction of a metallic terminal electron acceptor is coupled to energy generation. The solubility of metall oxides is often low under neutral conditions. Hence, *Shewanella* species have established the formation of an extended respiratory chain to the cell surface. The final step is believed to be catalyzed by outer membrane cytochromes (OMC). *S. oneidensis* genome analysis revealed five putative OMC genes, three of those with unknown function. Our goal was to compare the specificities of single OMCs towards different electron acceptors *in vivo*
As a first step, we constructed a deletion mutant deficient in all annotated OMCs and measured reduction rates with different soluble and insoluble electron acceptors. Surprisingly, the mutant retained low level metal reduction activities. *S. oneidensis* produces riboflavin as an endogenous electron shuttle [1]. Hence, it was tested whether the deletion mutant might retain reducing activity due to the catalysis of riboflavin reduction. While wild type cells reduced riboflavin with a rate of 0,54 µmol $mg^{-1}min^{-1}$, the deletion mutant showed no detectable activity. Therefore, the existence of a membrane permeable shuttle has to be hypothesized.
As a second step, we expressed single OMCs in this deletion mutant. Correct localization of proteins was assessed with a new photometric test based on the OMC riboflavin interaction. Metal reduction and growth rates were measured with different terminal electron acceptors. Our data confirm previous studies [2] that OmcB is the most important metal reductase in *S. oneidensis* whereas OmcA expressing cells were unable to sustain metal reduction rates differing from the deletion mutant.
Furthermore, we show for the first time that the OMC MtrF is a potent reductase whereas expression of the other two putative OMCs did not lead to an altered phenotype compared to the deletion mutant.

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MTP63
Nitrogen metabolism in *Streptomyces coelicolor*: HPLC-based analysis of intracellular amino acid pools

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In contrast to most other bacteria *Streptomyces coelicolor* contains two different glutamine synthetase genes: *glnA* and *glnII*, encoding for the functional glutamine synthetases GSI and GSII, respectively. Glutamine synthetases are responsible for nitrogen assimilation to form glutamine out of glutamate and ammonium under low nitrogen conditions. Under high nitrogen conditions glutamine is synthesised in two metabolic steps: The glutamate dehydrogenase (gene product of *gdhA*) converts 2-oxoglutarate and ammonium into glutamate.
To elucidate the function of *glnA*, *glnII* and *gdhA*, mutant strains of *S. coelicolor* M145 with deletions in the respective genes were constructed and growth curve experiments were performed for physiological characterisation under high and low nitrogen conditions. The intracellular amino acid pool was investigated by reversed phase HPLC-analysis:
During growth, changes in the total amino acid concentration were observed as well as different patterns in the composition of the intracellular amino acid pool. The total amino acid concentration and the growth phases correlate. The total amino acid concentration shows a biphasic development with a minimal concentration at the end of the exponential growth phase. Under nutrient-rich growth conditions the predominant amino acids are glycine, alanine and glutamate, under nitrogen-depletion glutamate and glutamine. In the *glnA* mutant nitrogen limitation results in an increased glutamine pool whereas in the *glnII* mutant the glutamine pool is strongly reduced. This indicates that GSI and GSII have different functions in *S. coelicolor* M145.

MTP64
An omega-amidase for alpha-ketoglutaramate is part of the pAO1-mediated nicotine catabolic pathway of *Arthrobacter nicotinovorans*

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Several members of the soil bacterial community can grow on nicotine [1]. In *Pseudomonas putida* nicotine is degraded to 2-hydroxy-5-succinoyl-pyridine, which is converted to 2,5-dihydroxypyridine (DHP) and succinate. 2,5-DHP is a central metabolite since nicotine, nicotinate, 2-hydroxy- and 3-hydroxypyridine catabolism converge at this stage. Further degradation of 2,5-DHP follows the maleamate pathway which is initiated by opening of the pyridine ring by a dioxygenase. In Arthrobacter nicotinovorans growth on nicotine is linked to the presence of the catabolic megaplasmid pAO1 [2]. Cleavage of the side chain of 2,6-dihydroxy-pseudo-oxynicotine by 2,6-dihydroxy-pseudo-oxynicotine hydrolase [3] releases γ-N-methylaminobutyrate and 2,6-dihydroxypyridine, which is hydroxylated to trihydroxypyridine (THP) [4]. THP may dimerize to form nicotine blue, or it may be degraded to intermediates of central metabolism. In analogy with the degradation of nicotinic acid by P. putida, the activity of a dioxygenase was assumed to be responsible for cleaving THP. Here we present evidence that ORF294 of the nic-gene cluster of pAO1 encodes an alpha-ketoglutaramate omega-amidase, which was purified and its enzyme activity analyzed. The presence of an omega-amidase in the pAO1 mediated nicotine catabolism suggests a hydrolytic cleavage of THP as an alternative pathway to the maleamate pathway of 2,5-DHP catabolism.

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MTP65
Assessment of murein and muropeptides with AnmK and MurK

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The bacterial cell wall is a huge cell surrounding macromolecule consisting of glycan strands cross-linked by short peptides that is essential for cell viability and shape. *N*-acetylmuramic acid (MurNAc) is one of two sugar components of the murein and unique to most bacteria. During bacterial growth in Gram-negative bacteria lytic transglycosylases release an anhydro form of MurNAc (anhydroMurNAc) from the cell wall. The nature of cell wall turnover products of Gram-positive bacteria is basically unknown. Therefore a highly sensitive detection of MurNAc and anhydroMurNAc is mandatory. We developed a new enzymatic detection assay based on phosphorylation with radioactively labeled ATP: MurNAc and anhydroMurNAc are phosphorylated by the kinases AnmK of *Escherichia coli* and MurK of *Clostridium acetobutylicum*, respectively, yielding MurNAc-6-P. Compared with the insufficient sensitive colorimetric Morgan-Elson reaction our phosphorylation assay offers the advantage to detect MurNAc and anhydroMurNAc at much lower amounts.

MTP66
Mixotrophic utilization of hexoses by mutants of *Ralstonia eutropha* H16 blocked in the Entner-Doudoroff pathway

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Ralstonia eutropha H16 is a strictly respiratory betaproteobacterium well known for its facultative chemoautotrophy. During autotrophic growth CO_2 is assimilated via the Calvin-Benson-Bassham (CBB) cycle with hydrogen or formate serving as energy donors. Heterotrophically the organism utilizes very diverse organic compounds, preferring organic acids such as pyruvate or succinate. Fructose and N-acetylglucosamine (NAG) are the only carbohydrates, gluconate, glucosaminate and 2-ketogluconate the only sugar acids metabolized. These sugar(acid) substrates are exclusively metabolized via the Entner-Doudoroff (ED) pathway. Most of the ED genes are encoded within six gene clusters including three *zwf* (glucose-6-phosphate dehydrogenase) genes encoded within the *nag* (*A0310-nagFECAB-zwf1*), the *frc* (*frcRACB-zwf2-pgi2-frcK*) and the *zwf* operon (*zwf3-pgl-glk-B2563-B2562*). Inactivation of *zwf2* or *zwf3* did not strongly affect growth on fructose or NAG, while a *zwf1* mutant strain grew very slowly on NAG. Surprisingly, a triple *zwf* mutant still showed very slow growth on both fructose and NAG but faster growing colonies appeared upon prolonged incubation on agar plates. Since *R. eutropha* lacks alternative glycolytic pathways we supposed that the CBB cycle should operate during hexose assimilation in these mutants. In this unexpected function of the cycle the transketolase reaction represents the entry point of fructose-6-phosphate, while CO_2 fixation is essential to regenerate the acceptor glyceraldehyde-3-phosphate. Dissimilation of carbon must involve a fully functional tricarboxylic acid cycle. Several lines of evidence supported this novel mode of obligate mixotrophic hexose utilization in these mutants.

MTP67

The two key enzymes in the 4-aminobutyrate fermentation pathway of *Clostridium aminobutyricum*

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Clostridium aminobutyricum ferments 4-aminobutyrate to ammonia, acetate, and butyrate. This pathway comprises 4-hydroxybutyrate CoA-transferase (AbfT) and 4-hydroxybutyryl-CoA dehydratase (AbfD). AbfT catalyzes the reversible transfer of CoA from several CoA-thioesters to 4-hydroxybutyrate. Its crystal structure without [1] and with butyryl-CoA revealed the butyryl moiety in an approximate cis-conformation close to the active site E238. This is consistent with trans-crotonate not acting as substrate. Site-specific mutagenesis, inactivation by borohydride, fragmentation at 70°C, and the ping-pong mechanism identified the formation of the glutamyl-CoA intermediate typically for class I CoA-transferases. AbfD catalyzes the reversible dehydration of 4-hydroxybutyrate to trans-crotonyl-CoA via a ketyl radical mechanism. Its crystal structure exhibited one $[4Fe\text{-}4S]^{2+}$ cluster coordinated by C99, C103, C299 and H292, and one FAD per subunit of this homotetrameric protein [2]. In the putative active site, Y296, E257 and E455 are located between cluster and FAD. Mutation of any of these seven amino acids abolished the activity except for Y296F, which retained 1.3%. The H292C/E and C99A mutants lost 99% vinylacetyl-CoA delta-isomerase activity as compared to that of the wild type. The crystal structure as well as stereospecific syntheses of D- and T-labelled 4-hydroxybutyrates and their enzymatic conversions demonstrated anti-elimination of the 3Si and 2Re-hydrogens with retention of configuration in the replacement of the hydroxyl group by hydrogen [3]. H292 may act as the base to abstract the 2Re-proton of 4-hydroxybutyryl-CoA or vinylacetyl-CoA.

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MTP68

Influence of a *nuoN* mutation on growth of *Pseudomonas* sp. strain Chol1 with the toxic steroid compound cholate

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Bacterial degradation and transformation of steroids is ecologically relevant and of biotechnological importance for the production of steroid drugs. As many steroids have toxic effects, their degradation is an energetic challenge for bacteria using such compounds as growth substrates because they have to invest energy for protection mechanisms. As a model system for degradation of toxic steroids we investigate growth of *Pseudomonas* sp. strain Chol1 with the surface-active bile salt cholate.
Transposon mutants of this strain were screened for altered growth with cholate. The mutant strain NE11 had an extended lag phase of up to 50 hours with cholate as substrate. The interrupted gene had high identity to *nuoN* from *Pseudomonas mendocina*, encoding for a subunit of the NADH:ubiquinone oxidoreductase Ndh-I of the bacterial respiratory chain and to Na^+/H^+ antiporters.
Defects in the cholate degradation pathway in strain NE11 could be excluded because cholate was completely degraded during exponential growth. However, acetate was excreted temporarily during the prolonged lag phase. The *in vitro* NADH oxidation rates were very similar in strain NE11 and the wild type. Inhibitor studies and native PAGE with activity staining indicated that NADH oxidation in both strains is performed by two different complexes, presumably NQR and Ndh-I. When intermediates of the TCA cycle were supplied as substrates, strain NE11 had no altered phenotype. Furthermore, increased Na^+-concentrations in the medium led to a significant decrease of the prolonged lag phase.
These results strongly suggest that the mutation affects the establishment of chemiosmotic gradients in strain NE11, which might involve Na^+. The long lag phase may be caused by an energetic deficiency or imbalance that temporarily prevents strain NE11 from growing with the toxic substrate cholate. We are currently investigating the mechanisms by which strain NE11 overcomes that energy limitation and resumes growth.

MTP69

Insights into energy conservation and metabolism of *Acetobacterium woodii* from its genome analysis

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The anaerobic bacterium *Acetobacterium woodii* uses a transmembrane electrochemical sodium ion potential for bioenergetic reactions [1]. This potential is generated during carbonate (acetogenesis) as well as caffeate respiration. How the Na^+ gradient is formed is still obscure. In order to find genes encoding Na^+-translocating enzymes and to extend the knowledge of the physiology, the genome was completely sequenced. There is only one replicon present, the size is 3.9 Mbp and the the G+C content is 39.5 mol%. Enzymes involved in substrate conversion, carbon flow and energy conversion will be discussed.

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MTP70

The cellular level of the glutamate-5-kinase ProJ of the moderately halophilic bacterium *Halobacillus halophilus* is dependent on the salt and chloride concentration of the environment

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The moderately halophilic, chloride-dependent bacterium *Halobacillus halophilus* switches its osmolyte strategy with the salinity in its environment by the production of different compatible solutes [1]. At higher osmolarities (up to 3 M) proline is dominant. The expression of proline biosynthesis genes (*proH*, *proJ* and *proA*) was clearly salinity dependent. In upshock studies, addition of chloride salts led to the highest accumulation of proline. On the transcriptional level, nitrate and gluconate were able to substitute chloride, and glutamate was even more effective. Since it is known that chloride dependent regulation in *H. halophilus* mainly exists on the translational level rather than the transcriptional level [2, 3], we addressed the question whether cellular levels of proline biosynthesis enzymes are dependent on the salinity of the medium. Western blot analyses with polyclonal antibody against ProJ revealed that the enzyme is mainly produced in the early exponential growth phase and maximal at salinities ranging from 2.0 to 3.0 M NaCl. ProJ levels were maximal at 2 M NaCl or $NaNO_3$, but only half in the presence of Na-gluconate or Na-glutamate. These data demonstrate an additional layer of regulation of proline biosynthesis on the translational level and/or protein stability in the moderately halophilic model organism *H. halophilus*.

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MTP71
Genetic analysis of putative methyltransferases specific for methylsulfide metabolism in *Methanosarcina acetivorans* C2A

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Methanosarcina acetivorans is able to grow with carbon monoxide (CO) as the sole energy source and synthesizes under this condition high levels of three homologous proteins, MtsD, MtsF and MtsH. These proteins show sequence similarity of > 50 % to each other and consist of a N-terminal corrinoid and a C-terminal methyltransferase 2 domain. Mutational analysis demonstrated that these three isoforms are required for methylsulfide metabolism in this organism because the triple mutant DmtsDFH is neither able to form dimethylsulfide (DMS) from CO nor methane from DMS. Complementing the mutant in trans with any of the Mts isoforms reverts this phenotype. It is therefore possible that one fused corrinoid/methyl transfer protein can alone mediate methylation of coenzyme M (HS-CoM) with DMS as the methyl-donor. However, it was proposed that the Mts system substitutes for another methyltransferase mediating methylation of HS-CoM with methyl-tetrahydrosarcinapterin as the methyl-donor, which is down-regulated under carboxidotrophic conditions. To address these issues MtsF was overproduced heterologously in *E. coli* but found to be insoluble. We therefore attempted to establish a homologous overexpression system in *M. acetivorans* to generate sufficient amounts of MtsF for biochemical analysis. The results of these efforts, together with initial characteristics of MtsF, are presented.

MTP72
Function of Ech hydrogenase in ferredoxin-dependent electron transport in *Methanosarcina mazei*

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Methanosarcina mazei is a strictly anaerobic methanogenic archaeon which is able to grow on a variety of substrates, like methylated amines, methanol, methylsulfides, or H_2/CO_2. Furthermore, it is able to utilize acetate in the so-called aceticlastic pathway of methanogenesis. This pathway has a severe ecological impact because it makes up for about 70 % of the biologically produced methane; this is important because methane is responsible for 18 % of the global radiative forcing, thus is contributing to global warming. An in-depth understanding of methanogenesis and especially of the aceticlastic pathway of methanogenesis is therefore crucial.
Organisms using this pathway to convert acetate to methane exclusively belong to the genera *Methanosarcina* and *Methanosaeta*. The two carbon atoms of acetate have different fates in the pathway: The methyl moiety is converted to methane whereas the carbonyl moiety is oxidized to CO_2; the electrons derived from this oxidation step are used to reduce ferredoxin. Reduced ferredoxin donates electrons to membrane-integral proteins, which transfer electrons to heterodisulfide reductase. A ferredoxin interaction has already been observed for Ech hydrogenase. To investigate whether Ech hydrogenase is the only means by which *Ms. mazei* channels electrons from reduced ferredoxin into the respiratory chain, a mutant lacking the Ech hydrogenase (*Ms. mazei* Δech) was constructed. The detailed analysis of the *Ms. mazei* Δech mutant showed decreased ferredoxin-dependent membrane-bound electron-transport activity, a slower growth rate and faster substrate consumption in comparison to the wildtype. Evidence is presented that a second protein whose identity is unknown oxidizes reduced ferredoxin indicating an involvement in methanogenesis from methylated C1-compounds and acetate.

MTP73

Will not be presented and published

MTP74
Growth of "*Aromatoleum aromaticum*" EbN1 in acetate-limited chemostats

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"*Aromatoleum aromaticum*" strain EbN1 was cultivated for the first time in acetate-limited chemostats under nitrate-reducing conditions. Chemostats are used to continuously cultivate bacteria under defined, constant and substrate-limited conditions (influx of medium equals simultaneous removal of culture broth). Under these conditions the bacterial population approaches a stable state (steady state). Changing the dilution rate (flow rate of the influent medium divided by the culture volume), therefore allows manipulating bacterial growth rates.
The growth behavior of strain EbN1 was characterized over a wide range of dilution rates by monitoring the cell number, residual acetate concentration, nitrate and nitrite concentrations as well as the DNA and protein content of steady-state cells. To achieve this, the flow rate of the influent medium was increased in several steps until a critical dilution rate of 0.25 h^{-1} was reached and the cells were washed out from the chemostat. The measured residual acetate concentrations in the culture followed Monod-type growth kinetics.

MTP75
Biochemical and kinetic characterization of the 2-hydroxyisobutyryl-CoA mutase, a key enzyme in methyl *tert*-butylether degradation by *Aquincola tertiaricarbonis* L108

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The coenzyme B12-dependent 2-hydroxyisobutyryl-CoA mutase catalyzes the reversible rearrangement of 2-hydroxyisobutyryl-CoA to 3-hydroxybutyryl-CoA. It is closely related to the well known isobutyryl-CoA mutase (EC 5.4.99.13). 2-hydroxyisobutyryl-CoA mutase has been detected in *Aquincola tertiaricarbonis* strain L108, where it appears to play a key role in methyl *tert*-butylether (MTBE) degradation by connecting the peripheral to the central metabolism. MTBE is a volatile, oxygen-containing, organic compound, which is predominantly used as a gasoline additive to reduce engine knocking by increasing of the fuel´s octane rating. Meanwhile it has become a widespread pollutant. Strain L108 utilizes MTBE as sole carbon and energy source, so its initiation for the MTBE decomposition could be a measure for remediation in the near future.
To study the biochemical and kinetic characteristics of the 2-hydroxyisobutyryl-CoA mutase it is appropriate to clone the genes for the large (*hcmA*) and small (*hcmB*) subunit of the enzyme into over-expression vectors being controlled by strong promoters. These constructs will be used for synthesis of the corresponding recombinant proteins in *Escherichia coli* extended by affinity tags for their purification.
We report here our efforts to express and purify the 2-hydroxyisobutyryl-CoA mutase (HcmA/HcmB) and to analyze its activity, viz. the conversion of substrate to product by an HPLC assay. Comparative results with cell-free extract of strain L108 indicate that the participation of further helper-proteins might increase the 2-hydroxyisobutyryl-CoA mutase activity.

MTP76
Suitability of different *E. coli* derivatives for heterologous co-expression of 2-hydroxyisobutyryl-CoA mutase of *Aquincola tertiaricarbonis* L108

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The application of anti-knock additives, such as methyl *tert*-butyl ether (MTBE) or ethyl *tert*-butyl ether (ETBE), to improve gasoline performance resulting in relevant contamination of the environment, requires the complete elucidation of the MTBE-pathway to reach a deeper understanding of the respective enzymes that actually realize the degradation of the persistent compounds exhibiting ether structure and tertiary carbon atom.
Strain *Aquincola tertiaricarbonis* L108 is one of the few strains that has the ability to grow on MTBE as sole carbon and energy source and its 2-hydroxyisobutyryl-CoA mutase appears to be one of the key enzymes in MTBE metabolism. This mutase accomplishes the isomerization of 2-hydroxyisobutyryl-CoA to 3-hydroxybutyryl-CoA thus linking degradation and general pathways.
On the strength of the past results it seems as if the actual mutase enzyme comprises two subunits, HcmA and HcmB. The characterization of this enzyme via affinity purification is under way, but over-expression, as well known for *E. coli*, might lead to several problems such as the formation of inclusion bodies, misfolding of proteins or difficulties due to uncommon codon usage. For that purpose, we aspire to apply low level-expression in different *E. coli* derivatives exhibiting particular features to synthesize a functional mutase enzyme. Additional findings suggest that there might be supplementary proteins or helper proteins required to express the full activity of the mutase. Therefore, we aim to elucidate the role of selected other proteins through individual co-expression in variable combinations in *E. coli*. Thereby, mutase activity will be studied and verified by means of whole cell assays.

MTP77
Mannitol modifies the expression of *siiE* and STM3690-91 in *Salmonella enterica* sv. Typhimurium

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Salmonella enterica sv. Typhimurium is a pathogen that causes gastroenteritis in humans and typhoid-like fever in mice. It is not clear, how the virulence could be regulated when the carbon source is changed. Therefore, we wanted to address whether the expression of the large non-fimbrial adhesin SiiE and the genes STM3690-91, that encode for a lipoprotein and the trimeric autotransporter SadA, changes when glucose is replaced by mannitol. Mannitol was used because it is taken up by 100% of *S.* sv. Typhimurium strains. *S.* sv. Typhimurium NCTC 12023 was used for construction of reporter and mutant strains. Insertion of Luciferase (*luc*) gen and mutations in phosphotransfer system for mannitol (PTS-Mannitol) were carried out by λ red system. *luc* reporter gen was inserted by replacing the complete coding sequences of *siiE* and STM3690-91. Kinetics were followed, in LB, PCN-Glucose 23,8mM (PCN-G) and PCN-Mannitol 23,8mM (PCN-M) media, during 8 h. for expression assays and 12 h. for growth kinetics. The aliquots were taken over the whole period and processed for measure of the luciferase activity. *S.* sv Typhimurium showed similar growth during the whole time in the three media. PTS-Mannitol mutant strains were unable to grow in PCN-M. Activity of *siiE::luc* fusion showed around 10^6 relative luciferase units (RLU) in LB and PCN-G. In contrast, expression of STM3690-91*::luc* only was around 10^4 RLU with the same media. Nevertheless, expression of STM3690-91*::luc* increased up to 10^5 while *siiE::luc* expression reduced until 10^5 RLU, when both reporter strains were cultured in PCN-M. These results suggest that the kind of the carbon source and not only physical-chemical proprieties can influence the expression of virulence genes *in vitro*. Further studies are in progress to get insight into infection models like invasion in eukaryotic cells when carbon sources are changed and what is the regulatory mechanism.

MTP78
Growth and PHA production of wild-type *Ralstonia eutropha* H16 using glucose and fructose as single carbon substrates

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Ralstonia eutropha H16 is a bacterial species of considerable industrial importance for the production of polyhydroxyalkanoates (PHA) as renewable and biodegradable substitutes of plastic materials.
The study presented here is part of a larger Systems Biology project on PHA metabolism using a combination of mathematical modeling and bioreactor experiments. In a series of cultivation experiments for deriving the initial data sets, some interesting observations were made about biosynthesis and degradation of PHA and particularly concerning the utilizable carbohydrate substrates. Cultivations were performed with either fructose and glucose as single growth substrates, respectively. While fructose is well known to be an appropriate carbon source for *R. eutropha* H16, the utilization of glucose by the wild-type strain was quite surprising. A deficiency of utilizing glucose is commonly considered as a drawback for the industrial application of the *R. eutropha* wild-type strain H16 (DSMZ 428) employed during our study. Hence, in commercial processes, mutated glucose-utilizing strains obtained spontaneously or after mutagenic treatment were applied previously to overcome this limitation. The unexpected growth of the wild-type with glucose was initially observed only after prolonged incubation (> 100 h) at high (2%) glucose concentrations but could be sustained by sub-culturing these cultures also at low (0.2 %) glucose concentrations. While the specific growth rate was much lower than with fructose, the final cell densities and biomass/substrate yields were similar with both substrates. Control experiments were performed to rule out obvious explanations for the unprecedented growth with glucose such as spontaneous mutants and contaminated cultures as well as to confirm the identity of the employed strain. After repetitive sub-culturing in glucose-free complex medium, glucose utilization was again severely attenuated. In conclusion, the observed growth pattern showed the characteristics of a reversible adaptive response to the presence of glucose. The effect proved to be reproducible with three independent strains obtained from different culture collections.
In addition to the unusual growth pattern, dynamic responses of PHA biosynthesis as well as degradation to variations of the ammonia/glucose supply are presented.

MTP79
Metabolome analysis of *P. aeruginosa* in a chronic lung infection model

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Pseudomonas aeruginosa is a ubiquitous environmental soil bacterium and an opportunistic pathogen of humans, animals and plants. It causes chronic infections in patients with cystic fibrosis (CF), chronic obstructive pulmonary disease and bronchiectasis.
The *P. aeruginosa* isolate TBCF10839 is a highly virulent strain from the lungs of a chronically infected CF- patient. It is pilin-deficient, produces large amounts of alginate and survives in polymorphonuclear leukocytes. Being a strong producer of virulence effector proteins, it causes substantial airway pathology in mice after intratracheal instillation. Transcriptome and proteome analyses indicated that TBCF10839 orchestrates many more metabolic and signalling pathways upon exposure to inanimate and animate stressors than the strain PAO1.
To gain insight into the utilized metabolic pathways during a chronic lung infection, we established an *in vitro* lung infection model. We analysed the metabolome and transcriptome of *P.aeruginosa* TBCF10839 in various growth phases. In an integrative approach we combined both data sets to a holistic picture of the adaptive pathway regulation of *P.aeruginosa* in a lung infection to identify key determinants for the chronic colonization of the human lung.

MTP80
Metabolism of adapted *Pseudomonas aeruginosa* isolates recovered from chronic CF lung disease

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In cystic fibrosis (CF), the emergence of *Pseudomonas aeruginosa* (PA) hypermutators drives the selection of isogenic PA variants that are efficiently adapted to the inflamed CF-lung environment.
In previous studies we screened chronically infected CF patients for hypermutable PA strains by a comparative proteome and transcriptome analysis. Investigation of sequential, isogenic non-mutator and mutator isolates revealed several genes that are significant up-regulated in late mutator strains. This suggests changes in the physiology of PA during CF lung survival.
Our special interest belongs to the isocitrate dehydrogenase isoenzymes in PA. There is evidence that *icd* and *idh* gene expression is increased under anaerobic conditions. Therefore we used a biochemical assay to characterize $NADP^+$-dependent isoenzymes. We analysed the specific enzyme activities of ICD and IDH under oxygen-limited conditions in LB as well as in a special medium called ASM that should mimic CF airway secretions *in vitro*. Interestingly, under anaerobic conditions in particular ICD and under aerobic conditions in particular IDH contributes to isocitrate dehydrogenase activity.
In the following we investigate the metabolic flux of ^{13}C-atoms in the metabolism of PA using stable isotopes. With GC/MS experiments we showed the incooporation of isotopes into amino acids. Surprisingly the late mutator strains synthesize new amino acids although the ASM contains a high amount of free amino acids. This indicates changes in the amino acid metabolism of PA.
Furthermore we performed NMR spectroscopy to get a detailed insight into the fatty acid metabolism of PA. We could confirm the results from GC/MS experiments by explaining the labelling patterns of amino acids in particular alanine, glycine, serine and asparagine acid. Beyond that we could show that PA incooporates acetyl-CoA into the nucleoside cytidine. In summary we present at the first time a metabolic pathway for fatty acid degradation in PA.

MTV01
Genetic analysis of carbon monoxide metabolism in *Methanosarcina acetivorans*

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Methanosarcina acetivorans is one of the few members of the methanogenic archaea shown to use carbon monoxide (CO) as the sole source of energy for growth. Carboxidotrophic growth of this organism is peculiar as it involves formation of acetate, formate, and methylsulfides besides methane. A protein homologous to aldehyde dehydrogenases was shown to be >10-fold more abundant under carboxidotrophic than under methylotrophic conditions. To address its role in CO metabolism of *M. acetivorans* the encoding gene was deleted from the chromosome resulting in a mutant unable to tolerate the presence of even small amount of CO. Complementing the mutant with the homologous gene in *trans* rules out polar effects of the mutation introduced and immunological analysis indicates that the protein is present at a rather low level in *M. acetivorans*. These findings suggest that the factor deleted is important in the presence of CO, but probably not involved in catabolism. Prolonged incubation of the mutant in the presence of CO selected a suppressor mutant, which tolerated CO better, but grew more slowly on CO, than the wild type. Analyzing the protein inventory revealed aberrant regulation of methyl-coenzyme M reductase and carbon monoxide dehydrogenase/acetyl-CoA synthase in the suppressor mutant. Together with its metabolite-forming properties a model was developed of how *M. acetivorans* regulates its metabolism in response to CO and funnels carbon into the formation of methane, acetate, formate, and methylsulfides.

MTV02
A bifunctional fructose 1,6-bisphosphate aldolase/phosphatase - the ancestral glucogenic enzyme

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Most archaeal groups and deeply branching bacterial lineages harbour thermophilic organisms with a chemolithoautotrophic metabolism. They live at high temperatures in volcanic habitats at the expense of inorganic substances, often under anoxic conditions, features that may reflect the conditions of primordial life [1]. These autotrophic organisms use different carbon dioxide fixation mechanisms generating acetyl-CoA, from which gluconeogenesis must start [2-4].
We show that virtually all Archaea as well as the deeply branching bacterial lineages (*Aquificales, Thermoanaerobacterales, Thermotogales, Chloroflexales-Dehalococcoidetes*, and the *Deinococcus-Thermus* group) contain a bifunctional fructose 1,6-bisphosphate (FBP) aldolase/phosphatase with both FBP aldolase and phosphatase activity. This enzyme is missing in most other Bacteria and in Eukarya and is heat-stabile even in mesophilic marine Crenarchaeota. Its bifunctionality ensures that heat-labile triosephosphates are quickly removed and trapped in stabile fructose 6-phosphate rendering gluconeogenesis unidirectional.
We propose that this highly conserved, heat-stabile and bifunctional FBP aldolase/phosphatase represents the pace-making ancestral glucogenic enzyme. This finding provides further support to the theory of a chemolithoautotrophic origin of life [1], in with glucogenesis preceded glycolysis [5].

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MTV03
The Stringent Control in *Staphylococcus aureus*

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In most bacteria nutrient limitation provokes the stringent control by rapid synthesis of the alarmones pppGpp and ppGpp. (p)ppGpp synthesis is linked to repression of rRNA synthesis and other physiological changes involving gene activation/repression, protein translation, enzyme activation and replication. Whereas many of the basic research was performed with <i>E. coli there is now growing evidence that in gram-positive bacteria there are fundamental differences in (p)ppGpp synthesis, regulation and molecular function. Sequence analysis reveals that in most firmicutes three (p)ppGpp synthetase (Rsh, RelP and RelQ) are present. Rsh enzymes are bifunctional enzymes composed of a sensory domain, a (p)ppGpp synthase and a hydrolase domain. In *S. aureus* the essentiality of the bifunctional enzyme Rsh can be explained by the missing hydrolase activity and thus the Rsh independent toxic accumulation of (p)ppGpp due to RelP and RelQ. This was shown by analysis of site-specific mutants (conditional rsh-knockout, rsh-synthase, relP and relQ) and combinations thereof in different S. aureus strains. (p)ppGpp and the nucleotide pool was determined by thin-layer chromatography and HPLC. The rsh-synthase mutant showed no detectable (p)ppGpp accumulation upon mupirocin induction, indicating that only Rsh is activated by uncharged tRNA. Because mupirocin induces accumulation of (p)ppGpp in a high and unphysiological manner and to exclude potential additional side-effects of the antibiotic mupirocin, the stringent response was also induced by depletion of essential amino acids in the growth medium. Induction resulted in accumulation of (p)ppGpp and ppGpp in the wild type but not in the rsh synthase mutants. The rsh mediated stringent control is analysed using wild type and the rsh mutants under stringent and relaxed conditions by microarrray analysis. Furthermore it could be shown that the rsh mutant is less virulent in an animal model of infection.

MTV04
Identification and Characterization of the Maltose Uptake System MusKEFG of *Corynebacterium glutamicum*

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Corynebacterium glutamicum, a Gram-positive bacterium known for its use in large-scale production of amino acids, co-metabolises most carbon sources. Moreover, addition of maltose to glucose containing media raises expression of *ptsG* (encoding the glucose specific EII permease of the PTS) and increases the glucose utilisation of *C. glutamicum*. However, a system catalysing maltose uptake has not been identified in *C. glutamicum*. The permease EII^{Glc} itself is not required for maltose metabolism; deletion of *ptsG* did not cause any decrease of maltose uptake rates.
We here present the identification of the ATP-binding cassette transporter encoded by *cg2708, cg2705, cg2704* and *cg2703* as the maltose uptake system of *C. glutamicum* by combination of data derived from the biochemical characterisation of maltose uptake with transcriptome data. Deletion of the genomic locus comprising ORF´s *cg2708* to *cg2703* indeed abolished maltose uptake. Furthermore inactivation of the single genes *cg2705* (encoding the maltose-binding protein MusE), *cg2704* and *cg2703* (encoding the permease components MusF and MusG) caused a complete loss of maltose uptake and inactivation of *cg2708*(encoding the ATP-hydrolyzing subunit MusK) drastically impaired maltose utilisation. Analysis of the transcriptional organisation of these genes by Northern Blot and RT-PCR indicated, that the components of the MusKEFG ABC-transport system are located on 3 different transcripts, the transcriptional regulation is under investigation.
From our findings we conclude, that maltose utilization proceeds via a pathway involving maltose uptake by the ABC-transporter MusKEFG, maltodextrin and glucose formation by a 4-α-glucantotransferase, glucose phosphorylation by glucokinase, and maltodextrin degradation via the reactions of maltodextrin phosphorylase and phosphoglucomutase.

MTV05
Pyrrolysine in $tRNA^{His}$ guanylyltransferase: an unusual amino acid in a usual place

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A key recognition element of $tRNA^{His}$ by the histidyl-tRNA synthetase is a G-1 base that is added to 5' end of premature $tRNA^{His}$ by the catalytic action of $tRNA^{His}$ guanylyltransferase. Failure to add the G-1 base leads to an inability to efficiently form His-$tRNA^{His}$ and decode histidine codons during ribosomal translation. While the enzyme has been extensively studies in yeast, the archaeal Thg1 enzyme is so far uncharacterized. Surprisingly, the *Methanosarcina acetivorans* Thg1 gene includes an in-frame TAG (amber) codon. While typically a stop codon, in mRNA transcripts of *M. acetivorans* and related species the UAG codon can be translationally read-through to support the incorporation of the rare 22nd genetically encoded amino acid, pyrrolysine (Pyl). So far only three Pyl-containing enzymes have been described in some detail. We here represent the characterization of *M. acetivorans* Thg1, verifying the *in vivo* incorporation of Pyl into the enzyme, the role of Pyl within this enzyme and biochemical properties of Thg1. Phylogenetic and biochemical analysis suggest that Pyl appeared as a result of neutral evolution, being of neither advantage nor disadvantage to the enzyme, indicating that Pyl can act as a regular amino acid.

MTV06
Riboswitch-mediated control of gene expression enhances L-lysine formation in *Corynebacterium glutamicum*

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Natural riboswitches are RNA receptors which typically reside in 5' untranslated regions of specific mRNAs. They are composed of two domains and act as genetic control elements. The aptamer domain selectively binds the target metabolite whereas the expression platform interprets the binding status of the aptamer domain and mediates changes in gene expression [1]. More than 20 distinct classes of natural RNA aptamers are currently known, recognizing a diverse array of fundamental metabolites including coenzymes, nucleobases, an aminosugar, as well as the amino acids glycine and L-lysine [2]. The ability of riboswitches to conditionally regulate gene expression has been exploited by several approaches [1,3].
In this study we investigate the potential of the L-lysine riboswitch of *Bacillus subtilis* as a metabolic engineering tool in L-lysine production with *Corynebacterium glutamicum* (~850,000 t/a) [4]. Northern blot analysis and enzyme assays indicated that the *B. subtilis* L-lysine riboswitch is functioning in *C. glutamicum*. The L-lysine riboswitch-dependent regulation proceeds through a termination/antitermination mechanism that results in enhanced gene expression at low L-lysine concentrations and inhibited expression at high L-lysine concentrations. Integration of the L-lysine riboswitch upstream of the citrate synthase gene *gltA* in a genetically defined *C. glutamicum* lysine-producing strain DM1800 derivative had no effect on the growth rate and enhanced $g_{lysine}/g_{glucose}$ by about 30%.

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MTV07
Towards the reaction mechanism of sulfur oxygenase reductases

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The soluble sulfur oxygenase reductase (SOR) is the initial enzyme in aerobic sulfur oxidation of the archaeon *Acidianus ambivalens*. The globular, hollow 24mer catalyzes the O_2-dependent sulfur disproportionation to sulfite and H_2S. The active site comprises a mononuclear non-heme iron center with a low reduction potential and a cysteine, which becomes persulfurated upon heat treatment. The active site is surrounded by a hydrogen bonding network probably contributing to the low reduction potential. Two pores provide substrate access to the active site. The first pore is formed by chimney-like structures at the fourfold symmetry axes of the outer shell, providing access to the inner cavity. The second pore provides entrance to each of the active site pockets separately. A highly increased enzyme activity was obtained in deletion mutants of the chimney-like structures. Mutation of Met residues placed at the entrance to the active site pocket resulted in a decrease or increase of enzyme activities, depending on the substitution. The reaction products presumably exit the sphere via polar channels located at the threefold symmetry axes. An increased enzyme activity was obtained when two outlet-forming residues at the end of the channel are replaced with alanine. In order to determine whether the active site iron undergoes a valence change during catalysis we exchanged it for other metal ions. The activity of a Co^{2+}-substituted SOR was similar to the wildtype Fe enzyme. An active Ga^{3+}-substituted SOR showed that a valence change is not required and that oxygen is activated rather by the substrate than by the metal. Recently, the genome sequence of the bacterium *Halothiobacillus neapolitanus* revealed the first *sor*-gene from a mesophile. The protein showed SOR activity after gene expression in *E. coli* between 20 and 90°C. The biochemical properties of this SOR will be discussed with respect to the general reaction mechanism.

MTV08
The *Allochromatium vinosum* DsrMKJOP transmembrane complex

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In the purple sulfur bacterium *Allochromatium vinosum* the degradation of sulfur globules, formed during the oxidation of sulfide or thiosulfate, is strictly dependent on the proteins encoded in the *dsr* operon. The DsrMKJOP proteins form a membrane-spanning complex proposed to be involved in electron transfer across the membrane [1]. Here, we report analysis of DsrMKJOP in *A. vinosum* and the production of individual components in *E. coli* and their biochemical characterization.
DsrJ is predicted to bind three hemes *c* with bis-His, His/Met and His/Cys coordination, respectively [2]. Cysteine 46 is the predicted heme iron ligand for the His/Cys coordinated heme. Site directed mutagenesis revealed that it is essential for the degradation of internally stored sulfur *in vivo*. The signalpepide of DsrJ seems to serve as membraneanchor in *A. vinosum*. When produced in *E. coli*, DsrJ appeared as a three heme cytochrome. DsrM and DsrP are predicted to be integral membrane proteins with five and ten transmembrane helices, respectively. While DsrM is predicted to carry two hemes *b*, it was so far believed that DsrP does not contain any cofactors. However, when purified from *E. coli*, both proteins were clearly identified as *b*-type cytochromes. Both proteins can be partly reduced with menadiol. Redox potentiometric analyses revealed a lower redox potential for the hemes in DsrJ as compared to those of DsrM implicating electron flow from the periplasm into the cytoplasm. DsrK is related to the catalytic subunit of heterodisulfide reductase of methanogenic archaea and is proposed to contain three [4Fe-4S] clusters. When produced in *E. coli*, DsrK is exclusively found in the membrane fraction. UV-VIS spectroscopy suggests the presence of FeS clusters in purified DsrK.

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[2] Pires *et al.* (2006) Biochemistry 45,249-262

MTV09
Catabolism of 3-sulfinopropionate, a sulfur containing structural analogue of succinate, in *Advenella mimigardefordensis* strain DPN7^T

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3-Sulfinopropionate (3SP) was identified as an intermediate during degradation of 3,3' dithiodipropionate in *Advenella mimigardefordensis* strain DPN7^T. 3SP is a structural analogue of succinate in which one carboxyl group is exchanged by a sulfino group. Previously, it was described as a degradation product of homohypotaurin, an inhibitor of nervous permission [1]. In the past, it was also considered as a promising antiradiation drug [2, 3]. Only little is known about its catabolism in bacteria, yet. Tn*5*::*mob* mutagenesis of *A. mimigardefordensis* strain DPN7^T yielded several mutants defective in 3SP degradation. Genotypic characterisation of these mutants identified four disrupted open reading frames (ORFs) with putative metabolic functions. These ORFs were assigned function on the basis of homologies with genes coding for a succinyl-CoA synthetase (SucCD), an acyl-CoA dehydrogenase (CaiA), an enoyl-CoA dehydratase (CaiD) and an alkanesulfonate monooxygenase (SsuD) homologue. In one of these mutants, accumulation of 3-sulfino-2,3-dehydropropionate in the culture supernatant was observed, if incubated in mineral salt medium with 3SP plus propionate as carbon sources. Based on these results, a putative degradation pathway was proposed. As confirmed by enzyme assays, 3SP is initially activated to the corresponding 3SP-CoA thioester by SucCD. Subsequently, CaiA could oxidize 3SP-CoA in an FAD-dependent reaction to the corresponding 3 sulfino-2,3-dehydropropionyl-CoA thioester. Addition of H2O to the double bond is probably catalysed by CaiD yielding 3-sulfino-3-hydroxypropionyl-CoA. In the final step the SsuD homologue could abstract the sulfur and release it as sulfite.

[1] Beart, P. M., and Johnston, G. A. R. (1973) J. Neurochem. 20, 319-324.
[2] Srivastava, P. K., and Field, L. (1975) J. Med. Chem. 18, 798-802.
[3] Sweeney, T. R. (1979) Survey of Compounds from the Antiradiation Drug Development Program of the U.S. Army Medical Research and Development Command, Walter Reed Army Institute of Research: Washington, DC.

MTV10
Characterization of Nitrate Respiration in the Genus *Streptomyces*

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Members of the genus *Streptomyces* are obligately aerobic, high-GC gram-positive, filamentous Actinobacteria that are found predominantly in soil and on decaying vegetation. Typically, they are characterised by a complex life cycle and secondary metabolism. Their life cycle includes growth as vegetative hyphae and the generation of aerial hyphae, which result in exospore production.
Although an obligate aerobe, the genome of *S. coelicolor* has three copies of the *narGHJI* operon, which encodes membrane-associated respiratory nitrate reductase (Nar), an enzyme normally associated with anaerobic metabolism in other bacteria. These enzymes possess a bis-molybdopterin guanine dinucleotide (bis-MGD) cofactor and comprise three subunits. Orthologues of *narGHJI* are present in other *Streptomyces* species, e.g. *S. scabiei*, *S. lividans* and *S. kanamyceticus*.
Using defined knock-out mutants we demonstrated that all three Nar enzymes of *S. coelicolor* are synthesised and active in different tissues. By measuring nitrite accumulation throughout the life cycle, together with the development of chlorate-survival assays (reduced to toxic chlorite by Nar) and the determination of Nar specific enzyme activity, we could show that these enzymes are not redundant. Surprisingly, two of the Nar enzymes (Nar 2 and Nar 3) were active during aerobic cultivation, whereas the Nar enzyme 1 was only active in anaerobically stressed spores. Triple mutants lacking all three Nar enzymes and a mutant unable to synthesise the bis-MGD cofactor were completely deficient in nitrate reduction.
Analysis of the nitrite-production profiles of *S. scabiei* and *S. lividans* together with previous observations [1] reveal that nitrate respiration in general, and the ability to carry out aerobic nitrate reduction, is widespread in this genus.

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MTV11
Reconstitution of quinone-depleted Na+-translocating NADH:quinone oxidoreductase (Na+-NQR) from *Vibrio cholerae* with quinones

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The Na+-translocating NADH:quinone oxidoreductase (Na+-NQR) from the marine and human pathogen *Vibrio cholerae* is a redox driven sodium pump catalyzing NADH oxidation and ubiquinone reduction [1]. The redox process is coupled to the export of sodium ions out of the cell, generating thereby a transmembrane electrochemical potential. Na+-NQR consists of six subunits, NqrA-NqrF, and contains one [2Fe-2S] cluster, two covalently bound FMNs, one noncovalently bound FAD, one riboflavin (RF) and one tightly bound ubiquinone-8 as prosthetic groups. Detergent exchange from dodecylmaltoside to N,N-Dimethyldodecylamine-N-oxide (LDAO) with His6-tagged Na+-NQR bound to the affinity column resulted in loss of the endogenous quinone from the enzyme. Subsequently, the quinone-depleted Na+-NQR was reconstituted with different quinones. Successful reconstitution of quinone-1 and quinone-2 was confirmed by quinone extraction and HPLC. Reconstituted Na+-NQR as well as quinone-depleted Na+-NQR showed high activity. It is concluded that endogenous quinone can be replaced by artificial ones. This paves the way for analysis of the quinone binding site in Na+-NQR.

[1] Tao, M., Casutt, M. & Steuber, J. Oxidant-induced formation of a neutral flavosemiquinone in the Na(+)-translocating NADH:Quinone oxidoreductase (Na(+)-NQR) from *Vibrio cholerae*. Biochim Biophys Acta. 1777, 696-702 (2008)

MTV12
A highly economic electron transport chain involving novel primary Na^+ pumps in the acetogenic bacterium *Acetobacterium woodii*

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The anaerobic acetogenic bacterium *Acetobacterium woodii* can grow by caffeate respiration that is coupled to the generation of an electrochemical Na^+ gradient across the cytoplasmic membrane [1]. Here we report the entire enzymology, electron flow and bioenergetics of this novel type of anaerobic respiration. Electron flow from ferredoxin to caffeate involves several enzymes and mechanisms of energy conservation: (I) soluble ferredoxin reducing oxidoreductases, (II) a membrane-bound ferredoxin: NAD^+-oxidoreductase (Rnf) [2], (III) a putative electron bifurcation that yields reduced ferredoxin via a acyl-CoA dehydrogenase/Etf complex [3], (IV) an initial activation of caffeate to caffeyl-CoA that yields pyrophosphate. Experiments using inverted membrane vesicles revealed that hydrolysis of pyrophosphate is catalyzed by a membrane-bound, ^{22}Na-translocating pyrophosphatase. Thus, energy stored in the anhydride bond is used to generate a transmembrane Na^+ potential. (V) After the initial activation step, subsequent activation of caffeate is by CoA transfer from hydrocaffeyl-CoA. In summary, caffeate respiration requires initial ATP-dependent activation of the electron acceptor but energy is saved by a Na^+ pyrophosphatase. In the steady state of caffeate respiration, caffeate activation by the CoA loop is without net energy input. The only ion (Na^+) pump in the pathway is the Rnf complex that is fueled by reduced ferredoxin generated by substrate oxidation or possibly electron bifurcation.
These findings underline the importance of the Rnf complex in caffeate respiration in *A. woodii* and in bioenergetically linking the cellular ferredoxin to NAD^+ pools.

[1] Imkamp, F. and Müller, V. (2002). Chemiosmotic energy conservation with Na^+ as the coupling ion during hydrogen-dependent caffeate reduction by *Acetobacterium woodii*. J. Bacteriol. 184: 1947-1951
[2] Biegel, E., Schmidt, S. and Müller, V. (2009). Genetic, immunological and biochemical evidence for a Rnf complex in the acetogen *Acetobacterium woodii*. Environ. Microbiol. 11: 1438-1443
[3] Herrmann, G., Jayamani, E., Mai, G., and Buckel. W. (2008). Energy conservation via Electron-transferring flavoprotein in anaerobic bacteria. J. Bacteriol. 190: 784-791

MTV13
Life without glycolysis: Construction of glycolytic mutants in Bacillus subtilis

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Glycolysis is a central metabolic pathway for the utilization of glucose and other sugars in most heterotrophic organisms. The main aims of glycolysis are the conservation of energy, the synthesis of precursors for the amino acid synthesis, and the feeding of the Krebs cycle.
The *B. subtilis* genome codes for around 4100 proteins. A study in *B. subtilis* revealed that 271 genes are indispensible for growth [1,2]. Among these essential genes are almost all genes that code for glycolytic enzymes. However, it was unknown why these genes should be essential, while other metabolic pathways might have the capability to compensate the loss of several glycolytic enzymes on rich media. Previously we have shown that glycolytic enzymes contribute to essential interactions in *B. subtilis*, as shown for the RNA degradosome [3]. In addition, we discovered that each glycolytic gene can be deleted and that even strains with combinations of mutations are still viable.
Furthermore, we found that a mutant lacking the gene *gapA*, that codes for the glyceraldehyde-3-phosphate dehydrogenase, is able to grow in minimal media with glucose as single carbon source. The reason for this surprising phenotype will be presented. In addition we will explain which conditions are necessary for the construction and growth of glycolytic mutants.

[1] Kobayshi *et al*., 2003. Proc Natl Acad Sci U S A 100: 4678-83.
[2] Thomaides *et al*., 2007. J Bacteriol 189:591-602
[3] Commichau *et al*., 2009. Mol Cell Proteomics 8:1350-60.

MTV14
The *Pseudomonas aeruginosa* metabolome

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Pseudomonas aeruginosa possesses broad metabolic capacity allowing this ubiquitous microorganism to successfully adapt to diverse habitats. Uncovering the complexity of regulatory processes that account for high metabolic versatility in *P. aeruginosa* has been the aim of many transcriptome and proteome studies. Metabolome analysis, as a third complementary approach, is a promising tool for evaluation of metabolic fluxes. Metabolomics integrates metabolic data into the global regulatory network.
In order to elucidate new aspects of metabolic versatility in P. aeruginosa, we investigated the metabolome profiles of reference strain PAO1 and strain TBCF10839 in exponential growth and stationary phase under six different carbon source conditions, i.e. succinate, citrate, glucose, cadaverine, tryptone and casamino acids. Analyses based on gas chromatography coupled to mass spectrometry led to the identification of 271 metabolites.
Strains PAO1 and TBCF10839 exhibited strong similarities in mode of growth; only few individual properties relate to distinct metabolic standards imposed by strain-specific lifestyles. The intracellular metabolic profiles of strains were found to be driven by nutrient-specific impacts, especially during the early growth phase. In contrast, strain-specific metabolism of secondary metabolites led to less congruence between strains during stationary phase. In both strains constitutively core metabolic activity was observed which was not affected by carbon source induced adaptation processes. Maintenance of a stationary state of core metabolism appears to be the key feature that enables metabolic versatility in *P. aeruginosa*.

MTV15
Investigations into the metabolism of *Legionella pneumophila*

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Legionella pneumophila is an aquatic bacterium that replicates within protozoa (amoeba), but can also infect human alveaolar macrophages causing Legionnaires' disease, an atypical pneumonia. Many virulence factors of *L. pneumophila* have been reported, but less is known about nutrition of the bacteria, especially inside host cells. When nutrients become limiting, a regulatory casdade triggers the differentiation from the replicative form, with high metabolic activity, to the transmissive form. *L. pneumophila* uses amino acids as primary energy and carbon sources; glucose although assimilated is thought not to be important for bacterial growth. However, genes involved in glucose catabolism are mainly transcribed during the exponential growth phase and are organized in several cistrons. Via ^{13}C-isotopologue profiling in combination with magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) we demonstrated the use of glucose for *de-novo* biosynthesis of several amino acids. We found high ^{13}C-incorporation rates for the amino acids alanine, aspartate, glutamate, glycine, and proline as well as for 3-hydroxybutyrate in *L. pneumophila*. The tricarboxylic acid cycle is complete and active. Furthermore, experiments with [1,2-$^{13}C_2$]glucose indicate the importance of the pentose phosphate pathway for glucose catabolism. Moreover, we present an experimental background for *in-vivo* studies of the bacterial metabolism inside *Acanthamoeba castellanii* host cells.

MTV16
The central carbohydrate metabolism of *Sulfolobus solfataricus* in response to temperature changes - The "SulfoSYS" project -

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The thermoacidophilic crenarchaeote *Sulfolobus solfataricus* has become an important archaeal model organism for physiology and biochemistry, comparative and functional genomics, as well as, quite recently also for Systems Biology approaches [1]. *S. solfataricus* optimally grows at 80°C and a pH of 2-4 [2]. The organism is a strict aerobe and grows heterotrophically on various organic compounds as sole carbon and energy sources (several sugars, amino acids and peptides; [3]).
Within the integrated SulfoSYS (*Sulfolobus* Systems Biology) project (http://www.sulfosys.com/; [1]), details of regulation and energetics of the central carbohydrate metabolism (CCM) in response to temperature changes are addressed by the assembly and integration of "-omics" (genomic, transcriptomic, proteomic, and metabolomic), kinetic and biochemical data of the CCM genes and enzymes. Standard operating procedures (SOPs) for all wet-lab and *in silico* techniques have been established [4] and applied in first experimental rounds comparing growth of *S. solfataricus* at three different temperatures (65, 70 and 80°C). The effects of temperature variation on the CCM network, especially the branched Entner-Doudoroff pathway [5] of *S. solfataricus* as well as first CCM models will be presented.

[1] Albers SV *et al.* (2009) Biochem. Soc. Trans. 37, 58–64
[2] Zillig W *et al.* (1980) Arch. Microbiol. 125, 259-269
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[4] Zaparty M *et al.* (2009) Extremophiles, Epub ahead of print, DOI: 10.1007/s00792-009-0280-0
[5] Ahmed H *et al.* (2005) Biochem. J. 390, 529-540

MTV17
Metabolite Profiling Uncovers Plasmid-Induced Cobalt Limitation under Methylotrophic Growth Conditions

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During growth with methanol cells of *Methylobacterium extorquens* AM1 carrying one of the expression plasmids pCM80 or pCM160 exhibit a large reduction in growth rate of about 50%, relative to the wild-type. A less pronounced growth delay during growth under non-methylotrophic growth conditions was observed; this suggested an inhibition of one-carbon metabolism rather than a general growth inhibition or metabolic burden. Metabolome analyses revealed an increase in pool sizes of ethylmalonyl-CoA and methylmalonyl-CoA of more than 6- and 35-fold, respectively, relative to wild type. This suggested a strongly reduced conversion of these central intermediates, which are essential for glyoxylate regeneration in this model methylotroph. These intermediates of the ethylmalonyl-CoA pathway have in common their conversion by coenzyme B_{12}-dependent mutases, which have cobalt as a central ligand. The one-carbon metabolism-related growth delay was restored by providing higher cobalt concentrations, by heterologous expression of isocitrate lyase as an alternative path for glyoxylate regeneration, or by identification and overproduction of proteins involved in cobalt transport through the outer membrane. Downregulation of this cobalt uptake system caused by different antibiotics used for plasmid selection is probably the underlying mechanism behind this phenomenon.

MTV18
Proteome and protein complexes of anammox bacteria

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Anammox bacteria combine ammonium with nitrite to produce dinitrogen gas. They can be applied in waste water treatment systems, and were shown to contribute significantly to loss of fixed nitrogen in the ocean. In order to gain more insight into the mechanism of anammox, the genome, transcriptome, proteome and protein complexes of the anammox bacteria Kuenenia stuttgartiensis were studied. The 4.2 Mb genome of Kuenenia stuttgartiensis was originally assembled after sequencing of appropriate BAC, fosmid and shot gun libraries (Strous et al 2006), and recently confirmed by high throughput sequencing. Protein extracts of Kuenenia single cells were analyzed after separation on SDS-PAGE gels, trypsin digestion and LC-MS/MS analysis. Protein complexes were isolated using blue native and SDS PAGE gels and maldi tof mass spectrometry. Of the 4664 genes in genome, more than 1100 could be matched to proteins in the proteome. These included the genes encoding catabolic proteins most likely involved in nitrogen and carbon metabolism of anammox : nitrate reductase, nitrite reductase, hydrazine synthase, hydrazine oxidase, and carbon monoxide dehydrogenase (Jetten et al 2009). Most of the proteome data could be confirmed by transcriptome analysis of anammox mRNA.

[1] Strous M *et. al.* (2006) Deciphering the evolution and metabolism of an anammox bacterium from a community genome. Nature 440: 790-794.
[2] Jetten MSM, *et. Al.* (2009) Biochemistry and molecular biology of anammox bacteria. Crit Rev Biochem Mol Biol 26: 1-20.

MTV19
Anaerobic toluene metabolism: Biochemistry of benzylsuccinate synthase and its activating enzyme

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Anaerobic degradation of toluene in *Thauera aromatica* is initiated by an unusual addition reaction of the toluene methyl group to the double bond of a fumarate cosubstrate to form the first intermediate (*R*)-benzylsuccinate. This reaction is catalyzed by the glycyl radical enzyme benzylsuccinate synthase. All enzymes required for the degradation of toluene to (*R*)-benzylsuccinate are encoded in the toluene-inducible *bss*-operon. The bssDCAB genes code for the subunits of benzylsuccinate synthase (BssA, B and C) and an additional enzyme (BssD) showing strong similarity with activating enzymes needed for radical formation in other glycyl radical enzymes. The three subunits of benzylsuccinate synthase are arranged in a $\alpha_2\beta_2\gamma_2$ composition, whereas the large α-subunits contain the essential glycine and cysteine residues that are conserved in all glycine radical enzymes. We present new results concerning the mechanism of benzylsuccinate synthase and a first characterization of its activating enzyme.

MTV20
New aerobic aromatic metabolism – degradation of phenylacetic acid

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Aromatic compounds represent the second most abundant source of organic substrates besides carbohydrates. We describe a widespread aerobic catabolic pathway in *Escherichia coli* and *Pseudomonas sp. Y2* for phenylacetic acid, a central key intermediate in the degradation of phenylalanine and many other aromatic substances [1, 2]. In this route, intermediates are processed as CoA-esters. The aromatic ring becomes activated to a highly reactive epoxide, which is isomerized to a seven-membered oxygen containing ring (oxepin) before undergoing hydrolytical ring-cleavage. Further steps resemble β-oxidation leading to the central metabolites acetyl-CoA and succinyl-CoA. Conserved genes encoding enzymes of this pathway are present in 22 % of all sequenced bacterial genomes so far. This includes members of Proteobacteria, Thermus/Deinococci and Gram-positive bacteria. The discovered strategy for aromatic degradation closes a gap in our understanding of how bacteria exploit inert substrates.

[1] Ismail, W. *et al.* (2003) Eur. J. Biochem. 270, 3047-3054
[2] Alonso, S. *et al.* (2003) Gene 319:71-83

MTV21
The role of geraniol dehydrogenase in anaerobic monoterpene degradation by the Betaproteobacterium *Castellaniella defragrans* 65Phen

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Monoterpenes are climate relevant components mainly emitted by trees, which plays a role in ozone production and aerosole formation after oxidation in the atmosphere. However, they are also present in soils, where microorganisms use monoterpenes as substrates for both, aerobic and anaerobic growth. *Castellaniella defragrans* , which metabolizes phellandrene, belongs to the latter one. The detailed degradation pathway still needs to be elucidated.
After completion of a genetic system for *C. defragrans* 65Phen we obtained a deletion mutant for a geraniol dehydrogenase involved in the monoterpene metabolism. Based on the position in a fosmid sequence, the gene was provisionally named ORF31 and the deletion mutant *C. defragrans* ΔORF31. Physiological growth studies revealed a retarded growth and a decrease in biomass production of the deletion mutant in comparison to the wildtype with phellandrene as substrate, but not with acetate. This may be explained by an intracellular accumulated geraniol pool having a toxic effect in cells of *C. defragrans* ΔORF31. Geraniol at high concentrations is toxic for the bacterium.
Cloning of ORF31 in the expression vector pET42a led to overexpression of the geraniol dehydrogenase in the host strain *E. coli* BL21 Star™ (DE3) under control of the T7lac-promoter. IPTG induction resulted in a 40 kDa protein, which correlated to the predicted size of 37 kDa for ORF31. Crude extract of *E. coli* BL21 Star™ (DE3) cells overexpressing ORF31 showed a geraniol-dependent NAD reduction, which not occurred in crude extract of host strain cells containing the original vector. We currently develop the purification of the geraniol dehydrogenase from the overexpressing strain for detailed enzymatic characterization.

MTV22
Properties of BamBC, the prototype of the tungsten-containing class of benzoyl-CoA reductases

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Benzoyl-CoA reductases (BCR) are key enzymes in the anaerobic degradation of aromatic compounds and catalyze the two-electron reduction of the aromatic ring to a cyclic dienoyl-CoA. The are two different classes of BCR enzymes: facultative anaerobes use a 3x[4Fe-4S] clusters containing ATP-dependent BCR, whereas in strictly anaerobic bacteria an ATP-independent BCR complex, encoded by the benzoate-induced *bamBCDEFGHI* genes is involved [1]. We report on the first isolation and characterization of BamBC, the prototype of the active site containing components of the ATP-independent class of BCRs from *Geobacter metallireducens*. The enzyme had an α2β2 composition and contained 0.9 W, 15 Fe, 2.2 Ca and 1.0 Zn per αβ module. EPR and cofactor analysis suggested the presence of a W-bis-MGD-pterin, 3 [4Fe 4S], and one [3Fe-4S] cluster per αβ-unit. The cofactors could only be reduced by dienoyl-CoA but not with dithionite or Ti(III)-citrate. Low potential viologens served as electron acceptors in the backward reaction suggesting that BamBC transfers electrons on an extremely negative redox potential. Unusual kinetic properties of BamBC are presented.

[1] Wischgoll *et al.* (2005), Mol Microbiol. 58(5):1238-52
[2] Kung *et al.* (2009), PNAS 106:17687-92

MTV23
Catabolic gene landscape of novel benzene degrading Pseudomonas strains

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Isolates capable of mineralizing either both benzene and toluene or only benzene harbouring catabolic genes were isolated from a benzene contaminated site in the Czech Republic. Two isolates designated Pseudomonas sp. strain 1YB2 and Pseudomonas sp. strain 1YdBTEX2 highly related to *Pseudomonas veronii* as deduced by 16S rRNA gene sequencing were chosen for further analysis. They are of particular interest because they harbour slightly different types of isopropylbenzene dioxygenases, where sequencing of a central gene fragment indicated them to be responsible for different substrate specificity and they also contain slightly different catechol 2,3-dioxygenases of a subgroup not previously associated with the degradation of hydrophobic aromatic pollutants and are devoid of those typically associated with isopropylbenzene dioxygenases. Thus, these isolates may carry novel gene organisations which have been selected for by the environmental pressures present in such a pollutant site and represent key players for degradation of benzene and toluene. To investigate those genes of interest, genomic DNA of the strains was isolated, a fosmid library prepared in pCC1FOS, screened for the presence of catabolic genes followed by sequencing of the catabolic operons revealing novel and different gene organisations. Expression studies are underway to confirm differences in substrate specificity of encoded isopropylbenzene-type dioxygenases. To further assess those possible determinants of adaptation of the strains to the contaminated site, a novel catabolome array was used to identify the catabolic gene landscape of both strains and their response to pollutant stress. This work presents strategies by the microbial community to adapt under pressure and transform pollutants in a manner which has yet to be described until now.

MTV24
Discovery of methanoferredoxin in *Methanosarcina mazei*: a new class of superoxide reductase

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Methanogens are generally considered to be sensitive towards aeration with oxygen. However, it has been suggested that methanogens are surprisingly oxygen stable and can indeed survive exposure to air for several hours. *Methanosarcina* appears to be the most aerotolerant genus among the methanogens. The ability to cope with oxidative stress is consistent with the finding that methanogens are widespread in habitats that are periodically exposed to air, like paddy soils.
The gene *mm0632* from *Methanosarcina mazei* was heterologously expressed in *E. coli* and the purified protein showed superoxide reductase activity, rapidly converting superoxide to peroxide. Superoxide reductase activity was determined in two assays. One assay used rubredoxin as an electron donor, while the other used cytochrome *c*. MM0632 exhibited superoxide reductase activity with either electron donor. Additionally, electron paramagnetic resonance analysis showed that MM0632 is the first superoxide reductase that possesses a 4Fe4S cluster. We propose the name methanoferrodoxin for this new class of superoxide reductase. This class is characterized by a $FeCysHis_4$ catalytic center and a 4Fe4S cluster as a second prosthetic group.

PRP01
Host range of vanA plasmids encoding transferable vancomycin resistance

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The most prevalent type of acquired glycopeptide resistance is the vanA cluster type and their reservoir is in *E. faecium*. vanA is mainly located on transferable plasmids and why prevalence is mostly limited to *E. faeciu*m is not known. In the present study we investigated *in vitro* transferability of pre-characterized vanA plasmids (n= 16; various clinical and non-clinical strains, different countries) into several enterococcal, lactobacterial, lactococcal, staphylococcal and bifidobacterial recipients. Filter-mating was performed with exponentially grown donor and recipient cells. Mating rates per donor and recipient cells were calculated. All experiments were performed in triplicate (3 filters) to limit processing and pipetting variations. Altogether 94 (x3) enterococcal matings and 102 inter-genus matings were performed (ermB instead of vanA plasmids [same plasmid group] were used for *S. aureus* matings). Results could be summarized as follows: Transfer is highest and if ever successful within the same species and far less frequent, if ever, beyond species barriers. None of the lactobacterial, lactococcal, bifidobacterial and staphylococcal recipients showed acquisition / maintenance of enterococcal resistance plasmids; there was only one exception - *E. faecalis* plasmid pRE25 (inc18 group) was successfully transferred into *S. aureus* RN4220RF with very low frequency. Selected enterococcal transconjugants were daily passaged for 4 weeks in liquid broth without antibiotics. Counts of resistant vs. susceptible cells were identical. Growth experiments in rich media did not show substantial differences between transconjugant and recipient cells.
In conclusion, *E. faecium* vanA plasmids showed a very narrow host range mostly limited to the same species. Acquired plasmids were stably maintained for generations in the new host also in the absence of antibiotic selective pressure and preliminary growth experiments did not show a fitness loss after plasmid acquisition.

PRP02
Comparative analysis of porcine MRSA ST398 isolates

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Objectives: Fifty-four MRSA ST398 isolates from diseased swine collected all over Germany were investigated for their genetic relatedness and their resistance and virulence properties.
Methods: Minimum inhibitory concentrations of 30 antimicrobial agents were determined by broth microdilution. Resistance and virulence genes were detected via a diagnostic DNA microarray or PCR. The isolates were subjected to SCC*mec* typing, *Apa*I- and *Sma*I-PFGE, and *spa* typing.
Results: All 54 isolates were tetracycline-resistant via the resistance genes *tet*(M)+ *tet*(K) (n=40), *tet*(M)+*tet*(K)+*tet*(L) (n=11) as well as *tet*(M), *tet*(L), or *tet*(M)+ *tet*(L) in single isolates. Among the 28 trimethoprim-resistant isolates 14 and nine harboured the genes *dfrK* and *dfrG*, respectively. Macrolide/lincosamide resistance was due to the genes *erm*(A), *erm*(B), and *erm*(C) in four, six, and 12 isolates, respectively, as well as *erm*(A)+ *erm*(B), *erm*(A)+ *erm*(C), and *erm*(T) in single isolates. The gene *fexA* was identified in the two chloramphenicol/florfenicol-resistant isolates. All eight gentamicin-resistant isolates carried the gene *aacA/aphD*. Fifty-three isolates harboured SCC*mec* type V elements while the remaining one carried no recombinase gene. All isolates were positive for haemolysin α and δ genes, but negative for *luk*F-PV and *luk*S-PV, *tst*, and genes for exfoliative toxins. One and three isolates were positive for the enterotoxin B (*seb*) and the enterotoxin K+Q (*sek*+*seq*) genes, respectively. Eight *spa* types were identified with t011 (n=39) being most common. All 54 isolates were non-typeable with *Sma*I, but exhibited six major *Apa*I-patterns with up to eight sub-patterns.
Conclusion: The MRSA ST398 isolates varied slightly in their virulence properties and *spa* types, but differed distinctly in their antimicrobial resistance pheno- and genotypes as well as their *Apa*I-PFGE patterns. These data underline the ability of ST398 to acquire genetic material which might increase antimicrobial resistance and virulence.

PRP03
A novel ABC transporter Vga(C) mediating resistance to streptogramin A antibiotics, lincosamides and pleuromutilins identified in a porcine MRSA ST398 isolate

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Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates often exhibit resistance to several classes of antimicrobial agents in addition to β-lactam antibiotics. However to date, pleuromutilin resistance has been observed very rarely among MRSA isolates. In this study, we analyzed the multiresistance plasmid pKKS825 of a porcine MRSA ST398 isolate.
Methods: Plasmid pKKS825 was transferred by protoplast transformation into *S. aureus*. The plasmid was sequenced completely by primer walking and analyzed for the resistance genes present. MIC testing was conducted by broth micro- or macrodilution according to the CLSI.
Results: Plasmid pKKS825 was 14,364 bp in size and carried three already known resistance genes: *dfrK* for trimethoprim, *tet*(L) for tetracycline, and *aadD* for kanamycin/neomycin resistance. These three resistance genes were organized in a gene cluster related to that recently described on plasmid pKKS2187. A fourth resistance gene, designated *vga*(C), was detected. It encoded an ABC transporter of 523 amino acids (aa). Structural comparisons identified closest similarity to the similar sized Vga(A) variants of staphylococci (62.3%-65.5% aa identity) and only 39.2% identity to Vga(B). The Vga(C) protein revealed the typical features of a class 2 ABC transporter. It conferred resistance to streptogramin A antibiotics (virginiamycin M1), lincosamides (clindamycin, lincomycin, pirlimycin), and pleuromutilins (tiamulin, valnemulin) as confirmed by comparative MIC testing.
Conclusion: This study describes a novel resistance gene *vga*(C) coding for an ABC transporter which exports streptogramin A, lincosamides and pleuromutilins. Moreover, the *vga*(C) gene was co-located on a 14,364-bp plasmid carrying a multiresistance gene cluster consisting of *aadD*, *tet*(L), and *dfrK*. This co-localization might facilitate the dissemination and persistence of *vga*(C) in the presence of a selective pressure by kanamycin, neomycin, tetracyclines or trimethoprim.

PRP04
Horizontal gene pool of Gram-positive pathogenic bacteria from ISS and CONCORDIA Station

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The aim of this study is to analyse Gram-positive (G+) isolates from confined environments such as the International Space station (ISS) and the CONCORDIA research station in Antarctica for antibiotic resistance genes, transfer (*tra*) genes, mobile genetic elements (MGE), the ability to form biofilms and transfer activity. 133 isolates from air and swab samples belonging to the genera *Staphylococcus*, *Enterococcus* and *Bacillus* were screened for 20 different antibiotic resistance genes, for *tra* factors encoded by *virB1*, *virB4* and *virD4*-homologous genes from several broad-host-range G+ conjugative plasmids and for relaxases of the pMV158 family by PCR. Plasmid DNA profiles from all the isolates were analysed via agarose gel electrophoresis. All the isolates were screened for biofilm formation in a microtiter plate assay. Most of the isolates contained 1-2 resistance genes but 11 of them harboured 4-6. In 33 staphylococcal isolates we detected 1-4 *tra* genes coding for different relaxases, ATPases and coupling proteins. Selected isolates were applied to biparental and triparental matings in continuous upflow biofilm reactors.

Transfer frequencies up to 10^{-6} were obtained. Some of the isolates affiliated to *B. cereus/thuringiensis*, *S. aureus*, *S. epidermidis* and *S. saprophyticus* harboured conjugative plasmids enabling mobilization of the pIP501-based *gfp*-labelled monitoring tool to *E. faecalis*. Isolates encoding the highest number of resistance genes belonged to the enterococci whereas those with most *tra* genes affiliated to the staphylococci. No MRSA was detected whereas one methicillin resistant (*mecA*) *S. epidermidis* (MRSE) was isolated from the CONCORDIA station. In three staphylococcal CONCORDIA isolates we detected the *vanB* gene together with *ermD* and *ermG*. Pulsed field gel electrophoresis of isolates with large plasmids and risk assessment of the generation of multiple resistant pathogens in these confined environments will complement the molecular studies.

PRP05

Physician antibiotics prescribing practice for urogenital infections in German hospitals and outpatient settings: first results of a national survey

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Objectives: Physicians' prescribing practice may be a factor contributing to antibiotic resistance. We aimed to explore physician prescribing behaviours for urogenital infections and antibiotics use in both hospital and outpatient settings.
Methods: We used data from a national cross-sectional survey of 10,610 physicians from stationary (S) and outpatient (O) settings in Germany exploring diagnoses and behaviour with antibiotics treatments. Data was also collected on attitudes about antibiotics, and influences that affect practice. The survey also solicited opinions about interventions to improve practice.
Results: Among survey respondents (n = 3,492; response rate = 33%), 66% reported that they consider to prescribe antibiotics daily, and 90% consider at least weekly. For urogenital diagnoses, 56% were treated with sulphonamide-trimethoprim and 29% fluorquinolones. Table 1 shows prescriptions for urogenital infections. Half (56%; 49% S, 60% O) of physicians diagnosing urogenital infections report they start an empirical antibiotic therapy 80% of the time or more, and 17% always do so (17%; S, 18% O). Over a third (38%; 59% S, 28% O) report that they require a pathogenic test result before starting therapy for urogenital infections 80% of the time or more, and 16% (10%; S, 20% O) never do so. Of urogenital diagnosing physicians 54% (40%; S, 61% O) report that in 1 out of 5 cases they changed from an empirical to a targeted therapy, once they had results of testing, and 14% (8%; S, 17% O) reported they never changed.
Conclusions: Physicians report often that they prescribe empirically with urogenital diagnoses, and infrequently change to a targeted therapy. Empirical therapy requires knowledge of resistance situations, and resistance data should be made readily available to those physicians who need it. Interventions highlighting the importance of diagnostics and guidelines for focused therapies should be available to physicians who prescribe antibiotics therapies.

PRP06

Antimicrobial Resistance Surveillance in Germany: First results for *E. coli, K. pneumoniae* and *P. mirabilis* from urine samples in ambulatory care 2008/09

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Germany expanded the scope of its national antimicrobial resistance surveillance system to the sector of ambulatory care. This presentation gives a first insight into resistance in the most frequent Gram-negative pathogens isolated from urine samples from outpatients in 2008/2009.
Data are taken from the Antimicrobial Resistance Surveillance (ARS) System. Analysis is based on non-duplicate isolates of *E. coli, K. pneumoniae* and *P. mirabilis* from urine samples in four laboratories with continuous data collection from January 2008 to October 2009. Antimicrobial susceptibility testing is performed by VITEK 2, results are evaluated according to CLSI guidelines. Proportions of susceptible isolates are calculated for ampicillin (AMP), ampicillin/sulbactam (AMS), piperacillin (PIP), piperacillin/tazobactam (PIT), cefotaxime (CTX), ciprofloxacin (CIP), co-trimoxazole (SXT).
The most frequent Gram-negative pathogens isolated from a total of 87.873 urine samples were *E. coli*, *P. mirabilis* and K. pneumoniae. In *E. coli* overall proportions of susceptibility for antimicrobials used in urinary tract infections as AMP, AMS, SXT and CIP vary from 54.6% (AMP) to 84.2% (CIP), in *P. mirabilis* this range spans from 64% (SXT) to 92.3% (AMS) and in *K. pneumoniae* proportions for selected antibiotics are all above 80%. Stratification by age groups shows significant lower levels of susceptibility for patients older than 60 for AMP, PIP, CIP and SXT in *E. coli* as well as in *P. mirabilis*. Regional differences with similar patterns are observed for CIP, SXT and PIP across species.
These first large-scale data from ambulatory care indicate that non-susceptibility of *E. coli* and to a lesser degree of *P. mirabilis* from urines to first-line antibiotics is highly prevalent, that it is even higher in patients older than 60 and that there are some regional variations. Interpretation of the data should consider that in ambulatory care settings specimens are mainly taken from pre-treated patients.

PRP07

Airborne Microflora in an Hospital Environment of University of Benin Teaching Hospital (UBTH), Benin City, Nigeria

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A study was undertaken to determine typical concentrations of airborne bacteria and fungi (microflora) in Teaching Hospital environment in Benin City in the tropical rainforest environment of Nigeria. Aerial sampling was conducted at various hospital wards each day. The air samples were collected thrice daily, that is, in the morning, between 8am and 10am, in the afternoon, between 12noon and 2pm and in the evening between 4pm and 6pm. Concentrations of airborne microflora exceeded available local guidelines for indoor quality in the accident and emergency ward, female ward, male ward, pediatric ward and the maternity ward, but not in the restricted wards like the theatre, intensive care unit and bacteriological laboratory. Results showed that the occupant density was the key factor influencing the levels of airborne microflora, while humidity was also observed as a factor, depending on the particular location with the hospital.
The concentration of airborne bacteria and fungi in the nine different hospital units varied from wards to wards. The bacterial population ranges from 3.0cf/m3 to 76.0cf/m3, with the highest bacterial population recorded in the accident and emergency ward. The fungal population ranges from 6.0cf/m3 to 44.7cf/m3, while the highest fungal population was recorded in the accident and emergency ward. The microflora characterized and identified, were representative of the normal microflora of the human body (skin, gastrointestinal tracts, respiratory tract) and the opportunistic pathogens. The microbial isolates included six bacterial genera, among which are, Staphylococcus aureus, Staphylococcus epidermis, Escherichia coli, Bacillus sp. and Proteus mirabilis, the fugal isolates included, Aspergillus sp, Penicillium sp., Mucor sp., Candida sp and Verticillium sp. The variations in hospital units in concentrations of total airborne microorganisms (bacteria and fungi) in the air of hospital environments were statistically significant ($p<0.001$). The concentrations of airborne microflora recorded in the hospital environment, specifically in the accident and emergency ward was significantly different from other wards ($p<0.001$), with the mean value of 40.0 and 72.2 for fungal and bacterial population respectively.
Keywords:Airborne, microflora, hospital environment, time, UBTH, Benin City, Nigeria.

PRP08

Antibacterial activity of Thai spice essential oils against Methicillin-resistant *Staphylococcus aureus* (MRSA)

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Methicillin-resistant *Staphylococcus aureus* (MRSA) has been considered as a major nosocomial pathogen which causes problems in both the community and hospitals. There have been many case reports about MRSA resistance to mupirocin and triclosan, the topical drugs that generally used for the prevention of MRSA transmission. Attempt to find a new antibacterial agent against MRSA was performed in this study. Thai spice essential oils distilled from *Citrus hystrix* DC. (kaffir lime) fruit peel, *Cymbopogon citratus* Stapf. (lemongrass) grass, *Cinnamomum zeylamicum* Nees (cinnamon) dried inner bark, *Ocimum basilicum* L. (sweet basil) flowering top of the shrub and leaves, *Alpinia galanga* (L.) Willd. (galanga) rhizome and *Zingiber officinale* Rosc (ginger) rhizome were studied their antibacterial activity against MRSA isolated from Thai people. Broth microdilution method was carried out to determine the susceptibility of 6 essential oils against this pathogen. Chemical constituents of the effective oil were identified by Gas Chromatography/Mass Spectrometry (GC/MS). The results revealed that cinnamon oil exhibited the lowest minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) at 0.024 and 0.024-0.048 µl/ml, respectively. Both MIC and MBC values of lemongrass oil were ranged between 25-50 µl/ml. MIC and MBC values of the other essential oils were found at 50 µl/ml or higher. The prominent activity of cinnamon oil might be affected by eugenol and cinnamaldehyde, the major components found in this essential oil. In conclusion, cinnamon oil possesses anti-MRSA activity and should be further studied the suitable concentration for topical anti-MRSA pharmaceutical product in the future.

PRP09

Characterization of penicillin-binding proteins involved in murein biosynthesis of staphylococci

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The murein or peptidoglycan sacculus is the stress bearing layer of bacterial cells, which consists of glycan strands cross-linked by short peptides. During growth and division of cells the murein network has to be divided without losing its strength. Lots of clinical resistant bacteria like vancomycin and methicillin resistant *Staphylococcus aureus* (VRSA and MRSA) strains show major changes in murein biosynthesis. Even though the murein layer is a major target for antibacterial treatment, the knowledge about its synthesis is still very limited, especially in Gram-positive cocci. The precursor lipid II is synthesized within the cytoplasm, but the actual formation of the murein network by transglycosylation and transpeptidation takes place at the outside of the cytoplasmic membrane and is mainly catalyzed by the penicillin-binding proteins (PBPs).
The aim of our studies is to gain deeper insight into the reactions and interactions of the proteins synthesizing the murein network. As a model we use *S. carnosus* to avoid the influence of protein A and other surface proteins present in *S. aureus*. Until now we could purify PBP1, PBP2, and PBP3 of *S. carnosus* and in addition PBP2A of *S. aureus* (MRSA-strain). The latter is responsible for resistance against β-lactam antibiotics. PBP4 of *S. carnosus* has still to be cloned. Furthermore antibodies against PBP1 and PBP3 were raised, to perform *in vivo* interaction studies by co-immunoprecipitation. For functional studies of the PBPs by *in vitro* murein synthesis we also created a PBP2 protein, whose transpeptidase domain was inactivated via site directed mutagenesis.

PRP10

MRSA status quo in the department of surgery at the Otto-von-Guericke-University in Magdeburg

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With regard to MRSA in surgery departments the perioperative infections are the main problems. Beside a well organized MRSA screening the analysis of the current status is a prerequisite for an effective MRSA management. Therefore, we performed a retrospective analysis on all MRSA patients in the surgery department during the last two years followed up by a prospective MRSA screening on all patients in the respective department. In total 11230 patients were included into the retrospective study. The MRSA rate was 20%, the average MRSA prevalence was around 0.85%, the incidence around 0.94 and the daily MRSA load at 2.65. Among a total of 96 MRSA patients 42% suffered from MRSA infection. The most MRSA were detected in samples from soft tissue infections (64%), followed from respiratory tract infections (11%) and blood stream infections (11%). The most existing admission diagnose was vascular diseases (26%) and cancer (25%). 33% of the MRSA patients were emergency patients, 29% were from outside clinics, and 47% were at least one time on the ICU. MRSA patients had a significant longer stay as compared to non-MRSA patients. During the prospective study screening detected a MRSA rate of 12%. 53% of the MRSA patients had infections. Cancer (35%) and soft tissue infections (15%) were the most prevalent admission diagnoses. 35% of the MRSA patients were emergency patients, 27% were from outside clinics. Again MRSA patients had a significant longer stay as compared to non-MRSA patients. A combination of nasal-, pharyngeal and wound swabs was sufficient to detect 95% of the MRSA patients. The retrospective and prospective studies have shown that the MRSA patients in the surgery department can be detected by the known risk factors. Nonetheless, the results clearly show that a regularly survey on the MRSA status quo is mandatory for limiting the transmission, the infection risk for MRSA, and to adapt the respective MRSA management.

PRP11

MRSA screening of hygiene seminar participants

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94 participants of a hygiene seminar were throat swabbed and screened by qPCR for MRSA using the commercial kit Onar®MRSA and by culture method using CHROMagar®. 5 participants (5.3 %) were found positive for MRSA with both methods. None of these colonized participants showed any clinical symptoms. All tested participants were busy in a hospital and especially the positive tested participants had frequent patient contact in a chirurgic ambulance.
Both screening methods showed comparable predictive values. Much shorter response and a 100 % sensitivity was provided by the qPCR method. Anyhow, culture was extremely helpful to confirm results of the multi-locus PCR and for strain characterization.

PRP12

Molecular study of Extended-spectrum β-lactamases Enzymes in *Pseudomonas aeruginosa* isolated from Central Hospitals of East Azerbaijan by PCR

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Introduction: Pseudomonas aeruginosa is one of the most important agents in nosocomial infection. They could be plasmid mediated resistant against beta-lactams. The most important factor involved in resistance is producing of extended- spectrum β-lactamases. This study was conducted to assay ESBL-producing strains of *Pseudomonas aeruginosa*.
Methods: Different samples were taken from inpatients of 5 hospitals in Tabriz, Iran. All of Gram-negative bacilli isolates were identified using conventional bacteriologic methods. They were tested for susceptibility and screening of ESBL-producing by Disk diffusion method and E-test, respectively. Plasmid DNA extraction was done by Kado and Liu technique. The presences of bla CTX-M1, bla CTX-M2 were studied by PCR.
Results: 786 ICU patients were studied, 240 of them infected by Gram-negative bacilli. The second prevalent bacteria after *Klebsiella pneumoniae* 92(25.6%) was *Pesudomonas aeruginsa* 62(17.3%). The susceptibility test showed 38%, 96%, 70% and 100% resistance against ceftazidime, cefitaxime, ceftriaxone and cefuroxime, respectively. By E-test, 49(78%) of strains were ESBL-producing. 64% of isolates harbored a single plasmid of 63kb. All of strains lacked either CTX-M-1 or CTX-M-2 gene.
Conclusion: *Pseudomonas aeruginsa* was one of the most prevalent bacteria considering as the second agent. Highest rate of resistance was showed against Cefuroxime and Tetracycline. Confirming no rules for suspicious genes by PCR, approximately 3/4 of strains were founded as ESBL producer.

PRV01
First Survey of Metallo-beta-Lactamases (MBL) in clinical isolates of *Pseudomonas aeruginosa* in a German University Hospital

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Metallo-β-lactamase (MBL)-producing *Pseudomonas aeruginosa* are an increasing public health problem in many industrialized countries world-wide, and therefore the development of efficient screening criteria gains importance in routine diagnostic microbiology laboratories.
This study was designed to develop improved screening criteria for the detection of MBL-producing *P. aeruginosa*, and by applying these criteria to determine the proportion of MBLs in clinical isolates of *P. aeruginosa* in a German University hospital. Based on the prevalence data found we further evaluated two phenotypic tests as confirmatory tests for the routine detection of MBL production.
Among a total of 489 consecutive non replicate clinical isolates of *P. aeruginosa* collected from June 2008 until May 2009 at the University Hospital of Würzbug, Germany, 68 of them showed reduced susceptibility to imipenem. Adding resistance to ceftazidime as further screening criterion for MBL production we reduced the number of isolates to be tested by PCR to 15.
Consecutive molecular analysis confirmed the presence of a VIM-1/4 encoding gene (blaVIM-1/4) in one isolate and a VIM-2 encoding gene (blaVIM-2) in seven isolates. Of note, molecular typing suggested that all VIM-2 producing isolates presumably originated from a single clone.
Based on these prevalence data, the MBL Etest and EDTA disk test used as phenotypic confirmatory tests for MBL detection in isolates with reduced susceptibility to imipenem and resistance to ceftazidime achieved sensitivities of 100% but showed specificities of 14% and 100%, respectively. Accordingly, the corresponding positive predictive values were 57% and 100%, respectively.
We suggest an initial screening of *P. aeruginosa* for MBL production by combined susceptibility testing of imipenem and ceftazidime, followed by a confirmatory EDTA combination disk test.

PRV02
Multiresistant-*Enterobacter cloacae* outbreak in a neonatal and paediatric intensive care unit associated with the use of multidose packaging of oral drugs, Germany 2009

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Enterobacter (E.) cloacae receives increasing attention as a nosocomial pathogen in neonatal and pediatric intensive care units (NICU). In July 2009 an infant died of sepsis due to a multiresistant *E. cloacae* clone in a 95-bed pediatric hospital. The clone was identified to cause bacteraemia in 2 other children and colonize a further 10. Hygiene measures were intensified for infection control. The state health authorities invited the Robert Koch-Institute to assist the outbreak investigation.
We conducted a retrospective matched case-control-study. A case was a child in the NICU with laboratory confirmation of the outbreak clone 1/5-5/10/09. Eligible controls were patients in the NICU at the case's diagnosis for >72 hours and swab-negative for the outbreak clone. Information was collected on demographics, length of stay, underlying diseases, and on medication, nutrition and procedures. Matched Odds Ratios (mOR) were calculated by bivariate and multivariable conditional logistic regression. Environmental investigations were conducted.
We identified 31 cases (28 colonized, 3 bacteraemic). 29 matched case-control-pairs were included in the study. Multivariable analysis revealed an association between *E. cloacae* diagnosis and the receipt of oral drugs at bed-side from multidose packaging (mOR=1.8/drug administered; 95%CI 1.17-2.85). No specific drug was identified; microbiological investigation of drugs was negative. A different *E. cloacae* clone was isolated from a glove package.
The outbreak of multiresistant *E. cloacae* was most likely caused by contaminated multidose drug packaging and transmitted via hands. No further cases occurred in the 6 weeks after protocols for handling oral drugs were changed (smaller packaging, patient-based storage, and limiting circulation to 1 week). Special hygiene attention needs to be applied to use of multidose packaging at bed-side level. Oral drugs from one container should only be administered to one patient.

PRV03
Practical infection control of *Enterococcus faecium* and *Pseudomonas aeruginosa* isolates: molecular typing with the DiversiLab™ System

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Due to drug resistant microorganisms, nosocomial infections are a major health threat to hospitalized patients. In order to identify nosocomial transmission, rapid and reliable typing methods are essential to adapt appropriate infection control measures. For many bacteria, pulse-field gel electrophoresis (PFGE) is still the "gold standard" for outbreak investigations. However, PFGE is laborious and difficult to standardize. The development of the DiversiLab™ System (Biomerieux) allows the standardization of repetitive-sequence-based PCR (rep-PCR) and offers the use of this method in routine infection control.
We used the DiversiLab™ System to analyze representative strains of Vancomycin-resistant *Enterococcus faecium* (VREF) and *Pseudomonas aeruginosa* as examples for Gram-positive and non-fermenting Gram-negative pathogens. Altogether, more than 60 clinical samples isolated from patients and hospital environment from 2004 to 2009 were investigated. Typing results were compared to PFGE and/or sequence-based typing methods to determine the discriminatory power and concordance.
Preliminary results indicate that rep-PCR based typing of VREF and *P. aeruginosa* used in this study is highly reproducible, fast, and easy to perform with a typability of 100%. Data analysis and interpretation on the DiversiLab™ platform is user-friendly. Referring to the analyzed isolates, the approach was slightly more discriminatory than PFGE and sequence-based typing methods.
The data demonstrate that the DiversiLab™ System offers a rapid and reliable laboratory method for molecular analysis of clinical VREF and *P. aeruginosa* isolates. The short hands-on time for typing and cluster analysis, the comparability and standardized quality of obtained results as well as the possibility to create and maintain databases is of great advantage. The platform is a useful tool for molecular surveillance and assists to implement appropriate measures in daily infection control.

PRV04
Risk factors for the acquisition of VRE in a German university hospital

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Objective: To analyse an increase of vancomycin-resistant E. faecium (VREfm) at Hannover Medical School, a 1 400 bed university hospital with 40 000 admitted patients per year. The proportion of VREfm rose from 1.2 % in 2004 to 20.5 % in 2006.
Methods: Genotyping of VREfm was performed by pulsed field gel elecrophoresis (PFGE): SmaI-restriction profiles linked at > 80 % using the Dice coefficient were considered as clonally related. PFGE results and conventional epidemiology were applied for transmission analysis. For risk factor analysis, a matched case-control study was carried out: Cases were patients with VREfm acquisition, controls patients with vancomycin-susceptible E. faecium.
Results: PFGE was performed on 171 isolates of 166 patients hospitalized on 30 wards and revealed 57 different genotypes. Sixty-one patients (37 %) with VREfm were in the general surgery unit and 38 patients (23 %) in the haematological oncology unit. In these units, 30 % of VREfm were due to patient-to-patient transmissions. Multivariate logistic regression analysis of 142 cases and 142 controls identified the presence of neutropenic days (OR 7.7, CI95 1.5 – 39.8, $p < 0.005$) and a hospital admission within the previous 12 months (OR 5.3, CI95 2.5 – 11.3, $p < 0.005$) as risk factors for acquisition of VREfm. Additional independent risk factors were use of glycopeptides antibiotics (OR 3.7, CI95 1.6 – 8.7, $p < 0.005$), mechanical ventilation (OR 3.4, CI95 1.1 – 10.1, $p < 0.005$) and a history of additional multidrug-resistant organisms (OR 3.4, CI95 1.4 – 7.9, $p < 0.05$).

Conclusion: Risk factors like immunosupression, previous hospitalization and mechanical ventilation indicate that patients with severe underlying diseases were mainly affected by VREfm. In these patients, the use of glycopeptide antibiotics was a main risk factor for VREfm acquisition. A substantial proportion of VREfm acquisition was due to transmission.

PRV05
Costs of nosocomial infections caused by vancomycin-resistant Enterococcus

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Background: Nosocomial infections due to vancomycin-resistant Enterococcus (VRE) increased considerably during the last years. The purpose of this study was to investigate the costs for nosocomial VRE-infections inside the German DRG-System.
Methods: A case-control study was performed comparing patients with nosocomial infections caused by VRE and by vancomycin-susceptible Enterococcus (VSE) between January 2005 and December 2008. 47 nosocomial cases with VRE-infections were matched to 47 controls with VSE-infections for admission and discharge in the same year, the minimum length of stay corresponding to time at risk of the case, Charlson comorbidity-Index ±1, stay on intensive care units (ICU) and non-intensive-care units as well as for the type of infection, which were defined using the CDC criteria.
Results: The median overall costs for cases with nosocomial VRE-infection were significant higher than for control patients (€ 57,817 vs. € 38,334; p=0,045). The median attributable costs for VRE-infections were € 12,979 (p=0,058). Furthermore, the multivariate analysis showed that cost were driven by VRE-infection (multiplicative effect (ME)=1,4; p<0,042), ventilation >500h (ME=3,3; p<0,001), and stay on ICU (ME=2,4; p=0,002). In contrast age over 60 years was a predictor for decreased costs (ME=0,7; p=0,034).
Conclusion: This analysis revealed that nosocomial infections due to VRE are associated with high costs for healthcare systems compared with VSE-infections. Therefore hospital personal should implement control measures to prevent the transmission of VRE.

PRV06
The use of Six Sigma for the analysis and improvement of infection control measures in handling of central venous catheters on a stem cell transplant unit

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Although intravascular catheters are indispensable for the management of seriously ill patients, their use puts patients at risk for local and systemic infectious complications. Measures to minimize the risk for infection depend on strict adherence to infection control and prevention measures during insertion and maintenance of intravascular catheters.
Here we report the results of a comparative study to minimize bacterial contamination of intravascular catheters in a stem cell transplant unit by using the business-based Six Sigma process improvement strategy to analyze the process of preparing and using saline solutions for the flushing of central venous lines.
The steps of the process and possible hazard points for contamination of the solutions were identified. Using real time simulation software the costs, working time and number of potential contamination events were calculated. The process was compared to an optimized process using prefilled saline syringes (BD Saline XS, Becton Dickinson). The Six Sigma analysis could reveal the potential for reducing patient risk and overall costs by implementing the use of prefilled saline syringes.
Especially in times of cost pressure in health care infection control measures have to be cost effective. Many reasonable measures aren't taken because of high costs at first sight. Tools like Six Sigma facilitate an in depth analysis of the real process costs, so the cost effectiveness of these measures can be demonstrated. Furthermore, the impact of improvement strategies can be recognized before they are implemented into daily practice.

PRV07
Germany Strategy on Antimicrobial Resistance (DART) – State of Implementation

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The increase of antimicrobial resistant pathogens complicates the treatment of infectious diseases and causes additional medical costs. One of the main reason commonly is inappropriate use of antibiotics. Therefore, the Federal Ministry of Health developed together with the German Ministry of Food, Agriculture and Consumer Protection, the Ministry of Education and Research (BMBF) and further 150 stakeholders in the public health sector a national action on prevention and control of antimicrobial resistance (AMR) in Germany (DE).
For the establishment of the strategy of (inter-) national activities, the shortcomings and needs in the area of AMR were investigated e.g. by interviewing experts in this field. In November 2008 DART was published with 10 aims and 42 actions in the human part, which will be implemented from 2008 - 2013.
Main implemented actions so far are:
- Expansion of surveillance systems of data on antibiotic resistance and antibiotic consumption and feedback of data to antibiotic prescribing doctors.
- Extending the notification on the detection of MRSA in blood or cerebrospinal fluid isolates since 1 July 2009.
- Establishment of a commission for improving the rational antibiotic therapy at Robert Koch Institute (RKI) by giving the impulse for the generation of independent, scientifically grounded recommendations for antibiotic therapy.
- Establishment of a centre for prevention and control of antimicrobial resistance at the RKI for coordination and consolidation of activities in the field of antibiotic resistance.
- Promotion of education, continuing education and training of health professionals in the field of antibiotic resistance by supporting a training program developed by DGHM and DGI.
- Strengthening regional cooperation between regional actors in the health sector by promoting of regional networks on prevention and control of antimicrobial resistance.
- Identifying the gaps in research on the emergence and spread of resistance and support of interdisciplinary research collaborations on zoonotic infectious diseases with a focus on antibiotic-resistant zoonotic pathogens by BMBF.

PRV08
Regional MRSA-Screening and benchmarking in the EUREGIO Twente Münsterland

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Since MRSA can be disseminated between hospitals serving one regional patient catchment area, successful prevention and control of MRSA infections requires concerted efforts including the regional benchmarking of hospital MRSA surveillance data.
All acute care hospitals in the German EUREGIO (10.800 patient beds, 343.000 annual admissions) have established an MRSA-network. Since 2007 they have implemented a synchronized strategy for screening of risk patients at admission and are reporting MRSA data to local health authorities. In 2007 and 2008 we assessed: the rate of nasal swabs/100 patients admitted, MRSA incidence, nosocomial MRSA incidence density, nosocomial MRSA-cases/1,000 MRSA days and the number of MRSA blood cultures (MRSAB). The rates calculated were compared to preliminary MRSAB notifications from North Rhine-Westphalia (NRW).
In 2008, in average 22.2% of all patients admitted were screened (increase from 2007, p<0.05). The MRSA incidence in 2008 was 1.47 MRSA cases/100 admissions (increase from 2007, p<0.05). The mean nosocomial MRSA incidence density in 2008 was 0.19 nosocomial MRSA-cases /1,000 patient days and remained stable from 2007 to 2008. In 2008, there were an average of 8.6 nosocomial MRSA-cases/1,000 MRSA-days (decrease from 2007, p>0.05). The number of MRSAB decreased from 4.3/100,000 inhabitants in 2007 to 3.4 in 2008 in the EUREGIO. An extrapolation of the MRSAB rates from

mandatory notification data for 2009 was 3.0/100,000 inhabitants for the EUREGIO and 5.8 for other regions in NRW.
Adherence to the synchronized screening strategy was measurable by increased screening in hospitals. The enhanced screening of risk patients resulted in a higher MRSA-incidence, since more carriers were detected at admission. Nevertheless the number of MRSAB decreased in the study period. The understanding of this MRSA-screening/MRSAB paradox should encourage other regional networks for concerted MRSA screening programs to reduce MRSA infections.

PRV09
Emergence of a new community-acquired MRSA ST45

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Introduction: Nosocomial infections caused by Methicillin-resistant *S. aureus* are a problem in hospital settings worldwide. But MRSA became also increasingly prevalent in community-acquired infections. These caMRSA cause diseases in healthy individuals with no exposure to health care facilities or other risk factors normally associated with acquisition of MRSA. caMRSA strains typically cause skin and soft-tissue infections, but can also lead to life-threatening diseases. Identifying new emerging caMRSA is a challenge in the changing epidemiology of MRSA.
Methods: The isolate was sent to the Reference Centre for further characterisation. The antimicrobial susceptibility, the presence of several resistance and pathogenicity associated genes, the SCC*mec*-type, spa- and multilocus sequence-type were analyzed. *lukSF-PV* was sequenced to determine the PVL sequence variation. PFGE after *Sma*I-digestion of the chromosomal DNA was performed for comparison of this isolate with epidemic nosocomial and community strains.
Results: The strain exhibited resistance to penicillin and oxacillin. No superantigenic toxin gene was detected, but the isolate was positive for *mecA, cna* and *lukPV*. SCC*mec*-typing revealed IVa. The sequence analysis of nucleotide variation in the *lukSF-PV*-genes indicated the H2 variant of PVL. Based on the spa-type t1510 and the sequence-type ST45 the isolate was clustered into clonal complex 45. The *Sma*I-macrorestriction pattern also corresponds to those of CC45, but it is different from patterns of widely disseminated haMRSA ST45.
Conclusions: This is the first description of a PVL-positive, caMRSA ST45. Methicillin-susceptible *S. aureus* ST45 is a frequent nasal colonizer of healthy humans, and MRSA ST45 is known as epidemic haMRSA. This refers to the potential for colonization and spread of *S. aureus* ST45. Therefore the emergence of caMRSA ST45 needs particular attention.

PRV10
Methicillin-Resistant *Staphylococcus aureus*: Two Cases of Transmission between Humans and Dogs

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Objectives: During Sep 2007-Jan 2009, dogs admitted to the Small Animal Clinic were screened for methicillin-resistant *Staphylococcus aureus* (MRSA). Swabs were taken from the nose, pharyngeal region and perineum. The MRSA isolates of the two cases for which sufficient background data were available were analyzed.
Methods: The MRSA isolates were characterized by multilocus sequence typing (MLST), *spa* typing, macrorestriction analysis with *Sma*I and *Apa*I, and antimicrobial susceptibility testing. By PCR the SCC*mec* type was determined and the presence of the PVL toxin genes *luk*S-PV and *luk*F-PV tested.
Results: In case 1, a 6-month-old dog carried an MRSA isolate of MLST type ST398 and *spa* type t034 which was non-typeable by *Sma*I but typeable by *Apa*I. The strain was PVL-negative, carried SCC*mec* type V and was resistant to β-lactam antibiotics, erythromycin, clindamycin, and tetracycline. The dog owner, a specialist veterinarian in swine diseases, showed nasal colonization by a strain which showed the same characteristics. In case 2, an 11-year-old dog was positive for an MRSA isolate of MLST type ST225 and *spa* type t014. The isolate was PVL-negative, harboured SCC*mec* type II and was resistant to β-lactam antibiotics, erythromycin, clindamycin, and enrofloxacin. The dog had regular contact to the dog owner's 85-year-old mother-in-law who lived in the same household and received nursing care at home because of an infected decubital ulcer on her foot. An MRSA isolate, indistinguishable from the one of the dog, was isolated from the decubital ulcer of the woman.
Conclusions: The most likely route of transmission of the MRSA ST398 isolate in case 1 was from pigs to the veterinarian and subsequently from the veterinarian to his dog. In case 2, the mother-in-law appeared to be the source of the MRSA ST225 isolate. MRSA ST225 was the second most frequently detected MRSA type among humans in Germany in 2007 and 2008 whereas this type has not been detected in dogs so far.

PRV11
Characterization of methicillin-resistant *Staphylococcus aureus* (MRSA) ST398 isolates from bovine mastitis

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Objectives: Twenty-five MRSA ST398 isolates from cases of bovine mastitis and two isolates from farm personnel collected from 17 dairy farms in Germany were investigated for their genetic relatedness, antimicrobial resistance pheno- and genotypes and virulence properties.
Methods: Antimicrobial resistance was determined by broth microdilution. Resistance and virulence genes were detected via a diagnostic DNA microarray and specific PCRs. The genetic relationships were determined by *Apa*I PFGE, *spa* typing, SCC*mec* typing and *dru* typing.
Results: Ten resistance patterns were observed with 11 (40.7%) isolates being resistant to β-lactam antibiotics and tetracyclines only. A variety of resistance genes was detected: the tetracycline resistance genes *tet*(M), *tet*(K) and *tet*(L), the macrolide/lincosamide resistance genes *erm*(A), *erm*(B) and *erm*(C), the aminoglycoside resistance genes *aacA-aphD*, *aphA3* and *aadD*, the phenicol resistance gene *fexA*, the trimethoprim resistance gene *dfrK* and the spectinomycin resistance gene *spc*. Nine *Apa*I PFGE patterns with up to three sub-patterns were detected. Three *spa* types (t011, t034 and t2576) and two SCC*mec* types (IV and V) were identified. Five different <k>dru</k> types were seen with dt11a being predominant. All isolates were negative for PVL, enterotoxin and exfoliative toxin genes, but positive for α and δ hemolysin, *agr* group 1 and capsule type 5. The isolates also exhibited a uniform MSCRAMM gene pattern including among others the
genes for clumping factor as well as fibronectin, fibrinogen, collagen and elastin binding proteins. The two human isolates were indistinguishable in their geno- and phenotypic characteristics from the mastitis isolates of the same farm.
Conclusion: As previously described for porcine MRSA ST398, MRSA ST398 isolates from bovine mastitis showed a high degree of variability regarding their *Apa*I PFGE patterns as well as their resistance pheno- and genotypes. However, they exhibited a uniform virulence gene pattern.

PRV12
Detection of a resistance gene cluster *erm*(T)-*dfrK-tet*(L) flanked by novel IS elements in an MRSA ST398 isolate

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Background: Porcine methicillin-resistant *Staphylococcus aureus* (MRSA) ST398 isolates are resistant to macrolides and lincosamides at varying frequencies. In the present study, the molecular basis of a macrolide-lincosamide-resistant porcine MRSA ST398 isolate was investigated.
Methods: The MRSA ST398 isolate showed resistance to erythromycin, other macrolides and clindamycin, but was PCR-negative for the macrolide-lincosamide resistance genes so far identified in staphylococci. Protoplast transformation into *S. aureus* RN4220 with selection on erythromycin was performed. Restriction fragments of the transformed plasmid were cloned and sequenced.

Results: Resistance to erythromycin was conferred by the rRNA methylase gene *erm*(T). This gene was located on the ca. 40-kb plasmid pKKS25, which also mediated resistance to trimethoprim and tetracycline via the genes *dfrK* and *tet*(L), respectively. Analysis of the *erm*(T) flanking regions showed identity to the *erm*(T) region of a *Streptococcus pyogenes* plasmid pRW35 in a stretch of 123 bp upstream and 139 bp downstream of *erm*(T). The *erm*(T) gene of pKKS25 was expressed constitutively due to a 57-bp deletion in the *erm*(T) translational attenuator. The genes *dfrK* and *tet*(L) were located downstream of *erm*(T). The region harbouring the genes *erm*(T), *dfrK* and *tet*(L) was flanked by two novel IS elements, designated IS*Sau10*. The element of 793 bp showed 87% or 88% identity to IS*431* or IS*257*, respectively.
Conclusion: This is the first report of the presence of *erm*(T) in an MRSA ST398 isolate. Since *erm*(T) has previously been described in streptococci and lactobacilli, the finding of *erm*(T) in an MRSA strain underlines the presence of a gene flux between these bacteria and staphylococci. The observation that the *erm*(T)-*dfrK*-*tet*(L) gene region was flanked by IS elements suggests the mobility of this resistance gene region.

PWP01
Is the flagellum of the probiotic *E. coli* strain Nissle 1917 the major adhesin *in vivo*?

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E. coli strain Nissle 1917 (EcN) has probably evolved into one of the best characterized probiotics and its therapeutic efficacy and safety have convincingly been proven. Although there are already some findings about the mechanisms of its probiotic effect (Schlee et al., 2007), not much is known about its adherence properties with regard to human surfaces.
In recent studies we were able to demonstrate that the expression of flagella is necessary for the successful adhesion of EcN to cryosections of human gut biopsies, as a *fliC*-deletion mutant couldn´t adhere anymore. Furthermore, a hyper flagellated variant of EcN adhered about twice as efficient to cryosections compared to the EcN wildtype (WT). In contrast, the flagella seem to play no role in adhesion to the human intestinal epithelial cell-lines Caco2 and T24, as the *fliC*-deletion mutant adheres to these cell lines as efficiently as the WT.
Surprisingly the type 1, F1C and curli EcN adhesin mutants are neither impaired in their adherence efficiency to cryosections nor to the cell lines employed.
Latest results indicated that mucin is the responsible structure for successful adhesion of EcN to the cryosections via its flagellum. This would explain the differences in the adhesion studies with the cell lines and the cryosections. The tested cell lines are not able to produce mucin, whereas this protein is most likely present in the cryosections. The hyper flagellated EcN variant adhered to mucin again much more efficient than the EcN WT, supporting the role of mucin as the major adherence receptor for the flagella of EcN. This notion is in accordance with the finding of EcN predominantly present in and on the mucus but not directly adhering to the epithelial cells in the gut of gnotobiotic rats and piglets after oral application of the probiotic strain. At the moment, further experiments are in progress to show the direct adhesion of the flagellum to mucin.

PWP02
Heterogeneity of first colonizing microorganisms in newborns

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Background: At birth the intestinal tract of vertebrates is sterile, but rapidly becomes colonized by bacteria. The establishment of the gut microbiota is considered a stepwise process with facultative anaerobes such as the enterobacteria, coliforms and lactobacilli first colonizing the intestine, rapidly succeeded by Bifidobacteria and lactic acid bacteria. The failure of a controlled succession may culminate in the risk of developing diseases later in life including obesity and allergy. Due to the steady increase in the later diseases we asked whether the early microbial colonization may be altered nowadays.
Methods: For this purpose we analyzed the stool samples from 20 newborns from day 1-3 after birth for the presence of aerobes and facultative anaerobes microorganisms by culture.
Results: 11 newborns showed no microorganisms on their stool on day 1, among them 3 remained sterile for a total of two days. Within the first three days of life we isolated *E. coli* from 11 babies, *Staphylococcus aureus* from 11 babies, coagulase-negative spp. from 16 babies, *Enterococcus spp*. from 3 babies, group B-streptococci from 4 babies, *Pseudomonas aeruginosa* from 3 babies and *Proteus spp*. from 3 babies as well. *Lactobacillus spp*. appeared on day 3 in the stool from 2 babies. Colonization of the intestine started 5 times with *E. coli*, 11x with *Staphylococcus spp*., 2x with Pseudomonas and 2x with B-streptococci. *E. coli* was able to stabilize in the presence of staphylococcus spp. but not vice versa.
Conclusion: Our data clearly show that early colonization of the intestine in newborns is nowadays altered in comparison to older reports and can be very heterogenous. The predominance of staphylococcus spp. as a first colonizer of the newborn intestine may be due to the hospital environment, an altered vaginal flora of the mother. In consequence, the interaction with the developing immune system of the newborn may be different in dependence of the first colonizer.

PWP03
Influence of Gram-negative probiotics on the intestinal barrier function – miRNAs as a new perspective for inflammatory bowel disease treatment

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The gastrointestinal tract harbours a complex microbial ecosystem, engaged in a continuous crosstalk with the host. The balanced relationship between intestinal epithelial cells (IECs) and gut microbes can be disturbed, resulting in the activation of the mucosal immune system which contributes to the development of inflammatory bowel diseases (IBD).
We used a T84 cell culture model to analyze the cellular responses of IECs co-incubated with the Gram-negativ probiotic strains *E.coli* Nissle 1917, a colonization mutant (EcN-M1) and the urogenic strain ABU (83972). Taking into account that IBD may develop after defects of barrier function and based on microarray data for co-incubated IECs we focussed on proteins (ZO-2, E-cadherin, Occludin) of the apical junctional complex (AJC).
We used transepithelial resistance (TER) of the T84 monolayer as a read-out for monitoring epithelial barrier function. Barrier function was enforced by probiotic *E.coli* and disrupted after EPEC infection (E2348/69). However, TER and barrier function could be reestablished by co-incubation with Gram-negativ probiotics.
By analyzing the expression of miRNA in IECs after co-incubation with probiotics we identified miRNAs that correspond to proteins of the AJC. We confirmed the participation of miRNAs in the regulation of the AJC proteins by transfecting T84 cells with miRNAs, miRNA-inhibitors and miRNA-mimics.The results provide evidence for an involvement of the identified miRNAs in the strengthening effects of probiotics as well as the disruptive effect of EPEC on the barrier function.
This study revealed modular responses of IECs specifically induced by Gram-negativ probiotic *E.coli* strains. Further insight into molecular mechanisms (e.g. miRNAs) might foster the development of new strategies for treatment of gastrointestinal diseases.

PWP04
Molecular mechanisms underlying maturation and semi maturation in murine dendritic cells

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Dependent on their structure and/or concentration, TLR ligands can generate different states of maturation of dendritic cells (DC). Two different states of maturation are well characterised (immature (iDC) and mature (mDC)), differing in migration behaviour, antigen processing and uptake and the ability of activating and polarizing T cells.

Recently a third phenotype, termed "semi mature" was discovered. It is characterised by a high capacity of antigen uptake, markedly reduced migration and an intermediate expression of co-stimulatory surface proteins and MHC class II. Particularly, these semi mature DC fail to activate T cells *in vivo* and *in vitro* and exhibit tolerance to re-stimulation. Differentiation to this DC phenotype is mediated by Interleukin-6 (IL-6). Since the antigen presenting machinery of DC plays a crucial role in T cell activation, we focused on the differences in MHC class II transport to the DC surface between iDC, mDC and smDC. The invariant chain (Ii) is a crucial regulator of MHC class II transport to the cell surface, as soon as Ii is degraded to a small peptide called CLIP, antigen derived peptide can be loaded on MHC class II followed by transport to the cell surface. We could prove that the intracellular amounts level of uncleaved Ii bound to MHC-II is rapidly decreasing within 24 hours in mDC, whereas iDC do not exhibit decreasing levels of uncleaved Ii even 72 hours after stimulation with LPS and smDC exhibit an intermediate reduction. This decrease is not due to reduced synthesis of the Ii protein because levels of Ii not bound to MHC class II remain constant in mDC, smDC and iDC. These findings correlate with enhanced enzymatic activities of cathepsin S (catS) in mDC compared to smDC and iDC, since catS ist the major protease that is responsable for Ii cleaving. It remains to be elucidated if this enhanced catS activity in mature DC is due to decreased levels of cystatin C, a prominent catS inhibitor.

PWP05
Distinct antimutagenic actions of probiotic *Escherichia coli* strain Nissle 1917 against different mutagenic substances

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Background. As shown previously, *E. coli* Nissle 1917 (EcN) exerts antimutagenic activity against some environmental and nutritional mutagens. Here, we compared the ability of living versus killed or inactivated cells of EcN to counteract the mutagenic effects of the directly acting mutagen hydrogen peroxide and the indirectly acting substance benzo(a)pyrene, which requires prior metabolic activation.
Methods. To detect mutagenic action of and antimutagenic activities against hydrogen peroxide (H_2O_2) the comet-assay, for benzo(a)pyrene [B(a)P] the Ames-test was used. H_2O_2 and B(a)P were co-incubated with either live, heat-killed, UV-light-inactivated or ultrasound-treated EcN bacteria or with cell-free spent supernatants of EcN cultures. Samples with H_2O_2 were also treated with proteinase K. After co-incubation and sterile filtration the samples were tested for remaining mutagenic activity. In case of B(a)P, also unfiltrated co-incubation mixtures were assayed.
Results. Mutagenic activities of H_2O_2 and B(a)P were dose-dependently inhibited by live EcN bacteria. Ultrasound-treatment did not affect EcN´s antimutagenic activity against H2O2 while UV-light abolished it. Heat-killed EcN bacteria and cell-free spent supernatants did not show any antimutagenic effect. Proteinase K did not affect the antimutagenic action of live EcN bacteria, but diminished the efficacy of ultrasound-treated EcN. In case of B(a)P, heat-killed and UV-light-treated EcN bacteria showed nearly the same antimutagenic activity when assayed after sterile filtration, while the antimutagenic effect was nearly completely gone in unfiltrated samples. Cell-free spent supernatants did not show any antimutagenic effect, whereas ultrasound-treated EcN exhibited a small effect, both in sterile filtrated and in unfiltrated samples.
Conclusions. Our results suggest that the antimutagenic action of EcN against H_2O_2 is due to enzymatic inactivation, whereas the effect against B(a)P is due to absorbtion of the substance to the bacterial cells.

PWP06
Improved genetic accessibility of a potential probiotic *Bifidobacterium bifidum* strain

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We recently demonstrated the good anti-inflammatory capacity of a potential probiotic strain of *B. bifidum* in two models of murine colitis. However, studies of this and other probiotic candidate strains of bifidobacteria at the molecular level are limited by the lack of molecular tools and low transformation efficiency.
To improve the genetic accessibility of *B. bifidum*, the transformation protocol was optimized for buffer conditions and electrical pulse settings by transforming the *E. coli-Bifidobacterium* shuttle vector pMDY23. To further improve the transformation efficiency, the restriction barrier to foreign DNA by restriction-modification (R-M) systems should be overcome. Using *in silico* analysis one Type I and one Type II R-M system were predicted in the genome of our *B. bifidum* strain. Their presence was confirmed by PCR, Southern Blotting and Sequencing and both systems were shown to be expressed by RT-PCR. Both methyltransferases were introduced for IPTG-inducible expression in a methyltransferase-negative *E. coli* host via chromosomal integration making use of the pREDI system (1) originally designed for markerless deletion. *E. coli-Bifidobacterium* shuttle vectors were propagated in this strain prior to transformation into *B. bifidum* in order to obtain plasmid DNA with a *B. bifidum* strain-specific methylation pattern thereby circumventing the restriction barrier to foreign DNA in this strain.

[1] Yu BJ, Kang KH, Lee JH, Sung BH, Kim MS, Kim SC. Rapid and efficient construction of markerless deletions in the *Escherichia coli* genome. Nucleic Acids Res. 2008 Aug;36(14):e84.

PWP07
New ways in Identification of human vaginal *Lactobacillus spp.*

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Background: The human vagina harbors microorganisms which represent a finely balanced ecosystem. In this regard one important feature of vaginal Lactobacilli is their protective role against pathogens. However, for maintaining and/or recovery of a balanced vaginal flora the correct identification of the respective *Lactobacillus spp.* is an absolute requirement. Moreover, knowledge about the heterogeneity within one group in relation to their distinctive properties is compulsory. However, identification of lactobacillus species leaves a lot to be desired. Therefore we intended to improve the identification and characterization of Lactobacillus species.
Methods: In total 86 lactobacillus strains isolated from human vaginal swabs were analysed. MALDI-TOF was done on cultured microorganisms. PCR was performed with specific primers of 16S-23S rRNA intergenic spacer region and it flanking 23S rRNA. For sequencing we used the 16SrRNA-, tuf-, and hsp60 genes. Furthermore, identification is performed by a combination of PCR and MALDI-TOF as well as PCR and mass-spectroscopy.
Results: Using molecular methods the most precise identification was obtained by sequencing the tuf-gene. In contrast, identification by 16S-23SrRNA specific PCR only sixteen samples could be identified as *Lactobacillus gasseri* and two as Lactobacillus crispatus correctly. Fifty-six samples reacted with two or more species-specific primers, ten strains remained without identification. MALDI-TOF is able to identify type strains.
Conclusion: Although lactobacillus spp. are frequently used in food industry and are more frequently involved in therapy options characterization of that microorganisms lacks standards. Our data clearly show that classical culture based and classical molecular methods are not able to differentiate correctly between the different lactobacillus strains. The combination between PCR and MALDI-TOF or mass spectroscopy seems to be the most promising technique.

PWP08
Heterogeneity within the different vaginal Lactobacillus species isolated from pregnant women

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Background: Lactobacilli form a critically important component of the vaginal ecosystem and are the predominant vaginal microorganisms in healthy premenopausal women. Especially during pregnancy they are protective against infection and preterm birth. However, only about 5 of >40 different Lactobacillus species isolated from human stool samples or added to food colonize the human vagina. Up to now there exist no data on the heterogeneity as well as the different functions within the Lactobacillus species isolated from the human vagina of pregnant women.
Methods: We analyzed vaginal swabs from a total of 120 healthy pregnant women at week 35–41 for the presence of Lactobacilli. Colonies with a phenotype indicating Lactobacillus species were isolated and identified by species-specific PCR, by 16SrRNA sequencing. Typing was performed by rep-PCR and terminal-restriction-fragment polymorphism (T-RFLP).
Results: Among the 120 patients around 30% harboured more than one Lactobacillus species. We mainly isolated *L. acidophilus, L. crispatus, L. gasseri.* Further analysis of the respective Lactobacillus species by rep-PCR and T-RFLP indicated a wide heterogeneity. In this regard, the 26 *L. gasseri* strains belonged to 3 different clusters each subdivided into 2–4 subclusters. Similar results were obtained for the additional Lactobacillus species.

Conclusion: Knowledge about the heterogeneity within the Lactobacillus species in correlation to the additional microorganisms isolated from the respective vaginal swabs is a prerequisite for the study of the respective functionality.

PWP09

Mitochondrial stress fuels endoplasmic reticulum unfolded protein response via double-stranded RNA-activated protein kinase (PKR) under conditions of chronic intestinal inflammation

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Background & Aim: Inflammatory bowel diseases including ulcerative colitis (UC) and Crohn's disease are immunologically-mediated disorders of the gastrointestinal tract. Recently, it has been shown that Endoplasmic reticulum (ER) and mitochondrial (mt) stress associated proteins (GRP78, CPN60) are induced in intestinal epithelial cells (IEC) of murine models of t cell-mediated colitis as well as UC patients under chronic inflammation. The aim of this study was to characterize the role of mt stress responses in IEC under inflammatory conditions.
Methods & Results: To study the specific role of mt stress responses we stimulated the IEC line Mode-K with the selective mt stress inducer OTCΔ. Additionally, cells were treated with Tunicamycin to induce ER UPR. Under mt stress, the double-stranded RNA-activated protein kinase (PKR) was identified to be selectively induced at the mRNA- as well as protein level. Chromatin immunoprecipitation (ChIP) revealed recruitment of the transcription factor (TF) AP-1 to the PKR promoter. In addition, PKR co-immunoprecipitated with eIF2α pointing towards a direct role of PKR in eIF2α phosphorylation after selective mt stress induction. Furthermore, both mt- and ER unfolded protein response (UPR) converged in the recruitment of the TF CHOP to the Cpn60 promoter. Interestingly, respiration and β-oxidation were reduced under ER- and mt stress suggesting metabolic changes in IEC under chronic inflammation. Finally, Western blot analysis of primary IEC from UC patients and the murine models confirmed increased expression of GRP78 and CPN60 associated with an induction of PKR.
Conclusion: Primary IEC from both human UC patients and mouse models of colitis show activated mt stress signaling. Our data demonstrate that mt stress responses enhance ER-associated UPR through PKR induction, eIF2α phosphorylation and CHOP recruitment to the CPN60 promoter. In conclusion, mt stress fuels pathology-relevant ER stress mechanisms under chronic inflammation.

PWP10

Ribosomal RNA-based profiling of bacteria in the axilla of healthy male indicates right-left asymmetry in bacterial activity

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The activity of the human armpit microbiota triggers the formation of body odor. As microbial activity is correlated with the cellular content of rRNA, we used - for the first time - differential 16S rDNA- and rRNA-based fingerprinting and cloning to identify particularly active members of the human armpit microbiota. rDNA and rRNA were isolated from swab samples taken from both armpits of ten pre-conditioned, healthy, right-handed male. Using 16S rDNA sequence data, the majority of peaks in armpit T-RFLP profiles were assigned to bacteria affiliated with well-known genera of skin bacteria: *Staphylococcus, Corynebacterium, Propionibacterium, Anaerococcus, Peptoniphilus*. The relative abundances of these groups were similar among the rDNA and rRNA samples, suggesting that all groups of armpit bacteria were apparently active, probably owing to the beneficial environmental conditions in the armpits (warm, wet, eutrophic). Surprisingly, the relative abundance of bacterial sequences affiliated with *Peptoniphilus* sp. was by far highest (20.1%) in the rRNA samples of the right armpits and significantly higher than in the rRNA samples from the left armpits (9.6%) and the corresponding right (3.0%) and left (4.5%) DNA armpit samples. Thus, bacteria affiliated with *Peptoniphilus* sp. might have been particularly active in the right axillas of the test persons. This might be due to the right-handedness of the test persons, which might cause different environmental conditions, e.g. sweat secretion, in the right axillas.
In conclusion, activity-based analyses appear highly suited to shed more light on the functionality of the human skin microbiota, e.g. its role in the formation of body odor.

PWP11

Effect of Lactobacillus species on *Candida albicans* biofilm formation

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Lactobacillus species express inhibitory properties against *Candida albicans*, the major fungal pathogen of humans and one of the most common causes of nosocomial bloodstream infections. Here we investigated the *in vitro* influence of Lactobacillus species on *Candida albicans* biofilm formation.
Materials and Methods. We used the Candida albicans strains ATCC 10231 and SC 5314 and the Lactobacillus strains *L. reuteri* DSM 20016, *L. johnsonii* DSM 10533, *L. rhamnosus* DSM 20021 and a clinical isolate of *L. rhamnosus*. All strains were used at concentrations ranging from 10^1 up to 10^7/ml. The effects of Lactobacilli on Candida were analysed in a time-and concentration-dependent manner. Metabolic activity of C. albicans cells in biofilms was monitored using the XTT reducing assay. Morphological changes were observed with phase-contrast microscopy.
Results. L.rhamnosus DSM 20021 and *L. johnsonii* DSM 10533 were able to reduce metabolic activity in *C. albicans* ATCC 10231 biofilms of more than 50%. In contrast, *L. reuteri* and the clinical *L. rhamnosus* isolate showed no inhibitory effects. However, at Candida concentrations of 10^4/ml or less, all Lactobacillus strains enhanced metabolic activity in the respective biofilms. Using the *C. albicans* SC 5314, all Lactobacillus species reduced metabolic activity over the whole concentration range of *C. albicans* under study. In this regard *L. johnsonii* reduced the metabolic activity up to 80%. The decrease was accompanied by a dramatically reduced biofilm formation in phase-contrast microscopy. Among the Lactobacillus strains under study only the *L. rhamnosus* strains promoted hyphae formation in both *C. albicans* strains.
Conclusion. Our results clearly show that a) Lactobacillus strains differ in their effects on *C. albicans*, b) *C. albicans* strains differ in their response to Lactobacillus strains which might be due to distinct gene expression.

PWP12
Factors affecting the host-specific intestinal microbiota composition

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Commensal bacteria are implicated in diseases associated with a Western lifestyle. Therapeutic concepts targeting the intestinal microbiota by e.g. probiotics are sometimes difficult to put into reality because of the host-specific composition of the gut ecosystem. The genetic background has been proposed to play a pivotal role in selecting bacteria colonizing a given host. However, the factors that possibly govern microbiota composition are largely unknown. To identify those factors, we associated two groups of germ-free mice differing in their genetic background (C3H and B10) with fecal bacteria from a single conventional donor mouse. Analysis of microbiota composition 12 weeks after association revealed a strain-specific colonization pattern. Gene expression analysis with a customized Affymetrix chip representing ~24.000 genes was applied to identify genes differentially expressed in the colon mucosa of the mice. Only changes in gene expression with a high statistical significance (p≤1,85E-03) are reported. The expression levels of the MHC II gene H2-Q1 (39.7-fold), the mucin gene Muc1, (1.9-fold), the ß-defensin gene Defb37 (3.3-fold), and Ang4 (26.1-fold), encoding a protein with angiogenic and antimicrobial properties were higher in C3H than in B10. In contrast, C3H mice displayed a higher expression of Tlr1 and Cd14 (4.2-fold, each) which play a role in antigen recognition, and of Slpi (29.8-fold), Lpo (11.3-fold), and Lyzs (2.8-fold) all of which encode antimicrobial substances. The ~70-fold higher expression of the antimicrobial Pla2g2c in C3H indicates that this gene is defective in B10 as reported for B6 mice with a very similar genetic background. Our results support the notion that MHC genes are important for an individual microbiota composition. We identified further candidates which possibly play a role in the host-specific nature of the gut ecosystem. The relevance of the differential gene expression is currently tested with a proteomic approach.

PWP13
Immune responses of endosymbiont-bearing carpenter ants

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Insects rely on innate defense mechanisms to combat infections. Whereas the immune system of *Drosophila melanogaster* is well characterized [1], much less is known in other insects, and in particular in social insects [2]. Thus, we focused on the characterization of the innate immune system of the carpenter ant *Camponotus floridanus*.
Serratia marcescens is regarded as a facultative insect pathogen. After ingestion by *D. melanogaster* the bacterium overcomes the intestinal barrier, enters the body cavity and kills the host within a few days [3]. To investigate the consequences of infection with this bacterium on hymenopterans, feeding and injection experiments were carried out with *C. floridanus*. The experiments showed that after oral application *S. marcescens* reaches the intestine, but the bacterium was cleared within 48 hours without increasing mortality of the animals. In contrast, direct injection into the haemocoel allowed massive proliferation of the bacteria causing rapid death of the hosts.
To specify the ant´s immune response on the molecular level, we used suppression subtractive hybridization (SSH) to identify genes that are transcriptionally induced in response to injection of dead *S. marcescens* into the haemocoel. Differentially expressed ESTs encode proteins that share significant sequence similarities with proteins from other insects known to be involved in immune reactions. Among these were factors involved in pathogen recognition, signal transduction, antimicrobial activity, or general stress response. A quantitative analysis of immune gene expression revealed different expression kinetics of individual factors.

[1] Feldhaar and Gross (2008), Microbes Infect., 1-7
[2] Schlüns and Crozier (2009), Myrmecological News 12, 237-249
[3] Nehme *et al.* (2007), PLoS Pathog 3, e173

PWP14
Analysis and comparison of bacterial diversity of healthy adults, mother´s milk and their babies

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The primary infant gut microbiota is described to consist of lactic acid- and bifidobacteria. It may be influenced by the genotype of the baby and/or microbiota of the mother in which the bacteria may be transmitted to the infant through breastfeeding or intestinal contamination. In this context, the aim of this study focused on the simultaneous analyses of the bacterial diversity of healthy adults, mother´s milk and their babies.
A total of 600 strains were isolated from faecal samples from 15 adults living in Syria, 15 faecal samples of breast-fed infants and samples from their mother´s milk, by using 8 specific culture media. All isolates were genotypically typed by RAPD-PCR and identified to the species level by 16S rDNA sequence analyses. 18 different species of *Lactobacillus*, *Enterococcus*, *Weissella*, *Streptococcus* and *Pediococcus* but no bifidobacteria were found. Most of the species were present in different RAPD genotypes. A total of 280 isolates displaying different RAPD patterns or originating from different sources were further characterized by MALDI-TOF Mass Spectrometry analyses. Whereas species identification by MALDI-TOF as compared to 16S sequencing showed an insufficient identification due to a limit of MALDI-TOF data base entries, differentiation at the strain level was possible.

Some species were unique for one of the three sources, while others were found in all sources. Identical genotypes of *L. plantarum*, *L. fermentum*, *L. brevis*, *E. faecium* and *P. pentosaceus* were found in the mother faeces, her milk and the corresponding baby faeces. The prevalence of *L. plantarum* present in these samples resembles the microbiota of fermented vegetables. These are frequently consumed in Syria. Therefore we suggest, that the identical genotypes found in all sample types are candidates for a possible transfer from mother to her baby, which can be influenced by the diet of the mother.

PWV01
Protective properties of lactic acid bacteria against enterohemorrhagic *Escherichia coli* (EHEC) infection in a cell culture model

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Infection with enterohemorrhagic *Escherichia coli* (EHEC) can cause bloody diarrhea, hemorrhagic colitis or the haemolytic uremic syndrome (HUS). Virulence of this food borne pathogen is related to the production of Shiga toxins (Stx1 and Stx2) as well as the ability to cause attaching and effacing lesions in the large intestine. Currently, no causative treatment of the life-threatening HUS is available. A preventative approach may be the ingestion of probiotic bacteria.
In the framework of this project, the interaction of 17 strains of lactic acid bacteria (LAB) and different EHEC strains in a HT-29 cell culture model was analyzed. As parameter for the infection the secretion of interleukin 8 (IL-8) of the infected cells was determined. In coinfection assays with the EHEC strain EDL933 (O157:H7) and different LAB strains, the IL-8 secretion of the cultured cells was reduced to 27 % by some strains in comparison to the single EDL933 infection. In further coinfection assays with EHEC strains of the serotypes O103:H2, O26:H$^-$, 0157:H$^-$ and O113:H21, different abilities of the LAB strains to influence the infection with the different EHEC strains were noticed. Therefore, the protective anti-inflammatory effect of the LAB is strain specific and also with regard to different EHEC strains.
As a second parameter, the activation of the transcription factor Nuclear Factor kappa B (NF-κB) of infected epithelial cells, resulting in IL-8 secretion was investigated. In coinfection trials, using a reporter gene assay, the NF-κB activation was reduced significantly by all tested LAB strains. In these experiments the LAB protected the epithelial cells, but there was no strain dependent protective effect. Thus it is assumed that the observed anti-inflammatory effect in IL-8 reduction is based on another signalling pathway. The four strains, which displayed the strongest effect in the IL-8 assay, will be investigated concerning their probiotic qualities in detail.

PWV02
The mechanisms of clearance of enteric pathogens by competitive commensal bacteria adapted to gut inflammation

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Background: >The mammalian intestine is colonized by a dense microbial community which protects against infection with enteropathogens (colonization resistance). Colonization resistance prohibits growth of *Salmonella serovar typhimurium* (S.T.) in the intestinal lumen after oral infection. However, if the microbiota composition is altered (i.e. antibiotic treatment), S.T. can colonize the gut lumen and trigger pronounced mucosal inflammation. We found that S.T. does benefit from triggering the host´s mucosal defense. Mucosal inflammation helps the pathogen to outcompete the microbiota 1, 2.
Objectives: S.T. generally represents 70-90% off all bacteria in the lumen of the inflamed gut. We noticed that 15-30% of all acutely infected animals tested harbored commensal *E.coli* strains in the gut lumen. We started to analyze this *E.coli* in more detail: We found that they, like S.T., profit from the host´s intestinal immune response. When S.T. triggers inflammation, these "competitor" bacteria grow up to high densities and out-compete S.T. in the gut lumen. Our data indicate that special members of the normal microbiota fulfill an important function within the gut ecosystem: in cases where all other defenses failed (i.e. acute gut infection by S.T.), some specialized bacteria "bloom" to high density and thereby reduce the pathogen burden in the gut. We sequenced the genome of this commensal *E.coli* and set up genetic screens to identify and understand the molecular features underlying pathogen overgrowth in the inflamed intestine.
Conclusions: We hypothesize that these ´competitive *E.coli*´ may provide a means for treating acute Salmonella infections and possibly other human enteric bacterial infections. Moreover, they may be useful for protecting against pathogen spread in lifestock (= zoonotic reservoir for human Salmonella infection) or susceptible human populations.

[1] Stecher, B., *et al.* (2007) PLoS Biol
[2] Stecher, B., and Hardt, W.D. (2008) TiM

PWV03
Bifidobacterium adolescentis protects from dissemination of *Yersinia enterocolitica*

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The *Bifidobacterium adolescentis* is an anaerobic, gram positive bacterium and belongs to the probiotics, which are by definition “live microorganisms that, when administered in adequate amounts, confer a beneficial effect on the health of the host” (FAO/WHO 2001). To investigate a possible protective potential of *B. adolescentis* in a gastro-enteritis we chose oral *Yersinia enterocolitica* infection of mice as pathogenicity model, which leads to a gastro-enteritis in mice. Therefore four different groups of SPF BL/6 mice were investigated: first mocks, second *Bifidobacteria* fed ones, third group was orally challenged with *Yersinia* and the fourth was first *Bifidobacteria* fed and subsequently *Yersinia* infected. Body weight and fitness of *Yersinia* infected mice were controlled and five days past infection mice were sacrificed. The *Bifidobaceteria* colonized group showed less weight loss compared to control group. Whole intestines were analyzed for dendritic cell subpopulations and T-cell subsets, as well were cytokines investigated. Further were colony forming units (CFU) of *Yersinia* in faces and spleen determined. *Bifidobacteria* fed mice showed same *Yersinia* CFU in feces, but in spleens *Yersinia* were only found in mice without the probiotic strain. Colonization with *Bifidobacteria* led to an increase in plasmacytoid dendritic cells and regulatory T-cells in the intestine compared to groups without, independent of *Yersinia* infection. Next were BL/6 mice first *Yersinia* infected and then pDCs depleted using intra venous injection of an anti pDC antibody, as control was the according isotype used. Mice lacking pDCs showed stronger weight loss and a higher *Yersinia* CFU in the spleen.
Colonization with *B. adolescentis* has modulatory effects on the immune system of mice by means of pDCs and regulatory T-cell occurrence, which are probably the reason to achieve a partial protection of the hosts against *Yersinia enterocolitica* infection.

PWV04
Response of plants and human immune cells to bacterial quorum sensing molecules of the N-acyl homoserine lactone type

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Bacteria produce small molecules as autoinducers to sense their environment and to optimize their gene expressions in a cell-density dependent manner, called quorum sensing. In Gram-negative bacteria, these signalling molecules are frequently of the N-acyl homoserine lactone (AHL) type.
Using biosensor constructs specific for certain types of AHL-molecules, the *in situ* production of these compounds could be demonstrated e.g. on root surfaces. Tomato plants, inoculated with AHL-producing Serratia liquefaciens MG1 bacteria, acquired considerable resistance towards the plant pathogenic fungus *Alternaria alternata*. In contrast, *Arabidopsis thaliana* responded to C6- and C8-HSL by altering the hormonal balance and increasing root growth. Using 3-oxo-C12- and 3-oxo-C14-HSLs, *A. thaliana* responded with increased pathogen defence against the leaf pathogen *Pseudomonas syringae* (Zuccaro and Kogel, personal communication). AHL-molecules were also demonstrated to be able to enter the roots and to be transported to the shoot in Arabidopsis and in barley, which obviously lack AHL-lactonases. However, many legumes possess active lactonases and thus AHLs were radidly degraded.
In the interaction of bacteria with human immune cells the activation of dendritic cells (DC) is of key importance since DCs educate T cells to mount the appropriate immune response. While lipopolysaccharides (LPS) of pathogenic bacteria activated DCs, Pseudomonas aeruginosa derived 3-oxo-C12-HSL inhibited DC activation, thus counteracting the immune response. This may explain in part why cystic fibrose patients are unable to clear pulmonary Pseudomonas aeruginosa infections (Binder et al., manuscript in preparation). The response of DC-cells to probiotic Lactobacillus and *Bifidobacterium spp*. is currently investigated. In conclusion, bacterial signalling substances take part in inter-kingdom molecular interactions of bacteria with eucaryotes, and are thus of high relevance for host immunity and well-being.

PWV05
Self-cleavage of the type III-secreted protein NopE1 is essential for physiological function *in planta*

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The type III-secretion system (T3SS) is a complex protein transport machinery, which was first identified in pathogenic bacteria. Within the Rhizobiaceae, T3SSs have been identified in *Bradyrhizobium japonicum, Rhizobium* sp. strain NGR234, *Sinorhizobium fredii* and others. The T3SS is supposed to transport proteins from the bacterial cytosol into the host cell. The role of the T3SS in the establishment of symbiosis is host-dependent (Krause *et al.* 2002).

The type III-secreted effector proteins NopE1 and NopE2 of *B. japonicum* are homologous but have no similarity to other characterized proteins. Nodulation assays revealed that the secreted proteins NopE1 and NopE2 act beneficial in symbiosis with soybean and *M. atropurpureum* but are detrimental for interaction with *Vigna radiata* cv. KPS2. NopE1 was shown to be expressed in symbiosis. Using the adenylate cyclase reporter the type III-dependent transport of NopE1 and NopE2 into the cytosol of nodule cells is shown (Wenzel *et al.*). In the supernatant of genistein-induced *B. japonicum* cultures, NopE1 was found in fragmented form (Süß *et al.* 2006). Biochemical analysis revealed that fragmentation of NopE1 is due to specific cleavage in the presence of calcium ions. Two conserved cleavage sites were identified by Edman degradation and further characterized by site-directed mutagenesis. Self-cleavage of NopE1 could be deactivated by exchange of conserved aspartic acid residues at both cleavage sites. Wild type NopE1 and a non-cleavable NopE1 variant were used for complementation of a NopE1-NopE2 double-mutant. Nodulation assays on the NopE-sensitive plant *V. radiata* cv. KPS2 revealed that protein processing is crucial for the effector function *in vivo* (Wenzel et al.).

[1] Krause *et al.* 2002. Mol. Plant-Microbe Interact. 12, 1228-1235.
[2] Wenzel *et al.* Mol. Plant-Microbe Interact. in press
[3] Süß *et al.* 2006. J. Biotechnol. 126, 69-77.

PWV06
The impact of arbuscular mycorrhizal colonization on plant above-ground metabolite profiles

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Biotic and abiotic factors influencing plant root activity can be expected to have metabolic consequences in all plant organs. The measurement of such consequences might allow i) to detect diagnostic marker patterns for various biotic or abiotic factors influencing root functioning, ii) to measure and compare the extent of respective above-ground effects and iii) to obtain a comprehensive understanding of the specific effects and their connection to below-ground processes. We have chosen GC-MS based metabolite profiling as our primary means to record organ-specific changes. We have applied this method to the model legume Lotus japonicus subjected to control treatment, high-fertilizer treatment and colonization by arbuscular mycorrhizal fungi. As a result from this experiment, we are able to define sets of above-ground metabolites allowing to distinguish between fertilized and non-fertilized as well as mycorrhizal and non-mycorrhizal plants.

PWV07
Symbiont transmission in bathymodiolin mussels from deep-sea hydrothermal vent and cold seep habitats

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Bathymodiolin mussels are endemic to marine habitats that are rich in reduced inorganic compounds such as hydrothermal vents and cold seeps. They harbor chemosynthetic bacteria in their gills which gain energy from oxidizing reduced sulfur compounds and methane. Obligatory associations between hosts and symbionts lead to mutual adaptations and, in some cases, to cospeciation. This has been observed in the chemosynthetic symbioses of vesicomyid clams in which the parental host generation passes the symbiotic bacteria to the offspring. In these assocations, the phylogenetic trees of hosts and symbionts are generally congruent, indicating cospeciation between the symbiotic partners. Bathymodiolin hosts are, in contrast, suggested to acquire their symbionts from the environment, and the degree of host-symbiont cospeciation is unclear. Interestingly, gill tissues of freshly settled juveniles have been reported to harbor bacteria. This may indicate that either larvae transport bacteria from their natal sites, but gradually replace them with local bacteria from the environment in order to adapt to new environmental conditions, or that freshly settled juveniles are infected by bacteria at a very early stage.
To better understand the acquisition of symbionts by the mussels and the evolutionary relationships between hosts and symbionts, *Bathymodiolus* spp. from diverse vent and seep sites were genetically and microscopically analyzed. Phylogenetic reconstructions of host (mitochondrial cytochrome oxidase subunit I) and symbiont (16S rRNA) genes were compared. Our phylogenetic data obtained from adult host specimens do not indicate cospeciation. In addition to phylogenetic analyses, the smallest juveniles were examined with fluorescence *in situ* hybridization (FISH) using phylotype-specific probes to find out if and when very small juvenile mussels are infected with bacteria and which organs are infected at which stage.

PWV08
Exchange of metabolites in phototrophic consortia

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The consortium "*Chlorochromatium aggregatum*" is a highly structured association between green sulfur bacterial epibionts and a central motile chemotrophic Betaproteobacterium. It represents the most highly developed type of bacterial symbiosis and is the first culturable model system to elucidate the molecular basis of symbiosis between different types of bacteria. The symbiotic exchange of metabolites between the two species was investigated by tracking the integration of ^{14}C-labeled CO_2 into their 16S RNA using magnetic capture. Based on the kinetics of carbon incorporation into rRNA, carbon is rapidly transferred from the epibiont to the central bacterium . The transferred metabolites were identified after seaparation of cells of the symbiotic partners by cesium chloride density gradient centrifugation and GC-MS. Glutamate and 2-oxoglutarate could be identified as candidates of carbon transfer. To verify the exchange of 2-oxoglutarate between the epibiont and the central bacterium, the integration of ^{14}C-labeled CO_2 into the RNA was also tested in the presence of non-labeled 2-oxoglutarate. When the effects of both compounds on viability were tested over a period of 30 days by Live/Dead staining, 2-oxoglutarate was found to significantly extend the viability of the central bacterium in the dark compared to glutamate or the negative controls. The data presented prove the theory of the epibiont being the power plant of the consortium, supplying its motor, the central bacterium, with energy and organic compounds, thus revealing a close symbiosis between the two different bacteria.

PWV09
Characterisation of bacterial associations of the human anterior nares

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The human anterior nares are the principle habitat for *Staphylococcus aureus* and approximately 20% of humans are persistently colonised by this pathogen. Although *S. aureus* colonisation of the nares is asymptomatic, nasal carriage plays a crucial role as a source of invasive infections in both community and hospital settings. Therefore, elimination of *S. aureus* nasal carriage seems to be one of the most straightforward strategies to prevent *S. aureus* infections. As interactions between microorganisms influence which species are able to persist in the anterior nares we recently analysed the microbial community composition of 40 healthy candidates by single strand chain polymorphism (SSCP) and a subset by 454-pyrosequencing. These analyses identified the core community of the anterior nares and indicated *Propionibacterium spp.*, *Corynebacterium spp.*, *Staphylococcus spp.*, organisms related to *Dolosigranulum pigrum*, members of the Insertae sedis XI family of the Clostridiales and uncultured actinomycetes to be abundant. Also, previously undiscovered co-colonisation patterns and natural variations in species composition were revealed. To elucidate the diversity and co-occurrence patterns across human populations in more detail, we now developed a terminal restriction fragment polymorphism (T-RFLP) analysis scheme to rapidly assess the community structures in a high throughput format. Reliability of the method was assured by analysis of type members of nasal communities as well as by validation of the results through SSCP. Fine-scale diversity was assessed through SSCP analysis of important subgroups. In the current presentation we will report on the analysis of the nasal microbial communities of 120 candidates, the stability of the community over time and important species-species associations, in particular, those found involving *S. aureus*.

PWV10
Identification of genetic loci in *Lactobacillus plantarum* that modulate the immune response of dendritic cells

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Dendritic cells (DCs) play a major role in orchestrating the responses of innate and adaptive cells to control tolerance and immunity to microbes encountered at mucosal surfaces. Lactobacillus are naturally present in the human intestinal tract and several species and strains have been evaluated for their probiotic activity. Conclusive evidence for the mechanisms underlying the beneficial properties of probiotics is lacking, but results obtained from *in vitro* studies and animal intervention models indicate a strong role for immunomodulation and enhancement of the epithelial barrier functions. In the small intestine DCs are known to sample microbes that gain access to the Peyer's Patches via M-cells but also directly across the epithelium by opening tight junctions and sending dendrites to the luminal side (Rescigno 2001). DCs are the main activators of naive T cells and their T cell polarising properties are largely governed by the nature of the microbial products encountered at mucosal sites.
We showed that the DC cytokine responses to several species of probiotics can be strikingly different and that significant variation is also seen at the strain level. This could account for the strain-dependent properties of probiotics reported in different clinical trials and animal models. Recently we identified gene loci in *L. plantarum* WCFS1 that modulate the immune responses of DCs. The cytokine levels induced by 42 different *L. plantarum* strains were correlated with the presence or absence of genes in each strain by comparative genome hybridization using the WCFS1 genome as a reference. The impact of these genetic loci on the immune response to *L. plantarum* was confirmed by the construction of gene deletion mutants in the WCFS1 strain. These findings contribute to our understanding of the strain-dependent variation in immune response to probiotics and have implications for selection of probiotics with specific immunomodulatory properties.

PWV11
E-cadherin, a mayor player at the gastrointestianl barrier, is regulated by Gram-positive probiotic bacteria

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The gastrointestinal tract harbours a complex microbial ecosystem that is engaged in a continuous crosstalk with the host, in this way maintaining in a balanced relationship between intestinal epithelial cells (IECs), immune responses and gut microbes. This balance can be disturbed leading to the activation of the mucosal immune system, a process that might contribute to the development of inflammatory bowel diseases (IBD).
We used the transepithelial electrical resistance (TER) of a T84 cell monolayer after co-incubation with Gram-positive probiotic bacteria as read-out system to monitor barrier integrity.
Based on DNA-microarray data and taking into account that defects of the intestinal barrier function facilitate the development of IBD, we focussed on genes encoding adherence junction (E-cadherin and β-catenin) proteins. Our results indicate that the barrier function is subject to modulation of different adherence junction proteins by Gram-positive probiotic bacteria.
For in depth transcriptional analysis we focussed on changes in the miRNA expression patterns following Lactobacilli co-incubation. Upregulation of hsa-mir-92a downregulates the expression of transcription factor SNAI2 and enhances E-cadherin expression.
Furthermore, we found that the phosphorylation of adherence junction proteins by different PKC isoforms (i.e. PKCδ/PKCζ) after L. gasseri co-incubation modulates the barrier function of epithelial cells. Accordingly the barrier function is subject not only to the presence but also to the activity of different PKC isoforms which are affected by different Gram-positive probiotic bacteria.
Our study revealed cellular responses of IECs specifically induced by probiotic Lactobacilli. Further insight into the underlying molecular mechanisms will foster the develop-ment of new strategies for the treatment of gastrointestinal diseases (e.g. IBD).

PWV12
E. faecalis Gelatinase contribute to the development of intestinal inflammation by impairing epithelial barrier function: role for bacteria-derived proteases in the pathology of IBD

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Background. The activation of endogenous matrix metalloproteinases (MP) plays an important role in the pathogenesis of chronic intestinal inflammation. The aim of our study was the investigation of bacteria-derived proteases in the development of IBD. For this purpose we focused on the zinc dependent MP Gelatinase (GelE) from *Enterococcus faecalis*.
Results. Monoassociation of gnotobiotic wild type (Wt) and IL-10$^{-/-}$ mice with the GelE producing *E. faecalis* strain OG1RF and two isogenic mutant strains that lack GelE expression (TX5264 and TX5266) revealed the contribution of GelE in the development of intestinal inflammation in a genetic susceptible host. The histopathological analysis showed a significant reduction of colonic inflammation in the absence of bacterial GelE in the IL-10$^{-/-}$ mice. This effect seems not to be a cause of an antigenic response triggered by GelE. E-Cadherin as important adhesion molecule and differentiation marker is completely abolished in the epithelium of the OG1RF colonized IL-10$^{-/-}$ mice suggesting that GelE impairs barrier integrity. Transwell experiments with concentrated conditioned media (CM) of E. faecalis OG1RF as well as purified proteolytically active GelE demonstrated a significant decrease of the transepithelial electrical resistance (TER) in human and murine colonocytes whereas the concentrated CM of GelE lacking strains TX5264 and TX5266 did not affect TER values. The loss of barrier function could be further demonstrated by ussing chamber experiments with Wt and TNF$^{\Delta ARE/Wt}$ mice. Purified GelE led to a significant decrease of TER in the distal colon and to an increased translocation of sodium fluorescein in the TNF$^{\Delta ARE/Wt}$ mice.
Conclusion. *E. faecalis* GelE contributes to the development of intestinal inflammation through the impairment of epithelial barrier function in a genetic susceptible host suggesting a role for bacteria-derived proteases in the pathology of IBD.

PWV13
E. coli Nissle 1917 inhibits T-cell induced colitis in Rag1-/- mice via TLR5 dependent trapping of T cells in mesenteric lymph nodes

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Background: E. coli Nissle 1917 is used as a probiotic to maintain remission in patients with ulcerative colitis. The protective mechanisms are still not clear. In an adoptive T-cell transfer model we studied the molecular mechanisms of EcN on inflammation.
Methods: Immuno-deficient Rag1-/- mice were fed orally with EcN and transplanted with naïve CD4+ T cells. Weight loss and inflammatory status of the colon were monitored. Repopulation of transferred T cells in MLN and colon were analysed by FACS and mRNA cytokine expression in mucosal scrapings was measured. As EcN induces β-defensin production via TLR5 we also used an EcN strain deficient for the flagella (ΔfliC) and additionally, fed RagxTlr5-/- with EcN.
Results: EcN was effective in reducing the T cell mediated inflammation in Rag1-/- mice by trapping the transferred T cells in the MLN. Administration of EcN ΔfliC reduced this protective effect, suggesting a TLR5 dependent mechanism. The protective effect of EcN was also abolished in RagxTlr5-/- mice.
Conclusions: EcN seems to alter the migratory behaviour of T cells, which accumulate in the MLN in a TLR5-dependent manner. Therefore, EcN treatment limits the infiltration of TH1 cells in the inflamed colon.

PWV14
Commensal microflora indirectly instructs epithelial expression of Reg3 genes via IL-22-producing lymphocytes of the lymphoid tissue inducer cell lineage

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We recently identified a cell population within the lamina propria of the small intestine characterized by its high expression of the transcription factor retinoic acid-related orphan receptor γ t (RORγt) and the expression of activating natural killer (NK) receptors such as NKG2D and NKp46. Similar to lymphoid tissue inducer (LTi) cells, these cells depended on the presence of RORγt for their development. Therefore, we have tentatively named these cells NKR-LTi cells. Both LTi cells and a fraction of NKR-LTi cells colocalized within the cryptropatches of the lamina propria of the small intestine and constitutively produced high levels of interleukin (IL-) 22. Interestingly, the constitutive production of IL-22 depended on the presence of commensal microflora. IL-22 is known to induce the expression of antimicrobial genes (e.g., Reg3 genes) in epithelial cells. Similar to germ-free mice, mice genetically lacking IL-22 or lacking IL-22-producing cells (RORγt-deficient mice) showed dramatically reduced expression of Reg3 in epithelial cells. Application of IL-22 into germ-free mice or mice lacking RORγt completely rescued epithelial Reg3 expression showing that IL-22 is necessary and sufficient in supporting epithelial Reg3 gene expression. Our data support the view that the commensal microflora indirectly instructs epithelial expression of Reg3 genes via IL-22-producing lymphocytes of the LTi cell lineage.

PWV15
Gut bacterial diversity in the TNF^{deltaARE} mouse model of ileitis

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Objectives: Microbes play important roles in inflammatory bowel diseases (IBD), including Crohn's disease. However, there is little data on gut bacteria involved in the onset and maintenance of chronic ileitis. Because many intestinal bacteria are still not cultured, we aimed at characterizing cultivable bacteria from the intestine of the TNF^{deltaARE} mouse model of Th1-driven ileitis. Also, endoplasmic reticulum (ER) stress is involved in IBD, as indicated by overexpression of the glucose-regulated protein (Grp)-78 in inflamed intestinal epithelial cells (IEC). Since molecular chaperones are evolutionary conserved and regulate immune responses, we hypothesized that surface eukaryotic-chaperone-like structures from gut bacteria influence IEC functions.
RESULTS. Two so far unknown bacteria were isolated from the intestine of TNF^{deltaARE} mice. The first strain was isolated from the ileal mucosa on a mucin-containing medium, hinting at possible close interactions with IEC. Based on comparative phenotypic and genetic analyses, the novel bacterium was named *Enterorhabdus mucosicola* gen. nov. sp. nov. and classified as an equol-producing risk-group-2 bacterium resistant to colistin and ciprofloxcin. The second new strain was obtained from cecal content after isolation on a selective medium containing amino acids. It was identified as a new member of the genus *Bacteroides*. Molecular work indicated that the species is dominant in the mouse intestine, independent of host genotype. Finally, we isolated bacteria from the cecum of a 25-weeks-old TNF^{deltaARE} mouse using DYNAL magnetic beads coated with mouse anti-Grp-78 antibodies. Antibody-free beads were used as control. Twenty-seven isolates were identified by 16S rRNA gene sequencing. Six strains, including four likely novel bacteria, occurred only in samples isolated on Grp-78 antibodies. They belong to the family *Coriobacteriaceae* or the genera *Enterorhabdus*, *Neisseria*, *Staphylococcus* and *Streptococcus*. Taxonomic and functional description of the isolates is underway, in particular with respect to their involvement in the regulation of immune and cell stress responses.

QSV01
QM-Aspekte in der PCR Routinediagnostik

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PCR-Verfahren zum Nachweis langsam wachsender, schwer anzüchtbarer oder durch Antibiose bereits abgetöteter Erreger sind inzwischen Standard im mikrobiologisch-diagnostischen Labor. In den letzten Jahren wurde die konventionelle PCR zunehmend durch moderne Real-time PCR-Verfahren ersetzt, die eine Quantifizierung ermöglichen und durch Arbeitsersparnis die Untersuchungdauer verkürzen.
Der Einsatz von Amplifikationsverfahren stellt wegen der Gefahr falsch positiver und falsch negativer Ergebnisse besondere Anforderungen an die Qualitätskontrolle. Das betrifft neben der Organisation des Arbeitsablaufes im Labor u.a. auch die Geräteüberwachung und Reagenzienkontrolle. Insbesondere bei der Verwendung von inhouse-Testen stellt dieser zusätzliche Aufwand neben der Routinediagnostik oft eine Herausforderung dar. Im Vortrag wird an Hand von Beispielen auf diese Thematik eingegangen und Vorteile der Real-time-PCR auch im Bereich der Qualitätskontrolle aufgezeigt.

QSV02
Quality assurance and inter-laboratory comparison assays in nucleic acid testing (NAT)

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Nucleic acid testing (NAT) has become a powerful tool for routine diagnosis of viral, bacterial and fungal pathogens. The increasing availability of commercial assays and the extensive automation of nucleic acid extraction and detection resulted in a broad range of applications with a tremendous variety of test formats. In addition the rising use of "in-house" assays expanded the field of NAT and many diagnostic laboratories started to introduce and perform PCR-testing. However, at the latest the introduction of quantitative PCR demonstrated the necessity to launch inter-laboratory comparison tests and quality standards, as it rapidly became clear, that considerable analytical differences between various methods, manufacturers and users were observed.
Varying nucleic acid extraction protocols revealed problems due to co-extraction of PCR-inhibitors, strong dependency concerning the efficacy of RNA- and DNA-isolation related to the type of sample or patient material and often non-reproducible efficiencies for example in the process of reverse transcription of RNA into c-DNA. Appropriate internal controls (IC) were introduced to monitor potential problems during nucleic acid extraction, purification and PCR-amplification. This led to a substantial improvement in detecting, addressing and resolving challenging questions and problems in PCR performance.
On the other hand new questions emerge as unintentional and objectionable interference of added ICs and -especially- low copy targets is widely discussed. Increasing cost pressure leads to extended application of multiplex-PCR-systems in order to detect different pathogens in one step and in one reaction tube. Nevertheless these assays are often characterized by complex analysis patterns and hard to interpret experimental data. In this context testing of pooled samples resulting in a substantial reduction of costs but also sensitivity is also controversially debated. The "optimal" PCR, a subject of the question asked?

QSV03
PCR based detection of *Coxiella burnetii* and *Francisella tularensis*: new series of INSTAND e.V. round robin tests

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Coxiella burnetii, the causative agent of Q fever, is responsible for periodic outbreaks in Germany. Besides subclinical courses, 50% of the infected persons develop acute Q fever with atypical pneumonia or hepatitis. Chronic forms – often associated with endocarditis – are rare. Due to unspecific or mild clinical symptoms clinical diagnosis is often delayed. In the first 10 to 14 days after infection, serological methods representing the gold standard in laboratory confirmation of clinically suspected Q fever (ELISA/IIFT) could be negative or not diagnostically conclusive, commonly a follow-up sample is needed. PCR detection of specific Coxiella DNA from different clinical materials (sputum, blood, serum) could support early diagnosis and reduce the time to confirm clinical Q fever cases. In chronic cases, PCR might be useful for controlling the activity of this disease form.

Tularemia caused by *Francisella tularensis*, has shown a growing importance in recent years in Germany. Serology is still a cornerstone of tularemia diagnosis, but may fail in acute severe cases because antibodies do not appear until day 7 to 10 after the occurrence of symptoms. Bacteriological testing is time-consuming (up to 12 days), difficult and prone to false negative results due to contaminant or physiological flora. Bacterial culture, if successful, poses a significant risk for laboratory infections. Since 2007, laboratory diagnosis using conventional or real-time PCR protocols is also recommended by WHO.
Both pathogens can cause community-acquired pneumonia but would be missed by commonly recommended empiric antibiotic regimens. For this reason direct detection of specific DNA would be of significant importance to guide antibiotic treatment if a standard regime has failed. The direct detection of *C. burnetii* or *F. tularensis* in clinical samples has to be reported according to the German Infection Protection Act (IfSG).

QSV04
Assessing the quality of medical laboratories: A follow-up analysis of proficiency testing results of accredited and non-accredited laboratories for bacteriologic infection serology

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Assessing the quality of medical laboratories remains difficult for clinicians and medical boards. Certification or accreditation is generally believed to result in higher analytical accuracy and diagnostic quality in the routine medical laboratory. Objective data to support this assumption, however, is lacking. Here, a follow-up analysis of 19 proficiency testing surveys on the quality of Lyme borreliosis-, syphilis, helicobacter-, and tetanus toxoid-antibody testing that were held in Germany between 2000 and 2009 were investigated for the performance of accredited and non accredited laboratories in infection serology. Reference test results were determined according to the INSTAND guidelines for the performance of proficiency testing surveys in infection serology. The successful performance and the mean accuracy of test results were compared for accredited and non-accredited laboratories. 5386 individual test results obtained from 660 laboratories including 84 accredited and 576 non-accredited medical laboratories were available for statistical examination. Our investigation will present significant findings concerning the individual influence of accreditation on the overall performance of laboratories throughout the proficiency testing surveys in some technically demanding disciplines of infection serology. Moreover, our findings will be discussed in the light of possible confounders such as the expertise of the individual laboratory or the manufacturer of the diagnostic test to answer the question as to whether the individual eminence of laboratories can really be measured and if there is a considerable impact of accreditation on the analytical quality of infection serology in the medical laboratory.

QSV05
Good cell culture practice GCCP: Standardization and Quality Control in Cell Culture Laboratories

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During the 3rd world congress for "Alternatives and Animal use in Life Sciences" 1999 in Bologna as well as in a first "Good Cell culture Practice Task Force Report" from the ECVAM 2002 scientists postulated guidelines to assure at least a minimum of standardization in cell culture laboratories. Regarding the central issues in this field we have to focus on the cells themselves, on liquid handling and finally on the operators. Especially during the last two years several papers reported on the appearance of wrong cell lines in several laboratories. Whereas most of all permanent cell lines available from regular cell banks like ATCC, ECACC and DMSZ are well characterized by their specific karyotype, their unique DNA-Profile and some other features most laboratories fail to control their cell lines during serial subcultivation using these parameters. But an important prerequisite of cell line research is the authenticity of the used cell lines because the mistaken identity of a cell line may lead to invalid conclusions.
Thus, it is not surprising that there is a great uncertainty with respect to the correct identity of cell lines. Aside from a simple mistake of cell lines it is well known that during extended propagation *in vitro* most cell lines behave unstable thus changing potentially their individual properties. Therefore GCCP demands for accurate cell banking using master banks and working banks like it is recommended in all cell culture handbooks. Identity testing can easily be performed in most (certified) genetic laboratories by Multiplex PCR or DNA-profiling. We just reported on the successful control of some prostate cancer cell lines from several research laboratories using this approach.
Another major problem appears during all steps of liquid handling. Since in most laboratories cell cultures were transferred out of the incubator during microscopic control and liquid handling, cells are subjected to changes in their environment. As a consequence - depending on the type of experiments - highly reproducible results can not be obtained. Only fully automated cell culturing with the aid of life cell imaging in combination with robotic technologies allows for highly standardized cell culture technique.
At least, contamination especially with mycoplasms seems to be another problem as a result of missing quality control. The widely used antibiotics and the only fragmentary control for the absence of mycoplasms with validated methods promote the occurrence of this type of contamination. Since a lot of validated techniques are available to test for mycoplasms it is unbelievable that many cell culture laboratories disclaim these controls.
In conclusion, the introduction of obligatory standards and quality control in the cell culture laboratory is imperatively required. But it looks like that even the above mentioned reports fail to convince scientists to introduce quality control in the cell culture labs.

QSV06
External Quality Assessment Schemes (EQASs) for Genome and Antigen Detection of New Influenza A(H1N1), Avian Influenza A(H5N1) and Seasonal Influenza

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External Quality Assessment Schemes (EQASs) are eligible to monitor the skills of laboratories and the suitability and reliability of commercial and in house tests. In Germany EQASs in virology are organized by INSTAND in cooperation with Charité Berlin, Robert Koch-Institute, German Association against Virus Diseases (DVV) and Society of Virology (GfV). EQASs for avian influenza A(H5N1) were introduced in Germany in 2006 and for new influenza A(H1N1) in August 2009. Nearly 190 laboratories (9 countries) received 8 samples with lyophilized new influenza A(H1N1), avian influenza A(H5N1), seasonal influenza A(H1N1 or H3N2) or influenza B virus per scheme. New influenza A(H1N1) and avian influenza A(H5N1) viruses were chemically inactivated.
The EQA scheme results revealed that *new influenza A(H1N1)* was reliably detected by specific genome detection tests. Only sporadic false positive results for influenza A(avian or seasonal) occurred. In contrast, rapid tests for antigen detection of new influenza A(H1N1) showed >20% false negative or borderline results.
Avian influenza A(H5N1) were detected falsely negative by genome detection tests when new strains were introduced. Rapid tests revealed a lower sensitivity for avian influenza A(H5N1).
For *seasonal influenza A and B viruses* most genome detection tests and rapid tests determined correctly.
In conclusion, these EQASs showed a good reliability of commercial and in house tests for the detection of new influenza A(H1N1). For avian influenza A(H5N1) some laboratories failed to detect this virus depending on the virus strain. Rapid tests for antigen detection have limited suitability for confirmation or exclusion of a case of new influenza A(H1N1).

RKP01
Toxoplasma gondii recombinant antigens for diagnostic of human and animal infection

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One of the most successful protozoan parasites is *Toxoplasma gondii*, the agent of toxoplasmosis. It probably infects all kinds of mammals and birds. The parasite exists in the stage of tachyzoites, bradyzoites and oocytes. The latter are only present in the primary hosts, cats and other members of the family of felidae. Each stage is known to express stage-specific antigens.

The aim of the present study was to investigate whether stage-specific antigens might be useful for improving diagnosis or for having prognostic implications. Firstly, we looked for antigens for detection of acute infection where tachyzoites are predominantly. Therefore, we identified possible markers for acute stage by 2D-Electrophoresis and analyzed them with MALDI-TOF. The MALDI-TOF analysis identified 20 from 47 analyzed spots as *T. gondii* proteins.
Furthermore, we recombinantly expressed five antigens identified by MALDI-TOF and seven known stage- and non-stage specific antigens in *E. coli*.
Afterwards, we evaluated the recombinant antigens in a lineblot assay regarding their ability for diagnostic of human infection and as prognostic markers forecast possible clinical manifestations.
In addition, eight recombinant antigens were also tested on turkeys that were experimentally infected either with oocysts or tachyzoites of *T. gondii*. The titer kinetics of the respective animals showed antigen-specific differences that may allow drawing a conclusion of the way of infection.

RKP02
Enhanced Surveillance of Invasive Infections by *Haemophilus influenzae* in Baden-Wuerttemberg

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The epidemiology of invasive disease caused by *H. influenzae* has changed considerably within the last decade. After successful implementation of the polysaccharide-conjugate vaccine against *H. influenzae* serotype b (Hib) in the 1990s, the incidence of invasive disease has decreased to values below 0.1/100,000 in 2000 in Germany. Nevertheless, since 2001 incidence rates have risen again to 0.18/100,000 in 2008 (data from the statutory notification system). This rise is mainly due to non-typeable *H. influenzae* (NTHi) strains, which do not express a polysaccharide capsule, among older adults. Reported incidence rates were particularly high in the state of Baden-Wuerttemberg (BW) with 0.34 cases per 100,000 inhabitants in 2008. This trend prompted a collaboration between KLHi and LGA with the objectives of comprehensive typing of isolates by KLHi and documention of disease manifestation by LGA. Out of 31 cases reported to LGA, 23 matching strains were received by the end of October 2009. Most strains processed at KLHi were NTHi (19 of 23, 83%). Serotypes b (3 of 23, 13%), and f (1 of 23, 4%) were additionally found. All strains were beta-lactamase negative. The mean and median age of patients was 54 and 69 years, respectively. The majority of isolates were cultured from blood (87%). All remaining isolates were grown from cerebrospinal fluid. Further typing by Multi-Locus-Sequence-Typing (MLST) revealed a highly diverse bacterial population, suggesting that invasive infections represent sporadic cases with no evidence for epidemiological links. Analysis of disease manifestation among notified cases will be performed at the end of 2009. In summary, invasive disease by *H. influenzae* in BW mostly affects the elderly, and is caused predominantly by highly diverse NTHi.

RKP03
Sequencing of the *Haemophilus influenzae* serotype e capsule locus

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Serotyping of *Haemophilus influenzae* (*H. influenzae*) isolates from invasive infections by slide agglutination using capsule specific antibodies is part of the core services offered by the consulting laboratory for *H. influenzae*. Additionally, the serotypes are confirmed by specific PCRs (Falla *et al.*, 1994). Whereas this method proved useful with five of the six serotypes (a-d and f), problems have been encountered by different laboratories, including our own, to detect serotype e using the established PCR.
To address this problem, we amplified the serotype e specific region II of the cap locus in *H. influenzae* strain ATCC8142 with primers derived from the conserved flanking regions I and III, as well as the published primers for *H. influenzae* e (Hie). We have identified a 11.5 kb DNA region comprising eight open reading frames. The genes show the highest homology with capsule synthesis genes found in *Pasteurella multocida* serogroup B and E, and partially also *Neisseria meningitidis* serogroup A. Consistently, the composition of the Hie capsular polysaccharide (Branefors-Helander *et al.*, 1980; Tsui *et al.*, 1980) matches that of *P. multocida* (Townsend *et al.*, 2001). This finding highlights the impact of horizontal gene transfer across genus borders for the evolution of bacterial capsular polysaccharides.
Finally, a set of specific primers was derived from the identified sequence for a reliable diagnostic Hie PCR. For validation, the new PCR will be tested with clinical isolates from our own strain collection and from international collaborators.

RKP04
How reliable is the Etest for susceptibility testing of Metronidazole for *H. pylori*

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Susceptibility testing of metronidazole is actually not approved by the FDA due to "variance in test results, lack of reproducibility and clinical relevance". In Maastricht III consensus report on "Current concepts in the management of *H. pylori* infection" metronidazole testing is not routinely recommended in clinical practice in contrast to clarithromycin testing because *in vitro* resistance to metronidazole may not accurately reflect *in vivo* resistance. In Germany, about 32% of *H. pylori* strains isolated from not-pretreated patients are resistant to metronidazole, which is a relevant problem because metronidazole is a recommended first line drug. Furthermore, phenotypic susceptibility testing is the only option for detection of metronidazole resistance because molecular mechanisms of resistance are not finally explained. The aim of our study is to evaluate the precision, reproducibility and repeatability of metronidazole testing using the Etest method. 50 randomly selected *H. pylori* isolates cultured from routine gastric biopsy specimens (no selection concerning resistance patterns, prior eradication therapies, patient data etc.) are actually under study. Preliminary results show, that Minimal Inhibitory Concentrations (MIC) determined by the Etest depends on the culture media and the McFarland turbidity standard used.

RKP05
Molecular characterization of *Salmonella enterica* serovar 4,[5],12:i:- from pig via pork to human

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In Europe a monophasic *Salmonella enterica* serovar Typhimurium (seroformula 4,[5],12:i:-) has been established among the ten most frequently isolated serovars in pig and human. An EU-monitoring study in 2006/2007 on the prevalence of *Salmonella* in slaughter pigs revealed that for Germany serovar 4,[5],12:i:- was the second most isolated serovar.
The aim of the study was to characterize the clonality, antimicrobial resistance and pathogenicity gene repertoire of this serovar. For that purpose various genotypic and phenotypic typing methods were applied to 148 selected serovar 4,[5],12:i:- strains isolated in 2006/2007 from pig primary production (lymph nodes), pork and humans in Germany.
Two major clonal lineages were found with 70% of the isolates assigned to phage type DT193 and 19% phage type DT120. Eighty-one percent of the strains were multi-resistant with the main resistance pattern ampicillin, streptomycin, tetracycline and sulfamethoxazol encoded by the genes $bla_{\text{tem1-like}}$, *strA/B*, *tet*(B) and *sul2*. Subtyping by MLVA and PFGE revealed a number of various clusters. Identical combinations of patterns were found in isolates from the primary production and pork, as well as clinical isolates.
Investigating the presence or absence of 102 representative genes with known contribution to pathogenicity of *Salmonella* by microarray analysis indicated no differences (two exceptions) between the isolates. There was also mainly no variation in the pathogenicity gene repertoire compared to 20 *Salmonella* serovar Typhimurium isolates.
In conclusion, the data revealed a high genetic relatedness to the biphasic *S. enterica* serovar Typhimurium. Consequently, it must be eradicated from the pig production chain, too, in order to disrupt the increasing transmission to humans.

RKP06

Recommendations for the diagnosis and treatment of Lyme borreliosis: What do guidelines tell us?

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Background: In Europe and the USA there is considerable public dispute in respect to suitable methods for diagnosis of Lyme borreliosis(LB), the choice of antibiotics as well as length of antibiotic treatment. Conventional diagnostics, notably serology and PCR, are thought to be unreliable. Therefore a broad array of tests is offered by laboratories - such as lymphocyte transformation test, visual contrast sensitivity test or a test on decreased CD57+/CD3- lymphocyte subpopulation - that are said to be more sensitive, more specific and valuable for therapy control. However, none of these tests is properly validated. Further, therapy protocols are distributed,that recommend long term antibiotic therapy, pulsed therapy or even antibiotics never proven to be effective in therapy of LB.
Methods: With the help of members of the European Union Concerted Action on Lyme Borreliosis European and American guidelines for diagnosis and/or treatment of LB were searched for in medical databases. Guidelines were compared in terms of laboratory diagnostics and treatment recommendations(antibiotics, dosage, possible combinations and length)for erythema migrans, neuroborreliosis and Lyme arthritis.
Results: Overall 11 guidelines - 9 from Europe, 2 from the USA - were identified and included in this survey. There is overall agreement that supporting laboratory data - antibody detection, less important PCR and cultivation - is essential for diagnosis of later-stage infection. No guideline recommended serological testing for erythema migrans. Other tests were not recommended.
Conclusions: Overall there are great similarities regarding antibiotic therapy between the various guidelines, with some minor differences in dosage and duration. Antibiotics frequently recommended for therapy are Doxycyclin, Amoxicillin, Ceftriaxon and Cefotaxim. Furthermore PenicillinG, Cefuroxim and Azithromycin can be used. Prolonged or multiple courses of antibiotics or combinations were not recommended.

RKP07

Serotype specific penicillin resistance of *Streptococcus pneumoniae* in Germany from 1992 to 2008

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Objectives: Only few data on penicillin resistance of *S. pneumoniae* broken down to individual serotypes and tracked over years are available globally. Although some reports have been published on penicillin resistance in Germany, no data are reported on serotype dependent resistance development over time.
Methods: A population and laboratory based surveillance study was conducted in Germany by the German National Reference Center for Streptococci (NRCS) to collect data about invasive pneumococcal disease in children < 16 years and adults ≥ 16 years. Isolates were identified using standard procedures including bile solubility and optochin sensitivity.
Results: In this study, 11,814 isolates with data on penicillin susceptibility available could be included from January 1992 to December 2008. 8,837 isolates (74.8 %) were from adults and 2,977 isolates (25.2 %) originated from children. Overall, the leading serotypes were serotypes 14 (16.5% of serotyped isolates), 3 (8.1%), 7F (7.7%), 1 (7.4%) and 23F (6.0 %). The nonsusceptibility rate of all isolates adds up to 5.5% (intermediate, 4.3%; resistant, 1.2%) when the CLSI 2006 guidelines were applied, and to 1.4% (intermediate, 0.2%; resistant, 1.2%) when using the CLSI 2009 guidelines. Generally, slightly higher resistance rates were observed among children than among adults. Serotypes contributing considerably to pneumococcal penicillin nonsusceptibility by a combination of frequency among invasive isolates and relatively high penicillin nonsusceptibility are 19A, 9V, 6B, 19F, 23F and 14. While the nonsusceptibility among serotype 19A isolates increased considerably over the years, the development of nonsusceptibility rates among the other serotypes is less and more ambiguous.
Conclusions: The present study describes the variations of penicillin resistance among pneumococcal serotypes. Especially serotypes 19A, 9V, 6B, 19F, 23F and 14 contribute to pneumococcal penicillin resistance, and, over the years, the increase of penicillin resistance of serotype 19A is remarkable.

RKP08

Comparison of commercially available immunoblot assays for the detection of antibodies to *Bordetella pertussis*

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Background: *Bordetella pertussis* continues to circulate in highly vaccinated populations. Cases of pertussis are mostly observed in very young infants, older vaccinated children, adolescents and adults. Apart from infants, the diagnosis of pertussis is based mostly on serological tests. Antibodies to *B. pertussis* antigens can be measured either by ELISA or immunoblots, and various commercial kits for both methods are available in Germany. As from 2009, a WHO reference preparation for human antibodies to various *B. pertussis* is available. We compared immunoblots from four different manufacturers, measuring isotypes IgG and IgG.
Materials and Methods: We used immunoblots from four different German manufacturers: Euroimmun (Lübeck) Microgen (Neuried) Viramed (Planegg) and Virotech (Rüsselsheim). All kits measured IgG and IgA-antibodies separately. Kits were used according to the manufacturers' descriptions, and the results were expressed as suggested by the manufacturers.
Reference preparations: 1st International Standard of Pertussis Antibodies (WHO) (NIBSC, Potters Bar, GB). Secondary standard (NIBSC) related to the 1st International Standard. Both references were used undiluted and in a 1:10 dilution. Reference preparations #3, #4 and #5 of the CBER/FDA (Bethesda,MD,USA).
Patient sera: Patient sera with high antibody titres (ELISA) or with a titre increase between acute and convalescent samples.
Sera from patients with suspected clinical pertussis, in which a laboratory confirmation could not be achieved.
Results: The sensitivity and specificity of all assays was sufficient, when the qualitative results of immunoblots and ELISAs using purified antigens were compared. The interpretation of a semiquantitive reading of the immunoblots, however, did not compare well to the quantitative results of the ELISAs.
Conclusions: Due to the lack of quantification of antibody concentrations, IgG and IgA immunoblots are of limited value in the serological diagnosis of pertussis.

RKP09

Invasive bacterial infections in the elderly: data from the IBI network

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Demographic changes affect the epidemiology of bacterial infectious disease. One task set by the Invasive Bacterial Infections (IBI) reference network has therefore been the exploration of pneumococcal, meningococcal, and Haemophilus influenzae infections in the elderly. Recent childhood vaccination recommendations might impact on disease rates and serotype distribution in this age group through the phenomenon of herd protection. The data from the reference network enabled us to unravel serotype distributions of IBI caused by the three pathogens. Since the introduction of childhood pneumococcal conjugate vaccines there have been drastic changes in the serotype distribution. Vaccine serotypes have almost disappeared among children suffering from invasive pneumococcal disease (IPD). Most likely due to herd immunity effects, the same is true for the elderly, despite of the fact that this age group is not vaccinated. Serogroup W135 and Y meningococci are rarely found in invasive meningococcal disease in Germany, but clearly exhibt an age shift to the elderly when compared to serogroup B and C meningococci. This phenomenon might reflect a decrease of immunity controlling asymptomatic carriage. According to statutory notification data of Haemophilus influenzae infections there has been an increase in invasive disease exclusively in the elderly. This increase is caused by non-typeable strains. In conclusion, the results of our survey demonstrate that herd immunity is an important factor for the prevention of IPD in the elderly, whereas this is not established for meningococci and Haemophilus influenzae due to differing transmission routes and serotype distributions across age groups. A change to "atypical" serotypes in the elderly needs to be considered for vaccine policies e.g. in splenectomized elderly patients.

RKP10

Nucleotide sequence varieties of the immediate early gene 62 in different german varicella-zoster wild-type strains

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Background: The open reading frame (ORF) 62 of varicella-zoster virus (VZV) contains numerous vaccine-related single nucleotide polymorphisms (SNP). Thus, this gene can be used to differentiate between the circulating wild-type strains and the Oka vaccine strain (vOka). We report sequencing data of the ORF 62 in different wild-type strains currently circulating.
Methods: The ORF 62 was analyzed by amplification of different fragments and sequencing in 86 different German wild-type strains with the known genotypes E1, E2 and M1.
Results: The genotype E1 strains contained three genotype-specific markers whereas only one genotype-specific marker was localized in the genotypes E2 and M1 strains. The genetic analysis of 37 E2 strains resulted in 17 strain variants, 11 of these were found in strains from varicella and 8 in strains from zoster. One E2 zoster strain had one vaccine mutation at the nucleotide position 107252. Thirty-one E1 strains were divided into 10 different genotype variants, 8 of them were associated with varicella and 3 with zoster. Only one genotype version resulting from a uniform ORF 62 pattern could be found in 18 genotype M1 strains.
Conclusions: VZV wild-type strains of the European genotypes E1 and E2 exhibit a high variability of the ORF 62 caused probably by a number of recombination events. The uniform pattern of the genotype M1 leads to the assumption that these strains were brought to Germany via few sources. M1 strains must have spread by a higher infectivity than the European E1 and E2 strains.

RKP11

Primary antibiotic susceptibility of *Helicobacter pylori* 2008-2009 - A European multi-centre study – The German situation

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The 3rd European *in vitro* antimicrobial resistance survey on *H. pylori* was launched in May 2008 with the National Reference Centre (NRC) for Helicobacter pylori as the German study centre. Aim was to assess the primary resistance rates in clinical isolates against clarithromycin, levofloxacin, tetracycline, rifabutin, metronidazole and amoxicillin in Europe. For susceptibility testing all centres used the same medium lots, Etest strips and protocol of Etest performance and reading. The NRC performed real time PCR for detection of resistance to clarithromycin (23S RT-PCR) from all gastric biopsies in addition to culture. Data were collected online and centrally processed.
In Germany, 430 gastric biopsies from previously untreated patients were investigated. A total of 197 biopsies were *H. pylori* negative (46%; culture and RT-PCR). In 108 biopsies RT-PCR was positive but culture negative (25%). MICs were determined for 125 isolates (29%). Primary resistance rates were 32% for metronidazole (MZ), 11% for clarithromycin (CLA), 18% for quinolones (QUI), and 2% for rifabutin. None of the strains showed resistance to amoxicillin and tetracycline. Dual resistance (MZ, CLA) was present in 4% and triple resistance (MZ, CLA, QUI) in 1% of the isolates. RT-PCR of the 108 culture negative biopsies revealed wild type in 70% and resistance-mediating clarithromycin mutations in 30%, with A2143G mutation most common.
Even in naive patients resistant *H. pylori* were found up to 32%. These primary quotes are comparable to our ResiNet data except for clarithromycin, for which an increase from 6% (ResiNet 2001-2008) to 11% (Multi-centre 2008-2009) was observed. This study supports the increasing demand for susceptibility testing before eradication therapy. The latter is now recommended already after the first treatment failure by the German S3-guideline on *H. pylori* infection and peptic ulcer disease.

RKP12

Will be not presented and published

RKP13

First description of carbapenem non-susceptible *K. pneumoniae* isolates from Germany harbouring the OXA-48 carbapenemase

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Objectives: Multidrug resistance in *Enterobacteriaceae* is of utmost clinical importance since no new antibiotics with activity against gramnegative bacteria will be introduced in the next five years. OXA-48 carbapenemases have been described as an emerging resistance mechanism recently.
Methods: Susceptibility testing was performed by Vitek 2 and Etest. Carbapenemase production was detected by a microbiological assay employing cell-free extracts and parallel testing of the effects of selective β-lactamase inhibitors. In addition a modified Hodge test was performed. PCRs for known carbapenemase gens followed by sequencing were performed. The number of β-lactamases was determined by isoelectric focusing. A pulsed-field gel electrophoresis (PFGE) was performed and plasmid transfer by transformation was tried.
Results: Four non-copy *K. pneumoniae* isolates from an universital hospital in Freiburg (Southern Germany) were tested because of elevated MICs in Vitek 2. The modified hodge test was positive in all isolates and the micorbiological assay demonstrated a carbapenemase activity which was not inhibited by EDTA, clavulanic acid, cloxacillin or 3-aminophenylboronic acid. Imipenem MICs varied between 1 mg/L and >32 mg/L, meropenem MICs were in the range of 0.38 mg/L til >32 mg/L. PCRs for KPC, VIM, IMP and GES were negative, whereas PCRs and subsequent sequencing showed the presence of OXA-48. Transformation of an OXA-48 harbouring succeeded; no resistance to non-β-lactam antibiotics was co-transferred. PFGE band patterns were related in all four isolates. By isoelectric focusing two β-lactamases with a pI of 5.4 and 7.2, respectively, were found.
Conclusion: This is the first description of OXA-48 in *K. pneumoniae* isolates from Germany. Some strains showed carbapenem MICs in the susceptible range, highlighting the difficulties in detection of this resistance mechanism.

RKP14

Establishment of a diagnostic network for commonly recognized and routinely unrecognized pathogens in human enteric infections

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During recent years, the number of notifiable cases with enteric infections in Germany caused by viruses, bacteria, or protozoa has been continuously increasing. Reported infections almost doubled from 251.000 cases in 2002 to 410.000 cases in 2008. Despite of this high number, the estimated number of unreported cases is still considerable, and the prevalence of most diarrhoea-inducing pathogens is largely unknown and needs to be established. Moreover, it remains to be determined whether the presence of specific subtypes or the combination of particular microorganisms may influence the clinical outcome of infections. Therefore, ten German consulting and reference laboratories (CL Haemolytic Uremic Syndrome; CL Molecular Diagnosis of Treponema; CL Whipple Bacteria; CL Norovirus; CL Rotavirus; CL Gastrointestinal Infections; Bi-national CL for Listeria; CL Adenovirus; NRC For Tropical Infections; NRC Salmonella and other Enteric Bacteria) started establishing the Network for Enteric Infections (NEI) for detection of both notifiable and in reportings not yet considered potential diarrhoeal pathogens. Stool samples are analyzed by the ten partners for the presence of about 30 different pathogens (adenovirus, *Aeromonas spp., Arcobacter spp., Brachyspira spp., Campylobacter spp., Clostridium difficile*, Cryptosporidia, *Cyclospora cayetanensis*, Dientamoeba, diarrhoegenic *Escherichia coli*, Entamoeba, *Giardia lamblia*, *Isospora belli, Klebsiella oxytoca, Listeria monocytogenes*, norovirus, *Plesiomonas spp*., rotavirus, enteric and typhoidal Salmonella, sapovirus, Sarcocystis, *Shigella spp*., *Tropheryma whipplei, Vibrio spp., Yersinia enterocolitica*), including subtype determination. Within the pilot phase from September 2009 till August 2010, the network project is coordinated by the NRC Salmonella and other Enteric Bacteria in Wernigerode and about 200 diarrhoea stool samples from hospitalized patients as well as from outpatients will be analyzed. Here, first results will be shown and experiences from the general network establishment procedure will be discussed.

RKP15

Evaluation of the Premi ®Test Salmonella for use in routine serotyping of Salmonella enterica

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The serotyping of *Salmonella enterica* strains is performed by agglutination test with sera according to the White-Kauffmann-Le Minor scheme. This scheme summarizes antigenic formulae of all known Salmonella serovars. The knowledge of the serovar is essential for all of the epidemiological and molecular investigations.
In order to establish new methods to substitute serotyping by comparable and timesaving procedures, DNA typing tests were developed.
One of these newly released multiplexed DNA typing tests is the commercial available testkit Premi ®Test Salmonella (DSM PremiTest B.V., NL). This system is based on microarrays from ClonDiag (Jena, Germany). The procedure can performed directly on samples of food, or environment as well as isolates grown on common bacteriological agar for salmonella.
Over a six-month-period it was used in the German National Reference Centre for Salmonella and other bacterial enterics in routine identification of Salmonella strains of human origin. 195 isolates within 66 serovars were investigated in parallel by classical serotyping and by the array.
Six isolates of 158 (65 different serovars) were detected by Premi ®Test with a false serovar (3.8%). 35 strains (22.2%) could be classified with a genovar.
Furthermore only 19 isolates within a collection of 37 S. Kentucky strains could be retyped with the correct serovar (51.4%).
The Premi®Test Salmonella offers an alternative to the classical serotyping for identification of the 10 most common Salmonella serovars and for screening purpuses. However, it is not suitable for replace the serotyping, because it failed in the detection of some emerging strains, such as monophasic *S. typhimurium* and rare serovars (including S. subspec. II to VI).

RKP16

German Network for Mycoses - Fungi in the elderly

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A previous pilot study performed at the University Medical Centre Goettingen demonstrated that the presence of fungi in the oral cavity of older individuals is associated with enoral symptoms.
Based on these findings, we hypothesize that the modified quality and integrity of the human skin and mucosa in the elderly might be associated with a significant increase of fungal colonization or infection. To test this hypothesis, different populations of older patients and individuals are compared with a control group of healthy medical students. Anamnestic data as well as data on antibiotic usage, personal hygiene and food habits are obtained and clinical inspection of the oral cavity and other anatomical regions of these study groups are done. Swabs from the oral cavities and nail specimens of the respective human individuals are investigated for the presence of fungi and their antifungal activities. Here, the first data of this interdisciplinary network project will be presented.

RKP17

Phenotyping Human Cytomegalovirus drug resistance mutations using a recombinant virus incorporating EGFP

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One essential prerequisite for genotypic drug susceptibility testing of human cytomegalovirus (HCMV) is the phenotypic characterisation of mutations identified in the viral protein kinase gene UL97 and the viral DNA polymerase gene UL54 regarding their quantitative impact on drug susceptibility. We developed a new method for phenotypic characterisation of UL54 mutations with regard to polymerase activity, viral replication and drug susceptibility.
To determine the most suitable viral indicator gene, the enhanced green fluorescence protein was C-terminally fused to the HCMV early-late protein UL83 (pp65) or the late proteins UL32 (pp150) and UL99 (pp28), resulting in three reporter viruses. The pp65-labelled virus proved to be superior to the other constructs due to its favourable signal-to-noise ratio and was therefore used to establish the optimum conditions for our assay.
The UL54 mutations E756K and D413E were introduced into vTB65g by markerless bacterial artificial chromosome mutagenesis. The drug susceptibility phenotypes for E756K and D413E were comparable to those previously reported. In addition, several UL54 mutations previously suspected to confer antiviral drug resistance – S291P, K415R, S692V, S695T, A972V and combinations – were found not to be relevant.
Furthermore, we found reduced replicative fitness for E756K recombinant virus by measuring fluorescence intensity as well as by conventional virus growth kinetics. Decreased fluorescence signals due to E756K and D413Eg at late times of infection suggested a reduced polymerase activity.
This new fluorescence-based assay is a highly reproducible method for the phenotypic characterisation of mutations potentially influencing drug susceptibility, viral replicative fitness, and polymerase activity of HCMV after marker transfer (Chevillotte et al., 2009. Antimicrob Agents Chemother 53:3752-3761). This study was supported by the German national reference network of the Robert-Koch-Institute.

RKP18

Molecular analysis of daptomycin-resistant *Staphylococcus aureus*

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Objectives: Daptomycin, a cyclic lipopeptide antibiotic active against a wide range of gram-positive organisms including methicillin-resistant *S. aureus* (MRSA), was licensed in Europe in 2006. Selection of daptomycin-non-susceptible *S. aureus* isolates had been demonstrated *in vitro* and resistance in clinical isolates is just emerging. We collected clinical daptomycin-resistant *S. aureus* strains (MIC ≥ 2,0 mg/l) sent to the German Reference Centre for Staphylococci from January 2008 to March 2009. Here we report on frequency of resistance, assignment of resistant isolates to clonal complexes and association with putative resistance mutations described so far.
Methods: All isolates sent to the German Reference Centre for Staphylococci were tested for their antibiotic susceptibility by broth microdilution and were assigned to clonal complexes by *spa*-typing and BURP analysis. For daptomycin-resistant isolates four genetic loci (*mpr*F, *yyc*FG, *rpo*B, *rpo*C) previously associated with daptomyin-resistance were investigated by sequence analysis.
Results: Among 4505 isolates investigated 18 exhibited daptomycin-resistance. Except 3 isolates all isolates were MRSA, predominantly belonging to clonal complexes CC5/ST225 and CC22. Neither in *yyc*FG nor in *rpo*C non-synonymous mutations were found. Only two isolates had mutations in *rpo*B, previously associated with rifampicin-resistance and both isolates revealed rifampicin-resistant phenotypically. 12 isolates revealed one to two non-synonymous mutations in *mpr*F, previously associated with daptomycin resistance *in vitro*. However, the remaining 6 isolates did not show any mutation in the loci investigated.
Conclusion: Results corroborate the role of *mpr*F-mutations in resistance development towards daptomycin; however further studies must elucidate additional mechanisms also contributing to reduced susceptibility.

RKV01

A new tool linking human cytomegalovirus drug resistance mutations to resistance phenotypes

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Drug resistant strains of human cytomegalovirus (HCMV) in patients at risk may increasingly develop into a problem in the clinical setting. Genotypic resistance testing is becoming the method of choice, but requires previous phenotypic characterisation of each newly found mutation. In order to facilitate the interpretation of the patient's CMV sequence data, a web based search tool was generated that links the sequence to a database containing all published UL97 (protein kinase) and UL54 (DNA polymerase) mutations and corresponding antiviral drug susceptibility phenotypes (http://www.informatik.uni-ulm.de/ni/mitarbeiter/HKestler/hcmv/index.html).
Although this approach still lacks proper clinical evaluation, it is reasonable to assume that HCMV drug resistance testing will provide relevant data for an adjustment of therapy and on prognosis of clinical outcome. HCMV drug susceptibility testing will become even more important once new drugs will be available for therapy allowing a wider choice of antiviral agents to treat HCMV disease. These topics will also play a pivotal role for optimising antiviral therapy of HCMV- and other viral diseases (Chevillotte *et al*., 2009. Antiviral Res. Epub ahead of print, doi:10.1016/j.antiviral.2009.10.004).
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RKV02

Effects of the pneumococcal conjugate vaccination on IPD in children and adults in Germany

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Objectives: Evaluation of the impact of the immunization of children <2y with pneumococcal conjugate vaccine (3+1 doses) which started in July 2006 on IPD among children and adults in Germany.
Methods: IPD cases in children <16y were reported by two independent sources: microbiological laboratories and pediatric hospitals. Age and serotype specific incidence rates were based on capture recapture estimates combining the two reporting sources. IPD cases in adults were reported by microbiological laboratories only. Serotyping was performed at the National Reference Center.
Results: For children <2y, in 2008/09, only 12 vaccine type cases were found on a total of 109 cases, compared to an average 88 from 133 cases per year in the period 1997-2006. This 86% reduction was seen for all 7 serotypes. Serotyped cases with non-vaccine serotypes increased (45 vs. 97). The incidence of IPD in children <16y decreased from 4.0/100,000 pre vaccination (1997-2003) to 3.2 post-vaccination (2007-2008). The reduction in incidence was attributed to significant reductions in children <2y (20.0/100,000 to 11.0). In this age group the incidence of all PCV7 serotypes was strongly reduced (4: 0.9 to 0.2; 6B: 1.7 to 1.0; 9V: 0.7 to 0.2; 14: 5.7 to 1.2; 18C: 1.0 to 0.3; 19F: 1.6 to 0.9; 23F 1.7 to 0.2). The incidence of non-vaccine serotypes remained stable. Among adults the fraction of IPD cases caused by vaccine types decreased from 45% to 25%, in Saxony even to 14%.
Conclusions: Three years after the introduction of PCV7, the reduction in reported cases of IPD with vaccine serotypes in children <2y was almost 90%. The incidence of IPD in children <2y was reduced by about 50%, whereas no significant increase in the incidence of non-vaccine serotypes was observed. This reflects the success of the vaccination programme. Higher serotyping rates in part explain the observed increase of reported cases of non-vaccine serotypes. Among adults, the first signs of a herd-immunty effect are visible.

RKV03

Prevalence of *Bartonella henselae* and *Borrelia burgdorferi* sensu lato DNA in *Ixodes ricinus* ticks in Europe

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Bartonella spp. can cause persistent bloodstream infections in humans and animals. Preliminary evidence suggests that ticks might serve as vectors for *Bartonella* spp. To determine whether *Bartonella henselae* is present in questing *Ixodes ricinus* ticks, we analyzed the prevalence of *B. henselae* DNA among tick stages compared with the prevalence of DNA from *Borrelia burgdorferi sensu lato*, the pathogen most frequently transmitted by ticks. *B. henselae* DNA was present with a prevalence of up to ~40% in tick populations sampled in four European sites (Eberdingen, Germany; Klasdorf, Germany; Lembach, France; Madeira, Portugal). The odds of detecting *B. henselae* DNA in nymphal ticks was ~14-fold higher than in adult ticks. No tick was found to be co infected with *B. henselae* and *B. burgdorferi* sensu lato. Taken together, our data indicate that ticks might serve as a vector for the transmission of *B. henselae* to humans.

RKV04
Typing of Romanian meningococcal isolates at the German reference laboratory for meningococci as part of ECDC/IBD-LabNet activities

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The European Monitoring Group on Meningococci, a society of national reference laboratories, and IBD-LabNet, a laboratory network funded by ECDC, were successful in harmonizing meningococcal typing in Europe. Nevertheless, countries with small numbers of cases of meningococcal disease and limited economical resources might face problems in implementing DNA sequence based typing schemes. To overcome this problem the IBD-LabNet suggested the formation of partnerships between countries. The collaboration between the German and the Romanian reference laboratories is presented here. A total of 30 strains from the years 2008 and 2009 were included in the study. Mean age of disease onset was 13 years (median: 4 yr). The isolates were characterized by serogrouping, penicillin MIC determination, PorA, FetA, and FHBP antigen sequence typing, MLST, and penA sequencing. Serogroups B and C dominated with a total of 24 isolates. Serogroup : PorA : FetA combinations were unique to Romania with no profile matching those encountered in Germany between 2002 and 2009, which is explained by geographic structuring. Only 8 of 30 isolates were penicillin susceptible with the rest being intermediate or resistant, respectively, of which 20 harbored an altered PenA allele. Astonishingly, there was a single dominant PenA allele associated with elevated Pen MICs and three distinct meningococcal finetypes. In contrast, previous reports and own data from Germany suggest a high genetic diversity of PenA alleles in isolates with elevated penicillin MICs. The paradox might be explained by a regional selective sweep of the particular allele. The study was limited by possible underestimation of the incidence of invasive meningococcal disease in Romania and laboratory surveillance constraints. Nevertheless, the project is an example for initiatives improving European data quality. The development of penicillin susceptibility should be carefully monitored in the future.

RKV05
Double - and triple - resistant *Helicobacter pylori* - an increasing problem in Germany - What are the best treatment options?

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Since spring 2006, all gastroenterologists sending *H. pylori* positive gastric biopsies receive upon request individual treatment recommendations for their patients, which are based on the isolates' resistance pattern and patient's risk factors. Three months later, data on the chosen treatment scheme and therapy outcome is collected. The aim of this ongoing study is to derive evidence-based recommendations for *H. pylori* eradication therapy with rescue schemes, and to find out which eradication therapy is most successful in a given pattern of antimicrobial resistance.
So far, the NRC gave 1044 treatment recommendations. Of 1013 questionnaires sent to gastroenterologists, 666 questionnaires (66%) were returned to the NRC, and 510 were complete and were included in data analysis. A total of 96/510 (19%) patients were still *H. pylori* positive after therapy, while 218/510 (43%) patients were treated successfully proven by histology, stool antigen test or urea breath test. Nearly the same number, namely 196/510 (38%) patients, did not present for post-treatment control or were not tested. Patients harbouring metronidazole and clarithromycin resistant isolates (n=230) were best treated with a therapy containing proton pump inhibitor (PPI) + levofloxacin + rifabutin (21 success/3 failures). In patients with triple-resistant *H. pylori* isolates (metronidazole, clarithromycin and quinolones; n=95) PPI + amoxicillin + rifabutin was most successful (22 success/4 failures). In both groups quadruple therapy (PPI + bismuthsubcitrate + tetracycline + metronidazole) failed in 43% (3/7; double-resistant strains) and 60% (6/10; triple-resistant strains).
Our data indicate that all rescue triple therapy schemes are effective in patients harbouring multi-resistant *H. pylori* strains. Reasons for inferiority of quadruple therapy are unknown, but might be due to a negative selection of difficult cases. The choice of therapy scheme should be based on susceptibility testing. Therefore, culture and susceptibility testing of *H. pylori* from gastric biopsies is strongly recommended already after the first treatment failure.

RKV06
Tracing the epidemic spread of MRSA based on single nucleotide polymorphisms

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Objectives: We have recently shown that MRSA may accumulate measurable genomic diversity upon epidemic spread. Here, we demonstrate that this diversity can be exploited (1) to reconstruct the spatiotemporal dynamics of epidemic spread and (2) to develop refined genotyping assays.
Methods: For mutation discovery, we applied dHPLC and genome sequencing by using next-generation technologies. For genotyping, we determined character states at a limited number of informative single-nucleotide polymorphisms (SNPs) by applying real-time PCR formats.
Results: Polymorphisms discovered in genomes from isolates that had been sampled through time allowed the precise dating of evolutionary and demographic events in the history of the MRSA clones investigated. Further, geographic spread was tracked based on the isolates´ ancestry. In addition, the determination of selected SNPs in a larger number of isolates enabled the identification of specific clones and provided insights into regional epidemiology.

TUP01
Quantitative drug susceptibility testing of nontuberculous mycobacteria using MGIT 960 and EpiCenter software

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Nontuberculous mycobacteria (NTM) are increasingly recovered as pathogens in the diagnostic laboratory causing diseases such as pneumonia, lymphadenitis, and soft tissue infections. There is thus an emerging need for standardized procedures to determine drug susceptibility. While future studies have to address the clinical predictive value of drug susceptibility testing (DST), i.e. by correlating phenotypic resistance levels and treatment response, a prerequisite for corresponding investigations is the possibility to exactly determine drug resistance levels. We assessed quantitative levels of drug susceptibility for *M. avium*, *M.intracellulare*, and *M. kansasii* by comparing BACTEC460 based DST with MGIT960/EpiCenter V5.53 equipped with the TB eXiST module. Extended comparative analysis on a range of susceptible and resistant clinical isolates allowed us to define the conditions for MGIT960 based quantitative DST and to develop criteria for interpretation. This resulted in a fully automated system for quantitative DST, which is compatible with expert systems for interpretation and electronic data management. Implementation of MGIT960 based quantitative DST using EpiCenter V5.53 TB eXiST module software will provide a wealth of standardized drug susceptibility data to correlate results from quantitative DST with clinical outcome.

TUP02
VNTR-typing of animal *Mycobacterium tuberculosis* complex isolates from Germany using 26 gene loci

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Germany has been declared officially free of bovine tuberculosis since 1997. Nevertheless, tuberculosis in cattle and tuberculosis infections of other animals are sporadically detected in Germany. The elucidation of the routes of spreading of the pathogen is of significant importance in order to control the transmission.

Variable number of tandem repeat (VNTR) typing is gaining more and more relevance in molecular typing of members of the *M. tuberculosis* complex (MTC). Altogether 26 VNTR loci have been recommended for typing MTC isolates of human and animal origin The present study was performed to select a reduced panel of most discriminative loci for animal isolates identified in Germany for future routine typing.
Forty-three according to our knowledge epidemiologically unrelated animal MTC isolates were typed using 26 loci. In addition, 55 isolates from different epidemiologically or geographically related tuberculosis cases were typed. The discriminatory index (DI) of each locus was calculated according to Gaston and Hunter showing marked differences of the DI of the different loci. On the basis of these results the cut-off value for „satisfactory" and „un-satisfactory" discrimination was preliminarily set at 0.6. Using VNTR-typing epidemiological relationship between cases could be hypothesized which were not realised before. At present a panel of ten loci can be recommended suitable for discrimination of animal MTC isolates in Germany.

TUP03
Novel targets for short-course tuberculosis therapy

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Mycobacterium tuberculosis (Mtb), the causative agent of tuberculosis (TB), is still one of the world´s leading killers among infectious agents. Eradication of TB is difficult due to the ability of Mtb to persist in a largely nonreplicating state within the host throughout lifetime - even in spite of months of antibiotic treatment. This often results in the development of recurrent diseases. The inevitable consequence of such an inadequate therapy is the emergence of multidrug-resistant (MDR) bacteria. Hence, the world desperately needs novel anti-mycobacterial agents that can shorten treatment duration and efficiently kill nonreplicating and MDR Mtb. In our work, we follow new strategies of screening for novel drugs against Mtb by focusing on drug targets which are essential for the survival of the pathogen within the host. We establish automated large-scale screening assays using the Mtb model organisms *M. smegmatis* and *M. bovis* (BCG strain), grown under stress conditions (e.g. oxidative stress), in low oxygen and nutrient concentration or in co-culture with immune cells (e.g. macrophages) to simulate the host´s conditions found in latent infections. Since commonly used screening methods assaying for inhibition of bacterial growth, antibiotics identified via those studies are typically more effective against replicating rather than nonreplicating persistent *Mycobacteria*. In contrast to this, our screenings are based on the detection of the viability of the pathogen. Therefor, we use luciferase reporter strains of our Mtb model organisms as well as redox-indicators like tetrazolium salts to measure the metabolic activity, and, thus, the viability state of the *Mycobacteria*. Using these assays, we screen the large collection of new, yet uncharacterised myxobacterial secondary metabolite extracts of the Helmholtz Centre for Infection Research for new anti-mycobacterial drug candidates.

TUP04
RamB (Rv0465c) represents a specific control factor of the glyoxylate cycle in *Mycobacterium tuberculosis*

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Mycobacterium tuberculosis is the causal agent of tuberculosis and claims more human lives each year than any other bacterial pathogen. *M. tuberculosis* generally is assumed to depend on lipids as a major carbon and energy source when persisting within the host. The utilization of fatty acids requires a functional glyoxylate cycle with the key enzymes isocitrate lyase (Icl) and malate synthase. The open reading frame Rv0465c of *M. tuberculosis* H37Rv encodes a protein with significant sequence similarity to the transcriptional regulator RamB, which in *Corynebacterium glutamicum* controls the expression of several genes involved in acetate metabolism, i.e., those encoding enzymes of acetate activation and the glyoxylate cycle. We show here that the *M. tuberculosis* Rv0465c protein can functionally complement RamB in *C. glutamicum* and that it binds to the promoter regions of *M. tuberculosis icl1* and Rv0465c. Construction and subsequent transcriptional and enzymatic analysis of a defined Rv0465c deletion mutant in *M. tuberculosis* revealed that the Rv0465c protein, now designated RamB, represses *icl1* expression during growth with glucose, and negatively autoregulates the expression of its own operon. Whole-genome microarray analysis of the *M. tuberculosis ramB* ($ramB_{MT}$) mutant and the wild type furthermore showed that apart from *icl1* and the $ramB_{MT}$ operon, the expression of all other *M. tuberculosis* genes involved in acetate metabolism remain unchanged in the mutant. Thus, $RamB_{MT}$ has a more specific regulatory function as RamB from *C. glutamicum* and is confined to expression control of *icl1* and the $ramB_{MT}$ operon.

TUP05
Molecular characterization of *Mycobacterium tuberculosis* lipoprotein LprF

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Lipoproteins are well known virulence factors of bacterial pathogens in general and of *Mycobacterium tuberculosis (Mtb)*, the causative agent of tuberculosis, in particular. Lipoprotein lipidation between Gram-positive and Gram-negative bacteria differs significantly as these are di- and triacylated, respectively. Little is known about the lipid anchor of mycobacterial lipoproteins. We reported recently that mycobacterial LppX, a lipoprotein involved in synthesis of cell wall components is triacylated (Tschumi et al. 2009), although mycobacteria are classified as GC-rich Gram-positive bacteria. We here exploited the model organism *Mycobacterium smegmatis* for the expression of *Mtb* LprF and characterized N-terminal modifications at the molecular level. LprF is a putative lipoprotein of *Mtb* involved in signaling of potassium-dependent osmotic stress. LprF is extensively modified in a mycobacterium-specific manner by a thioether-linked diacylglyceryl residue with one ester-bound tuberculostearic- and one C16:0 fatty acid and additionally by a third *N*-linked C16:0 fatty acid, and a hexose.

TUP06
Influence of mycobacterial infections on the function of human dendritic cells

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One of the most prevalent bacterial infections worldwide is tuberculosis caused by *Mycobacterium tuberculosis* and *Mycobacterium bovis*. Nearly 1/3 of the world's population is persistently infected. In most cases the primary infection remains asymptotic or with weak disease. 10 % of the infected people develop a disease with severe clinical symptoms. The live vaccine BCG (Bacillus Calmette-Guérin) is effective against severe forms of childhood tuberculosis, but is of limited protective capacity against adult pulmonary disease. An effective vaccine against both is still under investigation. The mycobacteria infect macrophages and dendritic cells (DCs). There have been many studies about the changes of macrophages after infection with mycobacteria but there is limited knowlegde about the infection of DCs and the functional and molecular alteration of these cells after infection with mycobacteria. Dendritic cells, which are the most potent antigen presenting cells play a central role in regulation and activation of the immune system. The analysis of the interaction between DCs and mycobacteria is of particular importance for the understanding of mycobacterial infections. In my PhD thesis, I use monocyte-derived human DCs and a non-virulent eGFP expressing Mycobacterium bovis BCG strain to analyse the effects of mycobacterial infections (collaboration with M. Singh, Dept.Genomeanalysis/Lionex GmbH). The infection of DCs by *M. bovis* BCG has been analysed with confocal and electron microscopy. After the uptake of bacteria dendritic cells mature and express characteristic surface markers, e.g. surface molecules to enable them to migrate to the draining lymph nodes and to present the antigens. Using flow cytometry, infected cells are characterized on the cell surface marker level and compared to uninfected and mature dendritic cells. I also performed microarray analyses to find out differences in the expression of genes involved in the immune response.

TUP07
Quantitative proteomics using metabolic stable isotope labeling for determining changes in mycobacterial protein composition

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Tuberculosis poses a global health threat with 2 million deaths per year and about one-third of the global population latently infected with *Mycobacterium tuberculosis*. To understand physiological behavior and adaptation of mycobacteria to stress conditions, e.g. antibiotic treatment, a proteomic approach was chosen, using *Mycobacterium bovis BCG* as model organism for *M. tuberculosis*. Recently, inhibitors of energy metabolism (phenothiazines and diarylquinolines) have come into focus as novel drug candidates. Diarylquinolines target the mycobacterial ATP synthase and show bactericidal activity both on dormant and replicating cells. Diarylquinoline lead compound TMC207 has the potential to shorten tuberculosis treatment and is in phase II clinical tests [1]. The present study primarily addresses the membrane proteome, since the diarylquinolines' target is in the membrane compartment. Membrane proteins perform essential physiological functions, e.g. energy metabolism and export of toxic substances, but they are often neglected in proteomic analysis due to their physico-chemical properties [2]. Methods for membrane protein analysis and quantification in *Corynebacterium glutamicum* have been developed and applied in our group. These techniques were successfully transferred to the related *M. bovis* BCG. Proteomic adaptation of *M. bovis BCG* to diarylquinolines is investigated using gel-based and gel-free sample preparation techniques prior to nLC-MS/MS. To quantify relative changes in protein abundance, a metabolically labeled internal standard [3], and label-free spectral counting is used [4]. Quantification of diarylquinoline-induced effects on the proteome will give insight into the global mycobacterial response to this novel class of antibiotic.

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TUP08
Population structure of multidrug resistant *Mycobacterium tuberculosis* strains from Germany

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Resistant and multidrug resistant (MDR, resistance to at least isoniazid and rifampin) *Mycobacterium tuberculosis* complex (MTBC) strains have emerged worldwide and represent a serious challenge for global tuberculosis (TB) control. Even more worrisome is the existence of extensively drug resistant (XDR, defined as MDR plus resistance to any fluoroquinolone and injectable drugs) strains that has been documented in nearly all geographical regions surveyed so far. Interestingly, high rates of MDR TB have been associated with particular phylogenetic lineages of the MTBC such as the Beijing genotype and the strong clonal expansion of particular MDR strains.
Resistance testing and molecular fine typing (24 loci MIRU-VNTR-typing and spoligotyping) was performed for 123 MDR strains obtained in the years 2006 and 2007 from patients living in Germany.
A significant number of MDR strains had further resistances e.g. to protionamide (34%), amikacin (13%), or ofloxacin (6%). Genotyping revealed that the majority of the strains belonged to the Beijing genotype (62%), followed by strains of the LAM (8%), Ural (7%), and Delhi/CAS (6%) genotypes. The overall rate of clustered isolates was 50%; however, the cluster rate was significantly higher in Beijing strains (66%) compared to non-Beijing strains (26%). More than 30% of all strains belong to just two major Beijing clones (94-193, 100-32).
The Beijing genotype, which has been shown to be a major cause of resistant TB in several high incidence settings, is also frequent among MDR strains from Germany. The high cluster rate and reduced population diversity among Beijing strains argues for a strong clonal expansion of particular MDR strains in countries of the former Soviet Union, where the majority of German MDR TB patients came from.

TUV01
Pathobiological variability of clinical *Mycobacterium tuberculosis* complex isolates

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Mycobacterium tuberculosis and other members of the *Mycobacterium tuberculosis* complex (MTC) remain a major cause of morbidity and mortality worldwide. Several studies based on spoligotyping, IS6110 fingerprinting and MIRU-VNTR typing demonstrated that the global population structure of MTC is defined by phylogeographical lineages and genotypes that are also associated with pathogenic differences. However, the influence of strain genomic variation on the outcome of infection and the clinical presentation are not completely understood.
In our study, we investigated growth differences of 15 strains of five different genotypes in comparison to the CDC1551 and H37Rv reference strains in liquid culture and in macrophages. Murine bone marrow derived macrophages were infected with liquid culture of different clinical isolates (MOI 3:1; 2x106 CFU/ml). Survival of strains in resting and activated macrophages was determined at different time points. Additionally, growth profile in 7H9 liquid media was analyzed.
Growth analyses of clinical isolates in liquid culture based on OD measurements showed no significant differences in comparison to CDC1551. Especially, we did not see a hypervirulent phenotype of strains of the Beijing genotype. On the contrary, growth curves in resting and activated macrophages show a larger variability in dependence of phylogenetic strain classification. Again, strains of the Beijing genotype did not show the highest growth rates in resting as well as in activated macrophages.
The complex interaction of the pathogen and the host seems to be genotype specific. Further analyses to determine the influence on the outcome of infection are in progress.

TUV02
Directed mutagenesis in 16S rRNA to reconstruct the *in-vivo* evolution of aminoglycoside resistance in *Mycobacterium tuberculosis*

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Drug resistance in *Mycobacterium tuberculosis* is a global problem, with major consequences for treatment and public health systems. As the emergence and spread of drug-resistant tuberculosis epidemics is largely influenced by the impact of the resistance mechanism on bacterial fitness, we wished to investigate whether compensatory evolution occurs in drug resistant clinical isolates of *M. tuberculosis*. By combining information from molecular epidemiology studies of drug resistant clinical *M. tuberculosis* isolates with genetic reconstructions and measurements of aminoglycoside susceptibility and fitness in *M. smegmatis*, we have reconstructed a plausible pathway for how aminoglycoside resistance develops in clinical isolates of *M. tuberculosis*. Thus, we show by reconstruction experiments that point mutations in the highly conserved A-site of 16S rRNA that (i) cause aminoglycoside resistance, (ii) confer a high fitness cost and (iii) destabilize a stem-loop structure, are associated with a particular compensatory point mutation that restores rRNA structure and fitness, while maintaining to a large extent the drug resistant phenotype. The same types of resistance and associated mutations can be found in *M. tuberculosis* in clinical isolates, strongly suggesting that compensatory evolution contributes to the spread of drug-resistant tuberculosis disease.

TUV03
Dissection of the lipoprotein synthesis pathway in mycobacteria

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Lipoproteins are a heterogeneous group of surface localized proteins, ubiquitously present in bacteria. They mature by post-translational modifications. In Gram-positive bacteria, lipoproteins are synthesized by the consecutive activity of pre-prolipoprotein diacylglyceryl transferase (Lgt) and prolipoprotein signal peptidase (LspA). In Gram-negative bacteria, an additional enzyme, apolipoprotein N-acyltransferase (Lnt), attaches a third fatty acid to the *N*-terminus. The genes of the lipoprotein synthesis pathway are essential in Gram-negatives but not in Gram-positives. Mycobacteria are classified as GC-rich Gram-positive bacteria. However, they are covered by a pseudo-outer membrane analogous to the outer membrane of Gran-negatives. We characterized the functions of mycobacterial Lgt, LspA and Lnt homologues by targeted generation of mutants in saprophytic *Mycobacterium smegmatis*, the vaccine strain *Mycobacterium bovis* BCG and virulent *Mycobacterium tuberculosis*. MALDI-TOF and MALDI-TOF/TOF analyses of purified lipoproteins revealed mycobacterium-specific modifications. Investigations in different infection models indicate an important role of the lipoprotein synthesis pathway in virulence of *M. tuberculosis*.

TUV04
Self-poisoning of *Mycobacterium tuberculosis* by inhibition of GlgE reveals a new class of drug target

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Mycobacterium tuberculosis (Mtb), the etiological agent of tuberculosis (TB), is the leading cause of mortality due to bacterial pathogens, claiming two million lives annually. The TB pandemic is fueled by the bacterium`s deadly alliance with HIV and the emergence of multidrug- and extensively-drug-resistant Mtb strains (XDR-TB) that are virtually untreatable with current chemotherapies. Thus, novel classes of bacterial targets very different from those of the antibiotics currently in use urgently need to be identified. We have discovered a novel trehalose-to-α-glucan biosynthetic pathway in Mtb comprising four enzymatic steps mediated by TreS, Pep2, GlgB, and GlgE, which is conserved in many other phylogenetically distant bacteria. Key enzyme is the essential maltosyltransferase GlgE capable of utilizing maltose 1-phosphate (M1P) as the activated donor substrate. By using traditional and chemical reverse genetics, we revealed a novel death mechanisms in Mtb based on suicidal self-poisoning by the accumulation of the toxic phosphosugar M1P, following GlgE inhibition. Microarray analyses revealed insight into the mechanism underlying M1P-induced cell death, demonstrating unexpected pleiotropic stress responses involving DNA damage, driven by a self-amplifying feedback loop representing a misled stress protection response. Moreover, the GlgE pathway exhibited a synthetic lethal interaction with the glucosyltransferase Rv3032 involved in biosynthesis of polymethylated α-glucans (methylglucose lipopolysaccharides), which are believed to play an essential regulatory role in fatty acid biosynthesis in Mtb, indicating the conditional essentiality of GlgE-pathway derivatives. Gene essentiality within a synthetic lethal pathway, revealing two novel independent and distinct synergistic death mechanisms, distinguishes GlgE from any other Mtb drug target described so far and provides the basis for the target-guided design of inhibitors effective against XDR-TB strains.

ZOP01
Evaluation of a commercial PCR kit for detection of EHEC directly from human stool samples

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Infections with enterohemorrhagic *Escherichia coli* (EHEC) represent a major cause of hemolytic uremic syndrome (HUS), the most common cause of acute renal failure in childhood. Shiga toxins (Stx1 and Stx2) and intimin, coded by *eae*, are believed to represent the major virulence factors of EHEC. Rapid and reliable methods for detection of EHEC are necessary for successful treatment. Thus, the aim of our study was to evaluate a commercial PCR kit for detection of EHEC bacteria directly from human stool samples.
Nineteen human stool samples from patients with different clinical symptoms, including HUS and diarrhea, and 4 asymptomatic patients were collected. DNA extraction from the stool samples was done by a commercial kit (Ingenetix) followed by a commercial PCR (Hain) for detection of *stx1*, *stx2* and *eae* genes. As reference the SOP of the Austrian Reference Centre was applied: Stool samples were enriched in EHEC direct medium (Heipha) at 37°C O/N following cultivation on a Sorbitol MacConkey agar plate. Finally cultured bacteria were investigated by PCR using suitable primers.
Among the 23 stool samples 3 were positive, the other 20 were negative for *stx1* with both methods. For *stx2* 4 of the 23 samples were positive, 19 negative with both methods. For *eae* the reference method detected 4 as positive and 19 as negative. One sample was false negative with the Hain PCR kit.
The commercial PCR kit of Hain is a very fast (only 6 hours of work) and reliable method for the detection of EHEC directly from human stool samples. However, the fact that isolation of EHEC bacteria is not required is also a disadvantage and will yield some false positive results as only after a culture yielding single colonies the co-occurrence of a shiga toxin positive E. coli with another one, carrying the *eae* gene, can be distinguished from the presence of an EHEC, carrying both virulence factors.

ZOP02
A genotypic variant of sorbitol-fermenting Shiga toxin-producing *Escherichia coli* O157:H- identified in patients and cattle

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Sorbitol-fermenting (SF) Shiga toxin (Stx)-producing *Escherichia coli* (STEC) O157:H- strains, originally identified in Germany, are emerging pathogens in Europe. The SF STEC O157:H- of the prototypic "German clone" harbors stx_2 gene, *eae* encoding adhesin intimin, and a large, ca. 120-kb plasmid (pSFO157) which contains EHEC-*hly*, *etp*, and *sfp* clusters encoding EHEC hemolysin, type II secretion system, and Sfp fimbriae, respectively. Here we characterize a new variant of SF STEC O157:H- which was associated with a family outbreak involving cases of hemolytic-uremic syndrome (HUS) and diarrhea in two siblings and a cattle as a source of the infection. While sharing multilocus sequence type (ST11) and chromosomal virulence genes (stx_2 and *eae*) with SF STEC O157:H- of the prototypic German clone, the variant SF STEC O157:H- isolates from both patients and the epidemiologically associated cattle harbor two plasmids of ~82 kb and 75 kb. Neither of these plasmids contains the EHEC-*hlyA* and *etp* genes, but the 75-kb plasmid harbors a complete *sfp* cluster. The striking plasmid differences from the prototypic SF STEC O157:H- facilitate further investigation of the geographic distribution, epidemiology and clinical significance of the new variant of SF STEC O157:H- .

ZOP03
Rickettsioses: Emerging zoonoses in Germany

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Tick-borne rickettsial diseases in humans are caused worldwide by obligate intracellular bacteria belonging to the spotted-fever group within the genus *Rickettsia*. Yet these tick-borne rickettsioses are among the most underdiagnosed vector borne diseases in Germany: Due to the variety of uncharacteristic clinical signs, they are not easily recognized. The clinical picture ranges from subclinical to fatal but may be difficult to differentiate from other febrile conditions without specific tests. Even to date diagnosis either is based on clinical findings, a record of tick exposure, and indirect detection of the pathogens. We briefly discuss modern diagnostic tools for important tick-borne rickettsial infections with emphasis on new molecular diagnostic assays.
Novel quantitative real-time polymerase chain reaction (qPCR) protocols facilitate genus specific, rapid, and sensitive detection of rickettsial pathogens. We developed and evaluated different assays as useful tools for epidemiological investigations in ticks and in human diagnostics and describe a rational diagnostic approach for the detection of tick-borne human rickettsioses which consists of qPCR, cell culture, multi-locus sequence typing and serology. Based on these methods we were able to detect and confirm several imported and even autochthonous cases of human rickettsioses in Germany.
Available data demonstrate the prevalence of at least seven different species of rickettsiae in Germany. In addition, our data show that rickettsioses account for a significant portion of febrile illnesses after travels to tropical and subtropical countries. Therefore, more attention should be drawn to *Rickettsia* species as zoonotic pathogens in Germany. Tick-borne rickettsioses have to be considered as a differential diagnosis in patients presenting with fever, headache and rash following a tick bite.

ZOP04
Imported and autochthonous rickettsioses in Germany

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Human rickettsioses occur worldwide and are caused by obligate intracellular gram negative bacteria belonging to the Genus *Rickettsia*. In the acute phase of rickettsial disease, serological studies are delayed, inconclusive, and often unhelpful. Unambigious diagnosis and definite identification of the causative agent can be obtained only by cell culture isolation or molecular biology assays.
We present several cases of febrile illness and skin manifestations due to rickettsial infections with or without travel history. Diagnosis was made on the basis of clinical, epidemiologic, molecular and serologic findings which we describe in detail.
A male patient attended a physician directly after the return from Zimbabwe where he had been bitten by ticks several times. Back in Germany he developed fever and skin lesions. By isolation in cell culture and molecular characterization the causative agent could be confirmed as *R. africae*.
A 26-year old female returned after a tree months' stay in Tansania and developed fever and fatigue within the first week of her return. The presumptive diagnosis of an acute dengue fever could not be confirmed, antibody-titres against rickettsia were tested negative. Two weeks later we received a follow-up specimen which was again tested for rickettsia antibodies. We found significant antibody-titers for IgM (1:256) and IgG (>1: 512) against epidemic typhus group rickettsia, indicating a case of murine typhus.
A 67-year old female, with no travel history, seeked medical advice one week after suffering a tick bite at the neck. Two days after the removal of the tick the patient noticed an ulceration of the biting-area and swollen cervical lymph glands. Serological findings and clinical presentation indicated a case of TIBOLA (tick borne lymphadenitis) caused by *R. slovaca*.
The number of diagnosed rickettsioses, in travelers but also in patients without any travel history, is increasing and we believe the prevalence of rickettsioses in Germany to be highly underestimated.

ZOP05
Ticks as Vectors of Spotted Fever Rickettsia in Germany

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In Germany, seven species of rickettsiae have been detected so far in ticks. The most prevalent Rickettsia (R.) species in Germany is *R. helvetica*. 5 to 10% of *Ixodes (I.) ricinus* and *I. hexagonus* ticks carry this rickettsial species. It is associated with aneruptive fever and potentially with myocarditis. *R. monacensis* is another rickettsial species found in *Ixodes ricinus*. It is prevalent in southern and south-eastern Germany and has been detected as a human pathogen in Spain, causing classical spotted fever. A third species causing spotted fever in humans is R. massiliae, which has also been detected by PCR in *I. ricinus* in south-eastern Germany. *R. felis* is a species which is transmitted by fleas. It causes flea-borne spotted fever. Human cases were described in the Region of Düsseldorf. Investigations conducted in southern Germany suggest a natural cycle of this species between rodents and their fleas or ticks. In ticks of the genus Dermacentor (D.), three species of rickettsiae have been detected so far in Germany. In earlier literature *R. slovaca*, the etiologic cause of tick-borne lymphadenopathy (TIBOLA) was described. The presence of this species was confirmed by recent detection in Dermacentor ticks from southern Germany and human cases of TIBOLA in Baden-Württemberg and Rhineland-Palatinate. *R. raoultii* is highly prevalent in *Dermacentor spp*. ticks in south-eastern and southern Germany with prevalence rates of > 20%. Its human pathogenicity has not been confirmed yetHowever, it seems also to be associated with TIBOLA in Europe. The third species, R. RpA4, has been detected in up to 20% of D. reticulatus ticks in south-western Germany. Its human pathogenicity is unknown. The medical importance of rickettsial infections in Germany may be increasing with additional knowledge on the distribution and prevalence of Rickettsia species in ticks in Germany.

ZOP06
Transmigration of African trypanosomes across an *in vitro* blood-brain barrier model

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The Blood-Brain Barrier (BBB) of mammals is the strictly regulated interface between the blood circulation and the central nervous system. It controls the exchange of substances and shields the brain from diverse toxins and pathogens in the blood flow. Composed of specialized endothelial cells, resulting from tight interactions with glial cells and pericytes, the BBB is a highly dynamic barrier system maintained by continuous induction processes. Here we describe the optimization of an *in-vitro* BBB model consisting of two immortalized cell lines used in a 24-well-insert system. Human endothelial like ECV304 cells were grown on an artificial extracellular matrix in coculture with C6 rat glial cells underneath mimicking a barrier being impermeable for the non-pathogenic *E. coli* HB101 in contrary to the meningitis causing *E. coli* K1. We demonstrate how this reproducible system can be used to analyze the transmigration process of *Trypansoma brucei gambiense* and *Trypansoma brucei rhodesiense*, the causative agents of a neglected disease: The Human African Trypanosomiasis (HAT). Both pathogens transmigrate the *in vitro* BBB with higher rates than the bovine pathogenic subspecies *Trypanosoma brucei brucei*. Once within the brain trypanosomes are also able to traverse the BBB in the opposite direction. We conclude that transmigration of the BBB is not a one-way process and the cerebrospinal fluid is possibly a reservoir for relapsing parasitemia.

ZOP07
A novel molecular typing approach for *Salmonella enterica* Serovar Derby based on virulence gene and tandem repeat analyses

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Salmonella enterica serovar derby is prevalent in pigs and a causative agent of gastroenteritis in humans. For investigating genetic relatedness and to study transmission from pig to human of *Salmonella derby*-isolates it is important to type them with molecular methods. The common multiple locus sequence typing approach, based on housekeeping genes, is not suitable for epidemiological purposes since the respective genes are highly conserved for *S. derby.* Therefore, sequencing of polymorphic virulence genes and tandem repeat loci, assumed to be more variable then housekeeping genes, was performed in order to subtype *S. derby*. Forty-five human and 39 animal isolates collected between 2006 and 2008 were investigated. The virulence genes sopA and sopD were present in all isolates and showed sequence variability, as did the two tested tandem repeat loci. The gene sopB was present in all but one strain and showed sequence variability, too. Comparison of the human and animal isolates of *S. derby* revealed similar distributions of the alleles for all five loci. Sequence analysis identified nine different sopA-alleles, three sopD-alleles and five sopB-alleles. Fragment analyses of the tandem repeat loci resulted in six alleles, varying in the number of the tandem repeat units. Sequencing of the tandem repeat loci showed that the fragments identical in size do not always have the same sequence. Thus nine alleles were detected due to the combination of fragment analyses and sequencing data. A combination of the alleles of all five loci investigated offered 15 different subtypes of *S. derby*. The conclusion of the study is that sequencing of sopA, sopB, sopD and the two tandem repeat loci is a promising approach for extended *S. derby* typing.

ZOP08
Isolation of Shiga toxin-producing *Escherichia coli* in petting zoos in Germany

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Little is known about the occurrence of Shiga toxin-producing *Escherichia coli* (STEC) in petting zoos in Germany. STEC/EHEC can cause bloody and non-bloody diarrhoea and the haemolytic uraemic syndrome (HUS) in humans, especially in children. Therefore an epidemiological study was performed in 56 petting zoos in Germany to analyze the prevalence of STEC in small ruminants and other petting zoo animals. The number of zoos was calculated with the central limit theorem and balanced between expenditure and level of confidence, so that we define a minimum of 50 zoos for sampling. To get homogeneous, comparable subgroups, we first stratified all zoos in wildlife-parks, amusement parks with animals and zoological gardens. In addition, Germany was divided in three regions, depending on the zoo frequency. The sample size in each zoo depended on the herdsize and was calculated with the central limit theorem. The study was performed between May to September 2008 and between May to October 2009. A total of 1567 faecal samples from 11 species (goat, sheep, chicken, pig, rabbit, guinea pig, donkey, fallow deer, kangaroo, cattle and horse) and 44 insects were collected. The samples were examined by immunomagnetic separation (IMS) for the serotyps O26, O103, O111, O145 and O157, followed by a multiplex-PCR (mPCR) for detection of virulence genes *stx*1, *stx*2, *hly*A and *eae*A. Nine STEC strains containing all virulence genes out of goat, sheep, pig and rabbit collected in four different petting zoos were isolated. Further studies by multilocus sequence typing (MLST) revealed, that the animal O157:H7 strains belong to sequence type 11 and have all characteristics typical for human O157:H7.

ZOP09
Salmonella serovar specific differences in host immune responses

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Salmonella enterica serovars cause infections ranging from asymptomatic carriage, gastroenteritis or systemic diseases with fatal outcome in a wide range of different host species. In addition to bacterial factors, host factors appear to play a major role in the adaptation of serovars to a certain host and the outcome of infections. Whereas cellular and humoral immune responses to *Salmonella* infections in general are becoming better understood, knowledge concerning differences in serovar-specific immune responses in different host species remains limited.
In order to characterize the differential immune responses of different host species to *Salmonella* during infection, we have developed human, porcine and chicken intestinal epithelia and macrophage cell lines containing chromosomal, NFκB-dependent reporter constructs for monitoring host cell responses to infection by *Salmonella* serovars. Infection with both broad-host range and host-adapted serovars are being used to determine *Salmonella* serovar- and host-specific NFκB activation to identify differences in host innate immune responses. In addition, we compared the expression of pro- and anti-inflammatory cytokines in human and chicken macrophages in response to infection with the same *Salmonella* serovars used in the NFκB assays.
The results showed that infection by broad host-range serovars elicits NFκB activation and cytokine profiles clearly distinct from those of host-adapted serovars. In addition, the host-adapted serovars *S. dublin*, *S. choleraesuis* and *S. gallinarum / pullorum* showed different immune responses compared to one another, suggesting that host-adapted serovars have developed different mechanisms which may contribute to their generally higher rates of systemic infections.
These and other results will be discussed with regard to host adaptation, virulence and pathogenicity and how they correlate with *in vivo* experiments.

ZOP10
Study on MRSA colonisation of therapy dogs and their owners

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Introduction: The use of dogs for therapy in health care facilities implements the question of colonisation with *S. aureus*/ MRSA from patients and their further transmission as already reported for sporadic cases in Northern America. Here we report results from a point prevalent study in Middle Europe.
Methods: Nasal swabs were taken from 39 therapy dogs, which are visiting in different long term health care settings in Austria and in South-West of Germany and also from 35 owners which accompany these animals.
Swabs were processed on sheep blood agar and in parallel on Chromagar MRSA. All *S.aureus* isolates were subjected to spa-typing and to antimicrobial susceptible testing by means mircobouillon delution assay.
Results: None of the dogs and and also none of the humans were positive for MRSA. Ten of the owners were carrying MSSA exhibiting no multiresistance and spa-types known from *S. aureus* as nasal colonizers of humans.
Conclusion:
Although *S. aureus*/MRSA are widely disseminated in long term care facilities, MRSA colonisation of therapy dogs and their human companions seems to be rare so fare in Middle Europe.

ZOP11
A mass spectrometry study on the expression of Shiga toxin receptors in human pancreatic cancer cell lines

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Pancreatic adenocarcinoma cause one of the highest mortality rates among human malignant tumors resulting from the aggressive tumor biology and the late diagnosis. Since no curative treatment is available yet, the development of new approaches for diagnostic and adjuvant treatments is urgently required in prospective cancer therapy.
Alterations in the composition of glycosphingolipids (GSLs) are known to occur on the cell surface of tumor cells. For various types of tumors an increased expression of the GSL globotriaosylceramide (Gb3Cer) has been observed which represents the specific receptor of Shiga toxin (Stx) produced by enterohemorrhagic *Escherichia coli*. In case of enhanced expression of Gb3Cer on pancreatic cancer cells it is obvious to develop a strategy for application of Stx as a therapeutic anti-cancer agent due to its high binding affinity to Gb3Cer on malignant cells.
We established a cell culture model employing several pancreatic cancer-derived cell lines originating from primary tumors with different characteristics and metastases. GSLs were isolated from *in vitro* grown cells and investigated with focus on Stx-receptor Gb3Cer. Lipid extracts were analysed by thin-layer chromatography combined with immunodetection using a specific anti-Gb3Cer antibody. The immunopositive Gb3Cer species were subsequently identified and structurally characterized by nanoESI-QTOF mass spectrometry. Structural heterogeneity was detected in the ceramide lipid anchors revealing Gb3Cer species harbouring fatty acids with different chain lengths ranging from C16 to C24. However, all pancreatic cell lines investigated so far express the Stx receptor Gb3Cer. Our preliminary data substantiate the visionary concept that patients suffering from pancreatic adenocarcinoma showing high expression of Gb3Cer might be considered as candidates for a Stx-based targeted cancer therapy.

ZOP12
APEC adhesin A (AadA), a new autotransporter adhesin, is widely distributed among avian and human ExPEC

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Using suppression subtractive hybridization we compared the avian pathogenic *Escherichia coli* (APEC) strain IMT5155 (O2:K1:H5; multi locus sequence type ST 140) isolated from chicken with the human uropathogenic *E. coli* (UPEC) strain CFT073 (O6:K2:H5; ST 73). In total, 28 different genomic loci were identified, which are present in IMT5155 but not in CFT073 or the non-pathogenic laboratory *E. coli* K-12 strain MG1655. One of these loci contained a gene encoding a putative novel autotransporter adhesin similar to yadA, which was provisionally designated apaA for APEC autotransporter encoding gene. The gene spans 3.498 bp encoding a putative 128-kDA protein. Adherence and adherence inhibition assays demonstrated a role for ApaA in adhesion to HEp-2 cells. The flanking regions of apaA contain sequences of mobile genetic elements, e. g. insertion elements and genes for transposases, indicating a putative horizontal spread of this adhesin gene among different strains. In accordance with this hypothesis, apaA was found to be present not only in extraintestinal pathogenic but also in non pathogenic commensal *E. coli* strains of human and animal origin. However, apaA was clearly associated with APEC strains: 54 % of the 214 analyzed APEC were positive for apaA, whereas only 5 % of 137 UPEC (uropathogenic *E. coli*) isolates, 4 % of human 29 SePEC (septicemic *E. coli*) and none of 25 NMEC (newborne meningitic *E. coli*) isolates were positive for apaA. Thus, apaA was significantly, but not absolutely, associated with isolates of poultry origin (83%).

ZOP13
Distribution of the genes encoding the subtilase cytotoxin among Shiga toxin-producing *Escherichia coli* (STEC)

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The subtilase cytotoxin, a novel AB_5 toxin identified recently on a large plasmid of STEC O113:H21, is considered a putative virulence factor of STEC. This is based on the ability of the purified toxin to cause, in a mouse model, extensive microvascular thromboses and necrosis in the kidneys, brain, and liver, which resemble lesions in patients with hemolytic uremic syndrome (HUS). However, the distribution of subtilase among STEC isolates and, in particular, its presence in STEC strains associated with HUS, is unknown. To gain a deeper insight into this topic, we investigated presence of the *subAB* operon in HUS-associated STEC ("the HUSEC collection") and in a broad spectrum of other STEC isolates from our collection using PCR. The *subA* and *subB* genes were found in two of 42 HUSEC strains, which were both *eae*-negative and belong to serotypes O76:H19 and O128:H2. None of *eae*-positive HUSEC strains belonging to the major serotypes associated with HUS (i.e. non-sorbitol-fermenting O157:H7/NM, sorbitol-fermenting O157:NM, O26:H11/NM, O103:H2, O111:H8/NM, O145:H25/H28/NM) possessed the subtilase genes. Among 72 other STEC isolated mostly from patients with diarrhea and belonging to 37 serotypes, the *subA* and *subB* genes were found in strains of 19 serotypes, including all tested strains of serotypes O22:H8, O40:H8, O91:H14/NM, O113:H4, and O128:H2. All *subAB*-positive strains were *eae*-negative. Our data demonstrate that the *subAB* operon occurs only in *eae*-negative STEC, which are usually associated with mild disease, and it is absent from major HUS-associated STEC serotypes. Our data thus do not support the role of subtilase as a virulence factors involved in the pathogenesis of HUS in the vast majority of STEC.

ZOP14
Interlaboratory Reproducibility of Multiple-Locus Variable-Number Tandem Repeat Analysis (MLVA) Typing for enterohemorrhagic *Escherichia coli* O157

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Enterohemorrhagic *Escherichia coli* (EHEC) of serogroup O157 are a highly pathogenic subgroup of Shiga toxin-producing *E. coli* and cause (bloody) diarrhea and the hemolytic uremic syndrome.
For molecular subtyping of EHEC O157, pulsed-field gel electrophoresis (PFGE) emerged as the gold standard in the last decade. However, PFGE turned out to be problematic in terms of its intra- and interlaboratory reproducibility due to the subjective nature of data analysis. Moreover, it is quite labor-intensive and not amenable for automation. To deal with these drawbacks other molecular methods were developed, among them multi-locus variable-number tandem-repeats (VNTR) analysis (MLVA). MLVA characterizes different VNTR loci of the bacterial genome through PCR amplification followed by fragment sizing and repeat unit number determination.
The current standardized PulseNet protocol for *E. coli* O157 includes eight VNTR loci. Apart from the recently published PulseNet reproducibility study using different sequencing platforms in ten laboratories, no other interlaboratory comparisons have been performed. We therefore investigated the interlaboratory reproducibility and applied the current PulseNet MLVA protocol by exchanging two collections comprising 50 blind-coded EHEC O157 isolates between our two laboratories (University Muenster, Germany and CDC, Atlanta, GA).
To calibrate fragment sizes, fragment data from the fully sequenced reference strain *E. coli* O157 EDL933 was used. Of 100 analyzed isolates, 98 were correctly MLVA-typed (98 %). In two cases only, the repeat number of one VNTR locus differed either by one repeat or gave no amplicon.
In conclusion, our results prove that MLVA data is highly reproducible and portable between laboratories which together with its rapid and reliable performance demonstrate the applicability of MLVA as an alternative for PFGE. Future studies will focus on an easier data transfer for tracing the spread and epidemiology of these foodborne pathogens.

ZOP15
Diagnostic procedures and molecular typing of *Coxiella burnetii*

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Coxiella burnetii (C.b.) is the causative agent of Q fever, a zoonosis that remains clinical asymptomatic in 50% of the cases or appears as atypical pneumonia or hepatitis in the acute form of the disease or as endocarditis in the rare chronic form (up to 2%).
Till now, serological methods are the gold standard in Q fever diagnostics. To this end, two serum samples at intervals of 1 to 3 weeks are required, especially in acute cases. With commercial available ELISA or IFA kits, specific antibodies (IgG and IgM) against phase II LPS can be detected in acute, those against phase I LPS in chronic cases.
In the recent years, PCR was used to detect C.b.-specific DNA from human specimens such as blood, bone marrow, sputum, placental material in acute or heart valves in chronic cases. But also for the DNA-detection from serum or plasma, PCRs were established as an appropriate addition to shorten the window period within early diagnostics and to estimate the activity of the pathogen in chronic cases. Most frequently, IS1111, coding for the transposase gene, is used as a multi-copy target region for highly sensitive diagnostic PCRs. Several commercial test kits utilize this element, permitting rapid detection by real-time PCR-techniques.
Because of its intracellular live cycle, the isolation of C.b. is only possible in cell culture. It is very difficult, time consuming and restricted to BSL3 laboratories. Since it is not as sensitive as PCR-techniques, viable cultures are more relevant for scientific investigations such as microbial sensitivity tests. A variety of molecular typing methods has been established to trace back infections, which might differentiate natural occurring outbreaks from a bioterroristic act, since C.b. is deemed to be potentially used as a biological weapon. Isolation and molecular typing play a subordinate role in diagnostic, but an important role in forensic questions.

ZOP16
Genome Sequences of Enterohemorrhagic *Escherichia coli* (EHEC) O26:H11 and O145:H28

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Enterohemorrhagic *Escherichia coli* (EHEC) is a highly pathogenic subgroup of intestinal pathogenic *E. coli*. EHEC can cause water/bloody diarrhea, hemorrhagic colitis and hemolytic uremic syndrome (HUS), which are the most common cause of acute renal failure in children worldwide. EHECs are transmitted by faecally contaminated water and food, raw meat products, raw milk or unpasteurized apple juice, yogurt or cheese. Molecular and cellular analyses have shown that in addition to Shiga toxins many other virulence factors are important for the host pathogen interaction. EHEC O157:H7 is the most frequently isolated serotype associated with HUS worldwide. In Germany however, in 2008 non-O157:H7 EHEC, among them O26:H11 and O145:H28 were the most common serotypes isolated from HUS patients. Because only little is known about the genome content of these serotypes, we investigated the genome content of two representative strains of these serotypes by whole genome sequencing. Currently, we have 5.43 Mb sequence information for the EHEC O26:H11 and 5.2 Mb for the EHEC O145:H28 isolate in 5 (O26:H11) and 23 (O145:H28) contigs. Moreover, we identified one plasmid in each isolate harboring genes also present in plasmid pSO157 from *E. coli* O157:H$^-$ strain 3072/96. Comparison of the O26:H11 contigs to the recently published genome of *E. coli* O26:H11 strain 11368 resulted in sequence similarities of 90%, of the O145:H28 strain to *E. coli* O157:H7 EDL933 gave 81% similarity. In total, ORF finding within contigs larger than 50 kb resulted in 4020 ORFs in EHEC O26:H11 and 4163 ORFs in EHEC O145:H28. After gap closure, future studies will focus on the comparison to other serotypes to understand the evolution of non-O157 EHEC and to discover common virulence traits and potential new targets for diagnosis and therapy of EHEC infections.

ZOP17
Immunological one-step differentiation and characterization of proteins with similar and overlaying molecular masses

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The identification of indicator proteins is important in the prevention of infection and transmission of diseases. Diagnostic assays have been developed but these are mostly restricted to the detection of single antigens. Thus there is great need for multiplex detection assays with simultaneous analysis of disease markers. The immunoblot technique is widely used for characterization and typing of proteins on the molecular level. Proteins of suspended tissues are separated on SDS-PAGE and the specific signals are detected by antibody recognition. The investigation reported here describes a one-step determination on immunoblots of the simultaneous detection of two indicator proteins, neuron specific enolase (NSE) and glial fibrillary acidic protein (GFAP), in tissues of the central nervous system and the simultaneous typing of prions using different antibodies which bind to various epitopes. Although NSE and GFAP overlap with similar molecular masses, the indicators are differentiated by immunological reactions followed by visualizing the different coloured specific protein bands which develop. The enolase is a brown band whereas the GFAP exhibits red colour. Another novel typing assay of one antigen detected by different antibodies requires two chemiluminescence substrates developing light signals which visualize the specific antigen signals. Prions represent the bands, the di-, mono- and nonglycosylated isoforms. Specific profiles are composed of the signal ratios of the isoforms detected by antibodies recognizing different epitopes. This one-step protein detection and quantification assay on immunoblots using two different antibodies forwards a direct identification and a direct typing of antigens preventing a high number of separated gel runs. Immunoblotting has proved to be a suitable assay for multiplex analysis of marker proteins possessing similar molecular weights and is therefore a suitable tool for application in food, veterinary and medical facilities.

ZOP18
Phenotypic manifold prions in human platelets and brains

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The agents causing transmissible spongiform encephalopathy are known as infectious prion proteins (PrP^{Sc}) which mainly accumulate in the central nervous and lymphoreticular system, and infectivity was also found in blood reported by cases of vCJD caused by blood transfusion. The molecular mechanisms leading to PrP^{Sc} induced transformation are poorly understood; however PrP^{Sc} is the result from conversion of the host encoded cellular prion protein (PrP^{C}) accompanied by a conformational change. PrP^{C} in brains and tissues are highly heterogeneous as results of expression and of presented glycoprotein profiles reflecting differences in the ratios of the di-, mono- and non-glycosylated protein signal intensities. We suggest that distinct PrP^{C} forms may be converted facile while others change cumbersome to PrP^{Sc}. However the basis proving this idea is to identify these various PrP isoforms within protein overlays of whole cell lysates. With a set of antibodies we quantitatively analysed human PrP^{C} derived from different brain regions and from platelets by densitometry to a single centrifugation step. We identified high and low soluble PrP^{C} isoforms with variable protein patterns. Using N-terminal binding antibodies the diglycosylated isoforms of high soluble PrP dominated in brain regions cerebellum, cortex and hippocampus, while nonglycosylated PrP of thalamus, pons and medulla oblongata resulted in highest immunoreactivity in the sediments. Brain PrP at the size of monoglycosylated proteins were intensely labelled in supernatants and pellets using C-terminal binding antibodies. In the case of PrP derived from platelets the protein profiles moved in favour of a dominance of the diglycosylated PrP bands. The identification of further PrP profiles as silent prions in whole cell preparations supports the idea of the existence of manifold prions which may lie dormant awaiting a change in the state of the host.

ZOP19
Mass spectrometric approaches for the characterization of bacterial toxin receptors

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The attachment of bacterial exotoxins to the outer surface of host cell membranes is the initial event of the infection process, followed by receptor-mediated internalization. Hereby, toxins bind to very specific targets, e. g. transmembrane proteins or glycosphingolipids (GSLs). Of the various bacterial toxins recognizing GSLs, cholera toxin (CT) from *Vibrio cholerae* and Shiga toxins (Stxs) from enterohemorrhagic *Escherichia coli* have been most intensively studied. Both toxins belong to the AB_5 family of bacterial toxins consisting of an enzymatically active A-subunit and a cell binding homopentameric B-subunit. After binding the toxins are internalized via receptor-mediated endocytosis. From recent studies it becomes obvious that only lipid raft associated GSLs are capable of efficient toxin uptake. Thus, the biophysical features particularly of the ceramide anchor may influence the toxins´ attachment to certain membrane domains and thereby affect toxin destination. Consequently, a detailed structural analysis of GSLs binding to CT and Stxs is necessary to understand the molecular reasons that may underlie the different toxin susceptibilities of cells expressing the CT and Stx receptors GM1 and globotriaosylceramide (Gb3Cer), respectively.
The aim of this study was to employ combined thin-layer chromatography (TLC) immunostaining and mass spectrometry technologies for the full structural characterization of bacterial GSL receptors. The immunodetected GM1- and Gb3Cer-species were analysed by direct TLC-IR-MALDI-o-TOF-MS (strategy 1). In another approach immunostained GSLs were extracted from the silica gel after TLC separation and applied to nanoESI-QTOF-MS and -MS/MS (strategy 2). These TLC-MS-joined procedures proved to be sensitive techniques offering a wide range of applications for the structural characterization of bacterial toxin receptors and may help to better understand the molecular mechanisms of toxin-host cell-interactions.

ZOV01
Molecular and evolutionary analysis of pathogenic *Escherichia coli* from human, animal and food

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Shiga toxin-producing *E. coli* (STEC) and enteropathogenic *E. coli* (EPEC) are able to translocate effector proteins via a type III secretion system (T3SS) into eukaryotic cells. Structural and regulatory proteins of the T3SS and at least seven type III effectors are encoded within a pathogenicity island, the locus of enterocyte effacement (LEE). Moreover, more than 20 families of non-LEE encoded type III effectors have been described in pathogenic *E. coli*. Here, we investigated the occurrence of 3 LEE-encoded and 16 non-LEE encoded effector genes in 137 STEC and EPEC strains, which originate from asymptomatic or diseased humans, animals, and risk food. The evolutionary relationship of strains was analyzed by multi-locus sequence typing (MLST). The occurrence of type III effector genes, subtyping of *stx* and *eae* were determined by PCR, dot blot hybridization and DNA-sequencing.
All analyzed LEE-encoded genes were present in 102 strains. Furthermore, 4 effector-carrying strains harbored no or only a part of the LEE. Thirty-one strains from human and food did neither contain the T3SS nor any of the determined effector genes. More than 85 % of type III effector-positive strains encoded *nleA*, *nleC*, *nleF*, *nleH1-2* and *espJ* or fragments of these genes, whereas *nleD* and *ospG* were only rarely detected. Clustering of strains into clonal complexes using MLST resulted in a quite uniform distribution of effector genes. Strains of clinical important serogroups within clonal complexes carried 10 to 15 of the analyzed non-LEE encoded genes and showed frequently a higher effector gene density than strains of other serogroups.
Beside depicting the virulence gene distribution in different evolutionary STEC lineages no explicit associations between certain effector genes and disease severity could be observed underlining the difficulties of the evaluation of a risk assessment with regard to STEC from human, animals and food, because of the high genetic diversity and presence of horizontal gene transfer.

ZOV02
Prevalence and Phylogeny of Genes Encoding immunoglobulin-binding protein G (*eibG*) in Shiga toxin-producing *Escherichia coli*

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Shiga toxin-producing *Escherichia coli* (STEC) constitute a heterogeneous group of bacteria that is abundant in the reservoir and in the environment. In humans they can cause diarrhea and the hemolytic uremic syndrome (HUS), the most common cause of acute renal failure in children. Whereas the serogroup O157 is predominant worldwide, in Germany non-157 serogroups caused most STEC infections in 2008, among these O26 and O91. EibG is one of the six *E. coli* immunoglobulin-binding (Eib) proteins and is also involved in the adhesion to epithelial cells. However, the prevalence and phylogeny of this non-fimbrial adhesin within a broad spectrum of STEC serotypes is unknown. We therefore determined the prevalence of *eibG* in a large collection of STEC strains and performed phylogenetic analyses based on *eibG* sequences and on multilocus sequence typing (MLST). In total, 440 STEC strains comprising 116 serotypes were investigated. None of the *eae*-positive (n = 183) but 37 of the 257 *eae*-negative STEC (13 serotypes) harbored *eibG* genes. Partial sequencing (510 bp) of the EibG-encoding gene resulted in 13 *eibG* alleles differing in up to 25.69% with effects on the amino acid composition in all alleles. Analysis of the genomic background of the *eibG*-positive strains using MLST gave 7 different sequence types (STs13, 33, 442, 656, 738, 745, 753). Investigation of the genetic relationship between the different *eibG* alleles and their host strains by comparing the phylogenetic tree of *eibG* with the STs revealed the presence of two different evolutionary scenarios of *eibG* in STEC: a clonal development of *eibG* within strains sharing identical STs on the one hand and the presence recombinational events within *eibG* because of similar or identical eibG alleles in strains exhibiting unrelated STs on the other hand. Future studies will investigate the functional aspects of the different *eibG* alleles and their role in pathogenicity.

ZOV03
Challenges of zoonoses epidemiology and control in Germany

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In Germany, a high proportion of the notifiable infectious disease cases are caused by zoonotic pathogens. Surveillance data provide a good basis for assessing trends, early investigation and control of outbreaks, and for risk factor studies. Since 2001, salmonellosis incidence declined whereas campylobacteriosis and listeriosis incidences increased.
Most reported outbreaks are caused by Salmonella. However, the highest case-fatality rates are observed in outbreaks of hemolytic uremic syndrome (HUS) caused by (sorbitol-fermenting) Shigatoxin-producing E. coli O157 and among cases of listeriosis. In many investigated outbreaks the responsible food vehicles or animal reservoirs could be identified. However, for HUS outbreaks major challenges regarding sources and risk factors of infection remain. Severe outbreaks by rodent-borne pathogens were also investigated. They were found to be linked to climatic factors, such as preceding heavy rainfalls in the case of leptospirosis.
We also used surveillance data for mathematical modelling. In one study STEC incidence in Germany was strongly associated with cattle density. This holds true for all STEC serogroups except O91 which mainly affects adults and appears to be mainly food-borne. Another study investigating the time-space distribution of campylobacter established a significant association between air temperature, humidity and human disease incidence.
Several examples illustrate that close collaboration between microbiology and epidemiology is essential for successful outbreak investigations and control of zoonotic diseases. Timely molecular typing of isolates from humans, food, and animals is important to identify outbreak strains and reveal associations. Molecular methods combined with epidemiologic data also help to determine extent and risk factors of outbreaks and allow to quantify the impact of different food vehicles and reservoirs relevant for human infection.

ZOV04
The Phylogeny, Bioinformatics and Amplicon Re-Sequencing of Zoonotic Pathogens (PBA-Zoo) Laboratory Information Management System

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Molecular typing of microbes is essential for the recognition of outbreaks and long-term evolutionary trends. Amplicon re-sequencing methods for typing are nowadays in widespread use. The establishment of a high-throughput DNA re-sequencing service requires a powerful laboratory information management system (LIMS). Currently there are only few and very expensive suitable commercial LIMS available. Therefore, we decided to implement such software in the framework of the BMBF funded PBA-Zoo project. A three-tier architecture was chosen for implementation of the PBA-Zoo LIMS. A PostgreSQL database stores all data and is accessible via a web-browser (www.pba-zoo.de). JSF (Java Server Faces) and Facelets are used for the front-end construction. A Hibernate library is used as middleware between the front-end and PostgreSQL database and thereby many other SQL-databases are supported. The PBA-Zoo LIMS functionality can be separated into three highly interconnected modules. The customer module is responsible for user, condition, and invoice management. It interacts with the customers; takes orders, controls the automatic notification of the user via e-mail, and makes the results available for download. The laboratory workflow management is part of the laboratory module. Every laboratory working stage (e.g. PCR or sequencing product purification) is configured here regarding sample and consumable processing. The working stages are integrated in a quality monitored process control where device in- and output lists are processed and samples and consumables movements are logged. Instrument specific characteristics are managed in the device module; e.g. the number of capillaries of the sequencer or the pipetting robot setup is stored in this module. It is planned to extend the functionality of the LIMS with statistical tools and a bidirectional application programming interface (API, SOAP based). The source code of the PBA-Zoo LIMS is available on request from the authors.

ZOV05
Leptospirosis – a re-emerging zoonosis in Germany?

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In the recent years we investigated several cases of leptospirosis in adults. Detailed medical history revealed in at least 4 cases swimming in the river Neckar as the most likely source of infection.
Noteworthy is especially one outbreak in 2006, when a young man contracted severe leptospirosis after participation at a triathlon competition in Heidelberg. Explorative assessment of possible risk factors and serological testing by the local and regional health authorities detected at least 6 participants that were tested positive for IgM antibodies. Five of them met the clinical definition for leptospirosis and three had been hospitalized due to the disease. Of course contact to several animals and leisure activities associated with exposure to fresh water are general risk factors for leptospirosis, but within this group of triathletes open wounds before swimming showed up as a significant additional risk factor.
In the literature we found a reported case of leptospirosis in a triathlete in the year 2000 (Neckar near Ludwigsburg). A very recent case of severe leptospirosis in September 2009 of a sportsman, who regularly swims in the Neckar, convinced us that the 2006 outbreak was not an isolated case.
As in some patients antibodies against several serogroups were found and cross reactivtiy is frequently observed in in-vitro testing, molecular typing of leptospiral strains should be advanced to obtain more epidemiological data on the transmission of the disease.

ZOV06
Profiling human and animal antibody responses to zoonotic infections by *Chlamydia abortus* using serological proteome analysis

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The obligate intracellular bacterium *C. abortus* is the causative agent of enzootic abortion of ewes (EAE) and can also be transmitted to humans. Life threatening infections and abortions have been observed in pregnant women after exposure to infected sheep or lambs. Bacterial proteins that are expressed in the natural host and recognized by the immune system may serve as diagnostic marker proteins and candidates for vaccination. These proteins may also represent virulence factors, which become targets of the immune defence by interacting with host cell components. In order to broaden the spectrum of reliable marker proteins for *C. abortus* infections and to identify putative virulence factors we screened *C. abortus* proteins in large scale using a combination of two-dimensional (2D) gel electrophoresis for separation of bacterial proteins from purified elementary bodies and Western blot analysis of the 2D maps using sera of naturally and experimentally infected sheep. Furthermore we profiled the antibody response of two human sera from pregnant women with serious *C. abortus* infections. Identification of about 40 immunoreactive proteins was performed by mass spectrometry based assays. Comparison of human and animal specific 2D immunoblots revealed similar profiles. Spot analysis identified not only already known chlamydial immunogens such as MOMP (major outer membrane protein) and the heat shock protein cHSP60 but also antigens which have not been described before. Interestingly also four “hypothetical proteins” predicted to be secreted by the Type III secretions system turned out to be recognized by the immune system and therefore represent potential virulence factors in chlamydial pathogenesis. Further characterization of these proteins is under way.

ZOV07
Population structure of methicillin-resistant *S. aureus* CC398

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Objective: In the last years more and more infections with methicillin-resistant *Staphyloccus aureus* (MRSA) appeared in human and veterinary medicine.
Since 2003 most notably the clonal complex 398 (CC398) is described in livestock and also in humans with exposure to colonized animals.
The emergence of this pathogen introduces the problem of host adaptation and pathopotency. Especially the evolutionary origin is of greater interest. We report about the analysis of CC398 population structure by using SNPs analysis.
Methods: We investigated the population structure of CC398 by mutation discovery at 123 housekeeping genes in 115 isolates from an international strain collection from Germany, Belgium, Austria, the United Kingdom, the USA, the Netherlands and Italy.
Isolates of humans (59), pigs (21), horses (12), cattle (9), poultry (5), dogs (4), stable surrounding (2), a goat and a cat were selected.
As detection method we used the dHPLC. Polymorphic PCR products were sequenced subsequently. Data analysis and the construction of a minimum spanning tree was performed by Bionumerics 5.1.
Results: On the basis of discovered polymorphisms we revealed details about the emergence and geographic spread of MRSA in commercially raised pigs and other animals and their relationship to MRSA causing infections in humans. So far we found SNPs in 1,5% of the genome.
Some isolates are clustered according to their geographic origin. Isolates from middle Europe seem to be more diverse.

Conclusion: There are obviously no different subpopulations of CC398 in humans and pigs. Whether isolates from horses represent a distinct lineage has to be established.
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ZOV08
Detection of *Rickettsia helvetica* in ixodid ticks collected from European hedgehogs (*Erinaceus europaeus*)

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The European hedgehog (Erinaceus europaeus) is a common natural host of Ixodes (I.) hexagonus and I. ricinus, both vectors of zoonotic pathogens including rickettsial agents. Several recent studies described the detection of rickettsiae in both ixodid ticks. Beyond that, Erinaceus europaeus, the reproductive host of *I. ricinus* and *I. hexagonus*, has been suggested as a reservoir of Rickettsia or Neo-Rickettsia species. In a study regarding the presence of rickettsiae in hedgehog ticks, we investigated a total of 1,287 ticks collected from 33 hedgehogs from different collection sites in Germany and the United Kingdom. In addition, DNA available from 11 tissue samples (heart, bladder, kidney, and collective samples) was investigated. The ticks were collected 2006 and stored in 96% ethanol until further investigation. Collected ticks included all developmental stages. Adult ticks were processed individually, nymphs and larvae were treated in pools of 5 and 10. All ticks were screened for rickettsiae by Real-time PCR targeting the citrate synthase (gltA) gene. This analysis revealed a prevalence of 34% Rickettsia-PCR-positive adult ticks, 44% positive nymph-pools and 37% positive pools of larvae. Sequencing of the ompB gene revealed 99%-100% similarity to *Rickettsia (R.) helvetica*. No significant difference was found between the prevalence at different collection sites or between tick species. These results indicate that rickettsiae are widespread throughout the study region. Two of 11 tissue samples were positive by screening PCR but further characterisation could not be performed. Our investigation reveals the first detection of *R. relvetica* in *I. hexagonus* ticks from Germany and confirms the occurrence of this rickettsial species in northern Germany. Furthermore, the results of this study provide evidence that European hedgehogs may play a role in the natural transmission cycle of R. helvetica.

ZOV09
Detection of *Rickettsia raoultii* in Ixodes and *Dermacentor spp.* from selected provinces of Mongolia

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From 2005 to 2009, 155 human cases of rickettsiosis in different provinces of Mongolia were reported to the National Center for Infectious Diseases with Natural Foci (NCIDNF), Ulanbaatar, Mongolia. These cases were diagnosed by clinical symptoms, serology and PCR. Data regarding Mongolian Rickettsia strains are scarce but of great importance for disease epidemiology, pathogenicity, and improvement of clinical and routine laboratory diagnostic tools. In a collaborative study, we aimed to determine the prevalence of Rickettsia spp. in Ixodes and Dermacentor ticks from selected provinces in Mongolia. Adult ixodid ticks (n=380) were collected by flagging and stored alive at 4°C until further investigation. In addition, 199 adult Dermacentor ticks were sampled. Ticks were processed individually and screened for the presence of Rickettsia by real-time PCR targeting. Identification of Rickettsia species was performed by sequence analysis of amplicons of the ompB gene. For strain isolation, cover slip cultures of Vero E6 cells were inoculated with gltA-PCR-positive tick homogenates and were incubated at 21°C. So far, 3 out of 100 ixodid ticks were Rickettsia-positive by PCR. In addition, 21 (8 m, 13 f) out of 22 Dermacentor ticks also revealed positive results. Analysis of ompB sequences revealed 99-100% similarity to Rickettsia raoultii given in GenBank (DQ365797, DQ365798,EU036984). Cell culture is still ongoing. To summarize, almost 100% of Dermacentor ticks investigated were positive for Rickettsia raoultii by PCR which implies a major role of Dermacentor in the transmission of Rickettsia to humans. In contrast, ticks of the genus Ixodes rather seem to be of minor importance for rickettsial transmission. Further studies on isolates from patients are necessary to clarify the medical importance of rickettsiae and rickettsial ecology.

ZOV10
Rodents as reservoir hosts for Rickettsiae?

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Rickettsiae are recognized as emerging infections in several parts of the world. In Southern Germany a widespread distribution of Rickettsia (R.) helvetica in Ixodes ricinus ticks was reported. Further PCR-based results indicate endemicity e.g. of R. monacensis, R. felis, R. massiliae, R. raoultii and R. slovaca. However, in Germany the natural reservoir hosts of rickettsiae have not been investigated in detail yet. This study was set up to provide information on the occurrence of spotted fever group (SFG) rickettsiae in small mammals in Southern Germany. In summary, 256 rodents and insectivores were collected in from 2004 to 2009 in Lower Bavaria, South-Eastern Germany. Using R. conorii and R. helvetica-specific immune fluorescence assays a sero-reactivity of vole and mouse sera or transudates was shown. Nucleic acids isolated from ears and liver tissues were screened for Rickettsia-specific NA by a pan Rickettsia Real time PCR (rtPCR) specific for the citrat synthase gene. Real time(rt)-PCR positive samples were found in ear tissue, rtPCR of liver tissues revealed no positive results. Rickettsia species determination was performed using a PCR targeting the partial OmpB gene and revealed the presence of R. felis and R. helvetica. This is the first description of R. felis in rodents that is usually described in cats and associated fleas. In the next two years we will collect more longitudinal data to further investigate the occurrence of Rickettsia in small mammals in an altitude-dependent temperature-climate gradient in these areas of Lower Bavaria. This work is embedded in the VICCI-network and supported by the Bavarian Ministry of Health.

ZOV11
Seroprevalence of IgG against Rickettsiae of the Spotted Fever Group in Forestry Workers in the State Brandenburg, Eastern Germany

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Rickettsiae are obligate intracellular, Gram-negative bacteria. Among them are important human pathogens e.g. *Rickettsia (R.) prowazekii* (louse-borne typhus), *R. typhi* (flea-borne typhus), *R. rickettsii* (Rocky Mountian Spotted Fever), *R. conorii* (Mediterranean Spotted Fever) and many others. In Germany at least seven different rickettsial species have been detected so far, of which at least five are confirmed human pathogens. Seroprevalence data in Germany are completely lacking.
We therefore tested 533 sera of forestry workes from ten different districts in Brandenburg, Eastern Germany. Sera were tested by an indirect immunofluorescence assay (IFA)(Fuller Laboratories, Fullerton USA) against *R. conorii* at a dilution of 1:64. Due to cross-reactivities within the spotted fever group we assume to detect positive sera against spotted fever group rickettsiae other than *R. conorii.* Reactive sera were re-tested with IFA slides against *R. conorii* from an other manufacturer (Virotech, Rüsselsheim, Germany).
A total of 231 sera (43.3%) were found reactive in both of the immunofluorescence tests. However, prevalence rates ranged for the different forest districts from 21% to 69%. The highest prevalence rate was found in forestry workers of the Biosphere Reservate Schorfheide, north-east to Berlin, while in regions west to Berlin lower prevalence rates were detected.

The data presented are among the first ones available on seroprevalence against spotted fever group rickettsiae in Germany. The high percentages of positive sera indicate a high infection rate among the potential risk group of forestry workers. According to the available data, *R. raoultii*, a species suggested to cause tick-borne lymphadenopathy syndrome (TIBOLA) and *R. helvetica*, the cause of aneruptive fever, are highly prevalent in Dermacentor and Ixodes ticks in parts of Eastern Germany. Further studies have to elucidate whether infection with rickettsiae may be of medical importance of humans.

ZOV12
Antimicrobial resistance of Staphylococci isolated from dogs with otitis externa

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Staphyloccocus sp. considers human and animal pathogen. Even though *S. aureus* consider the main pathogen of the genus, also *S. intermedius* and Coagulase negative Staphylococci (CnS) are commonly accepted as facultative pathogens. Companion animals such as dogs can be reservoir for resistant strains. Total 39 strains of Staphylococi isolated as the only pathogen from cases of canine otitis externa were identified based on the morphological and biochemical properties, with the API Staph system (biomeriux) and with the autoSCAN-4 System (Dade Behring). The antibiotic susceptibility test was performed with disk diffusion method according the CLSI guidelines for strains isolated from animals and with the autoSCAN-4 System (Dade Behring). Also all strains were examined with multiplex PCR for the presence of genes specific for Staphylococci, *S. aureus*, and for antibiotic resistant: mecA, aacA-aphD, tetK, tetM, erm(A), erm(C), vat(A), vat(B), vat(C).
From the total 39 strains, 6 were identified as *S. aureus* (15,38%), 22 as *S. intermedius* (56,41%), 4 as *S. sciuri* (10,25%), 3 as *S. xylosus* (7,69%), 3 as *S. simulans* (7,69%)and 2 as *S. epidermidis* (5,12%). None of the Coagulase positive Staphylococci was resistant to Methicillin (neither *S. aureus* nor *S. intermedius*). Regarding the CnS, four strains were resistant to Methicillin (carrying the mecA gene) and belong to the species of *S. sciuri* (2), *S. xylosus*(1) and *S. epidermidis*(1). Regarding resistance phenotype to antibiotics, resistance to Penicillins (62%) and Tetracyclines (38%) was the highest observed and followed by resistance to macrolides (23%) and aminoglycosides (13%). The multiplex PCR was not able to detect all the genes encoding resistance, regarding Tetracyclines and Macrolides. Companion animals should be consider as resistance strains reservoir regarding Staphylococci.

SPV01
How small RNAs orchestrate post-transcriptional regulons

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Small noncoding RNAs (sRNAs) have been a fast-growing class of post-transcriptional regulators of bacterial gene expression, and have come to rival transcription factors in terms of both number and range of regulatory activity. Such RNAs are typically 50 to 250 nucleotides in length, and are expressed from the intergenic regions of bacterial chromosomes. Where studied in detail, most sRNAs were found to act on *trans*-encoded mRNAs to modulate their translation and stability. The bacterial Sm-like protein, Hfq, plays a key role in this type of post-transcriptional regulation.
This lecture will give an overview of sRNA targets, mechanisms and functions in the model pathogen *Salmonella typhimurium*. I will focus on how single sRNAs can select multiple mRNAs for repression or activation to rewire gene expression at the post-transcriptional level. This will include emerging concepts of conserved "RNA domains" and target regulation by 5' terminal pairing, as well as new mechanisms of mRNA repression at the site of translational initiation or downstream in the coding sequence.

SPV02
From inorganic to organic - Carbon fixation in bacteria

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The fixation of inorganic carbon into cell material is a prerequisite for life. In the phylogenetic tree early branching lineages of the Eubacteria and Archaebacteria harbor thermophiles that thrive in volcanic environments on inorganic substrates, mostly under microoxic or anoxic conditions. Such prokaryotes may serve as models for primordial metabolism, which requires the synthesis of building blocks from inorganic carbon. In none of them the Calvin-Benson-Bassham cycle appears to operate in CO_2 fixation. Instead, other autotrophic pathways are functioning that have in common the formation of acetyl-CoA from CO_2 [1].
In the green non-sulfur photosynthetic Eubacterium *Chloroflexus aurantiacus*, a hydroxypropionate cycle is functioning in carbon fixation. In Crenarchaeota, two other cycles are operating. Aerobic Sulfolobales and probably autotrophic marine Crenarchaeota use a hydroxypropionate/hydroxybutyrate cycle. In these two cycles, acetyl-CoA/propionyl-CoA carboxylase functions as the key carboxylating enzyme. In contrast, the anaerobic Thermoproteales and Desulfurococcales use a dicarboxylate/hydroxybutyrate cycle. This cycle uses pyruvate synthase and phosphoenolpyruvate carboxylase as carboxylating enzymes. The common traits and the benefits of the different pathways will be discussed.
These autotrophic pathways require gluconeogenesis to start from acetyl-CoA or pyruvate. Most Archaebacteria and some deep branching eubacterial lineages harbor a novel bifunctional and heat-stabile fructose 1,6-bisphosphate (FBP) aldolase/phosphatase. Its bifunctionality ensures that heat-labile triosephosphates are quickly removed and trapped in stabile fructose 6-phosphate rendering gluconeogenesis unidirectional. This highly conserved bifunctional FBP aldolase/phosphatase may represent the pace-making ancestral gluconeogenic enzyme indicating that the Embden-Meyerhof-Parnas pathway evolved in the direction of gluconeogenesis.

[1] Berg *et al.* Nature Rev. Microbiol. submitted (2010)

SPV03
Major autolysins in Gram-positive bacteria: Maturation, Function and Structure

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The bacterial murein represents a rigid envelope that maintains the cell shape and counteracts the hydrostatic pressure of the cytoplasm (turgor). Especially in Gram-positive bacteria, cellular processes such as cell growth, cell division, competence or protein secretion are dependent on a constant turn-over and dynamic remodeling of the cell wall catalyzed by the coordinated activity of peptidoglycan (PG) synthesizing and hydrolyzing enzymes. These hydrolyzing enzymes, commonly referred to as autolysins, are classified according to the PG bond they hydrolyse: *N*-acetylmuramidases, *N*acetylglucosaminidases, *N*-acetylmuramyl-L-Ala amidases and endopeptidases. Well-studied bacterial autolysins are the staphylococcal major autolysins (AtlA and AtlE), the N-acetylmuramoyl-L-alanine amidase of *Streptococcus pneumoniae* (LytA), or the *Listeria monocytogenes* autolysins (Auto and Ami). All these enzymes are not only involved in many important physiological functions but also contribute to pathogenicity. Here, the focus lies on the maturation, function and structure of the staphylococcal autolysins in comparison with autolysins from related Gram-positive bacteria.

SPV04
Translation on demand: Control of bacterial heat shock and virulence genes by RNA thermometers

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RNA molecules play a central role in gene regulation in all three domains of life. The expression of many bacterial mRNAs is controlled by the formation of complex structures in their 5'-untranslated region (5'-UTR). Riboswitches and RNA thermometers are such built-in sensory elements that control the fate of mRNAs in response to environmental conditions. Both are comprised of complex RNA structures that undergo a conformational change when a certain chemical or physical signal is present. Typical RNA thermometers control translation initiation of heat shock or virulence genes by forming a secondary structure that traps the ribosome binding site (RBS). An increase in temperature to 37°C (virulence genes) or higher (heat shock genes) destabilizes the structure, liberates the RBS and permits formation of the translation initiation complex. Recent findings suggest that RNA thermometers are more widely used than initially anticipated. Different classes of RNA thermometers will be introduced. ROSE (Repression Of heat Shock gene Expression) elements control translation of small heat shock genes in many alpha- and gamma-proteobacteria. The molecular basis for temperature sensing has been revealed by NMR spectroscopy. FourU-type RNA thermometers are characterized by a stretch of four uridines that pair with the SD sequence at low temperatures. A novel class of RNA thermometers was discovered in cyanobacteria. It revealed that shuting-off heat shock protein synthesis in the recovery phase is an important, previously unrecognized physiological function of RNA thermometers.

SPV05
Life in the inflamed intestine, *Salmonella* style

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Salmonella enterica serotype Typhimurium elicits acute neutrophil influx in the human intestinal mucosa within one or two days after infection, resulting in inflammatory diarrhea. In contrast, no overt symptoms are observed within the first one or two weeks after infection with *S. enterica* serotype Typhi. The capsule encoding *viaB* locus is a S. Typhi-specific virulence factor that enables this pathogen to escape detection by TLRs, thereby preventing the host from mounting responses that would normally prevent bacterial dissemination from the intestine. The mechanism by which S. Typhi alters host responses is in part due to altered expression of virulence genes. The TviA protein integrates a new regulatory input into a conserved regulatory network, the RcsB regulon of S. Typhi, thereby altering expression of genes encoding flagella, the Vi antigen and T3SS-1 in response to osmolarity. These changes in gene expression have profound consequences on host pathogen interaction at the intestinal mucosa.

SPV06
New medicines for tuberculosis (NM4TB)

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Tuberculosis (TB) is one of the oldest infectious diseases known to man and has infected one third of the world´s population. As a result, someone dies from the disease every 15 seconds and over 20 million more people will lose their lives to TB in the next decade. Although directly observed short course chemotherapy (DOTS) is available to treat the disease, this treatment is old, slow and inefficient by the current standards of the pharmaceutical industry. Furthermore, the efficacy of the DOTS programme has been severely compromised in certain regions by the emergence of multidrug- and extensively drug-resistant strains of *Mycobacterium tuberculosis*. This new menace is of particular concern to Europe and the countries of the former Soviet Union.
In an integrated approach to discover new TB drugs, we have been pursuing a new class of antimycobacterial compounds known as the benzothiazinones (1,3-benzothiazin-4-ones, BTZs). The BTZ kill *M. tuberculosis in vitro*, *ex vivo*, and in mouse models of TB and are exceptionally potent. Using genetics and biochemistry, we identified the enzyme decaprenylphosphoryl-β-D-ribose 2´-epimerase as a major BTZ target. The inhibition of this critical enzyme activity abolishes the formation of decaprenylphosphoryl arabinose, a key precursor that is required for the synthesis of the cell-wall arabinans, thereby provoking cell lysis and bacterial death. The most advanced compound, BTZ043, which is currently in the late preclinical stage, is a candidate for inclusion in combination therapies for both drug-sensitive and extensively drug-resistant TB.

SPV07
Rational Vaccine and Biomarker Design for Tuberculosis

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Tuberculosis (TB) is a major health threat with 9 million new cases and 2 million deaths annually. One third of the word's population is infected with the causative agent *Mycobacterium tuberculosis*. This pathogen causes chronic infection which transforms into disease in the minority of infected individuals. The vast majority contains infection in distinct foci termed granulomas. As long as *M. tuberculosis* is contained in these lesions, disease does not develop. However, the pathogen persists in a dormant stage and can be reactivated at a later timepoint. Control of M. tuberculosis is a result of a sophisticated crosstalk between different parts of the cell-mediated immune response which is orchestrated in the granuloma. Of critical importance are CD4 T cells which activate anti-bacterial capacities in macrophages. The cytokine interferon-gamma (IFN-γ) plays a particular role in macrophage activation. In addition, other populations, notably CD8 T cells are involved in protection.
What can we learn for rational vaccine design? Novel vaccination strategies either focus on subunit vaccines or viable attenuated vaccines. Subunit vaccination strategies are based on the assumption that one or few antigens suffice for an efficient immune response. Hence, the identification of protective antigens represents an essential prerequisite for the success of this type of vaccines. These subunit vaccines are considered on top of BCG. Viable attenuated vaccines are based on the assumption that multiple antigens are required for efficacious protection against M. tuberculosis. These vaccines are thought to replace BCG. Rational vaccination strategies performed in this laboratory focus on improved r-BCG. We constructed an r-BCG strain which expresses listeriolysin but is devoid of urease. This vaccine candidate induces better protection than wild-type parental BCG in experimental animals. The vaccine strain has proven to be safer in immunocompromised mice. The candidate is currently in a phase I clinical trial. It is tempting to speculate that a prime–boost scheme comprising prime with improved r-BCG and boost with the most efficacious subunit vaccine candidate will provide optimal protection. Identification of a biosignature that allows distinction between infection/protection and infection/disease in TB could speed up efficacy testing of vaccines in clinical trials. With support from the Bill & Melinda Gates Foundation, we have embarked on the definition of biomarkers which allow definition of a biosignature in TB which could speed up efficacy testing of vaccine candidates in clinical trials.

SPV08
Activities of the National Reference Center in the fight against Tuberculosis

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The German National Reference Center (NRC) is part of a worldwide laboratory network, which is a cornerstone for effective tuberculosis (TB) control. Within this, it is one of the 26 Supranational Reference Laboratories for Drug Susceptibility Testing appointed by WHO/IUATLD, since 1995. The NRC cooperates with national (RKI, Robert Koch Institute; public health offices; GTZ, Gesellschaft für technische Zusammenarbeit; KFW, Kreditanstalt für Wiederaufbau; DAHW, Deutsche Lepra- und Tuberkulose-Hilfe) and international centers/organizations (WHO; MSF, Médecins Sans Frontières; ICRC, International Committee of Red Cross; USAID, U.S. Agency for the International Development; FIND; The Foundation for Innovative New Diagnostics). A further focus is the activity as consultant for WHO, MSF, GTZ/KFW, ICRC, e.g. for the set-up of TB laboratories and drug resistance surveillance in Armenia, Azerbaijan, Bosnia-Herzegowina, Kazakhstan, Kyrgyzstan, Moldova, Serbia, Turkmenistan, Uganda and Uzbekistan.
In general, quality control is getting higher significance in the laboratory diagnostics during the last years. The NRC is also engaged in this field as coordinator of the external quality assurance in the field of mycobacterial diagnostics, which is organized by INSTAND e.V. Düsseldorf. Since 2008 the NRC is responsible for the preparation of samples for microscopy, primary isolation, identification of mycobacteria, and drug susceptibility testing of *M. tuberculosis*.

The day-to-day work of the NRC comprises primary isolation of mycobacteria with all recently worldwide established methods, identification of all mycobacterial species with classical and molecular methods, and susceptibility testing. Approx. 12,000 specimens and strains per year are investigated. Evaluations of new diagnostic tests are one main focus in the NRC. Especially new molecular based methods for the detection of *M. tuberculosis* strains from cultures or identification of resistant strains were tested concerning their proficiency and applicability.
Research of the NRC is also focussed on the topics "variability of clinical *M. tuberculosis isolates*", and "epidemiology of tuberculosis". Clinical *M. tuberculosis* isolates show a considerable phenotypical as well as genetic diversity. Consequences of this variability concerning their relative fitness, transmissibility, and severeness of the disease have been investigated.

The epidemiology of TB, combining molecular and classical methods, represents a continuous focus of research. Applying molecular strain typing techniques, studies have been carried out to analyse the epidemiology of TB in the different settings in Germany (like Hamburg, Schleswig-Holstein, Baden-Württemberg) and abroad (like Uzbekistan, Moldova, Serbia). Key questions are the population structure of M. tuberculosis complex strains in the different areas and possible interactions between metropolitan and more rural settings.

SPV09
Regulation of *Yersinia pseudotuberculosis* virulence factors during different stages of the infection

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Enteropathogenic bacteria, including *Yersinia pseudotuberculosis*, which alternate between different ecological niches outside and inside their mammalian hosts, use sophisticated environmental signal sensing devices to adjust virulence gene expression. Thermal shifts and changes in the nutrient availability encountered upon host entry and during the infection process lead to dramatic changes in the gene expression profil of *Y. pseudotuberculosis*. In the very early stage of an infection, the bacteria synthesize a special set of virulence factors, e.g. the pilus adhesin PsaA and the internalization factor invasin, that promote initial colonization and subsequent penetration of the intestinal epithelial layer. These genes are activated by the virulence regulator RovA, which acts as an intrinsic protein thermometer. Thermal upshifts from moderate temperatures to 37°C lead to a reversible conformational change of the autoactivator, which interferes with its DNA-binding function and renders it more susceptible for proteolysis by the Lon protease. This process is dependent on the growth phase of the bacteria, and amino acid residues in the vicinity of the central DNA-binding domain are important for the proteolytic susceptibility. Expression of the rovA gene is also under the control of the post-transcriptional carbon storage regulator system (Csr) implicating the differentially regulated small RNAs CsrB and CsrC. Influence of the Csr system is indirect, depends on the nutrient content of the growth medium, Hfq and the nucleoid-associated protein YmoA and occurs through the LysR-type regulator RovM which inhibits *rovA* transcription. Shortly after uptake and transcytosis of the intestinal layer, the bacteria switch their genetic program. They repress synthesis of the very early virulence factors and induce expression of another set of later-stage virulence factors that are important for the colonization of subepithelial lymphatic tissues and dissemination to liver and spleen. Activation of these genes is only promoted at 37°C and upon host cell contact and requires the AraC-like regulator LcrF, whose expression is controlled by a FourU-type RNA thermometer.

SPV10
Pathogenesis of pneumococcal infections

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Streptococcus pneumoniae is a human pathogen and a major contributor to morbiditiy and mortality world-wide. Despite being a sometimes devastating pathogen pneumococci are also common colonizers of the upper respiratory tract. Antibiotic resistance in this bacterium is emerging creating treatment problems. Pneumococci can be divided into at least 91 serotypes and several clonal types. Virulence depends on both serotype and clonal type and many virulence factors have been described for pneumococci. Also the interaction with the innate immune system is important for disease development. Conjugated vaccines covering some of the 91 pneumococcal capsular polysaccharides have been introduced in several countries childhood vaccination programme and the effect of vaccination is now being studied.

SPV11
Bacterial manipulation of the host ubiquitin system

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The ubiquitination of proteins is a central eukaryotic regulatory mechanism controlling multiple biological processes such as programmed cell death, cell cycle progression, and signal transduction. Bacterial factors have recently been identified that exploit these pathways is several ways: functioning as E3 ubiquitin ligases or regulators of such ligases, targeting proteins for deubiquitination, possessing sequences conveying differential half-lives in the host, or serving themselves as substrates for mono-ubiquitination for purposes such as localization within the eukaryotic cell. We will consider several of these examples from pathogenic bacteria, focusing on recent structural work that defines the mechanisms of several microbial E3 ligases.

SPV12
Molecular Activities of New Cell Wall Biosynthesis Inhibitors

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Inhibitors of bacterial cell wall biosynthesis represent the economically most important group of antibiotics. Besides beta-lactam and glycopeptide antibiotics, there are many other antibiotic compounds acting on one of the many molecular targets available in this biosynthesis pathway. Although commercially less successful or just experimental, these compounds may represent new lead structures for future drug development; moreover, detailed analysis of their molecular modes of action yields new mechanistic insights into the function of the highly dynamic cell wall biosynthesis machinery. We recently identified the lipopeptide antibiotic friulimicin to form a stoichiometric complex with the membrane carrier bactoprenylphosphate, a target that had not been identified before. Since bacto-prenylphosphate also serves as a carrier of building blocks for teichoic acid and carbohydrate capsules, friulimicin appears to cause multiple cell surface stresses. Remarkably, some innate immunity host defense peptides, the most abundant and permanently used antibiotics, also turned out to interfere with the synthesis of a functional peptidoglycan network. For example, the fungal defensin plectasin binds with high affinity to the bactoprenol-linked cell wall building block, Lipid II, and prevents its incorporation into peptidoglycan. Also, human beta defensin 3 (hBD3) is able to interact with Lipid II, albeit with less affinity as compared to plectasin. However, hBD3 gains potency by additionally pertubating membrane bilayer functions. The concept of combining antibiotic activities has also been verified in recently developed glycopeptide antibiotics which, in addition to Lipid II sequestration, impair the lipid bilayer or have additional binding sites for the cell wall precursor.
Recent review article: T. Schneider, HG Sahl: An oldie but a goodie - cell wall biosynthesis as an antibiotic target pathway. Int. J. Med. Microbiology, 2010

SPV13
Anti-virulence strategies for novel antibacterial; drugs and diagnosis

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In recent years mounting problems related to antibiotic-resistant bacteria have resulted in the prediction that we are entering the preantibiotic era. A way of preventing such a development would be to introduce novel antibacterial agens with modes of action distinct from conventional antibiotics. Recent studies of bacterial virulence factors and toxins have resulted in increased understanding of the way in which pathogenic bacteria manipulate host cellular processes. This knowledge may now be exploited to develop novel antibacterial agens that disarm pathogenic bacteria. The type III secretion system (T3SS) is known to be a potent virulence mechanism shared by a broad spectrum of pathogenic Gram-negative bacteria that interact with human, animal and plant hosts by injecting effector proteins into the cytosol of host cells. Diseases, such as bubonic plague, yersinosis, shigellosis, salmonellosis, typhoid fever, sexually transmitted chlamydia and diarrhoea largely depend on the bacterial proteins injected by the T3SS machinery. Recently a number of T3SS inhibitors have been identified using HT-Screening-based approaches. One class of inhibitors, the salicylidene acylhydrazides, has been subjected to chemical optimization and evaluation in several *in vitro* and *ex vivo* assays in multiple bacterial species including *Yersinia spp., Chlamydia spp., Salmonella spp.* and *Pseudomonas aeruginosa*. Reports published up to date indicate that T3SS inhibitors have the potential to be developed into novel antibacterial therapeutics. This will be discussed. Further, the above mentioned strategy requires that the infecting pathogen is accurately diagnosed prior to the onset of therapy. To meet this demand we are employing metabolomics as a diagnostic tool. Results will be presented indicating that an infection can be diagnosed from a blood sample within hours after that the sample has been taken.

SPV14
Turned on for degradation - dysregulation of Clp protease by acyldepsipeptide antibiotics

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New natural product derived acyldepsipetides (ADEPs) are highly active against important nosocomial Gram-positive pathogens (e.g. staphylococci, enterococci, and streptococci) with antibacterial *in vitro* activities surpassing those of many marketed antibiotics and impressive efficacy in rodent models of bacterial infection [1, 2].
The ADEPs act on an unprecedented target, the bacterial caseinolytic Clp protease, and thoroughly reprogram its activity [1].
ADEPs bind to the ClpP core of the proteolytic complex and abrogate its interaction with cooperating Clp-ATPases [3]. The Clp-ATPases perform an indispensable function in the proteolytic process. They transport protein substrates to ClpP and unfold them for entry through the tiny substrate entrance pores of ClpP. Without its partner ATPases the isolated ClpP is not able to degrade oligopeptides and proteins.
By preventing the interaction between the two partners, ADEPs affect all physiological functions of the Clp-protease in generl and regulatory proteolysis. At the same time ADEPs bestow independent proteolytic activities to the isolated ClpP core [3]. Nascent polypeptides at the ribosome represent one important target for activated ClpP along with central components of the cell division machinery.
Recently elucidated crystal structures of ClpP from *Bacillus subtilis* alone and in complex with ADEPs provide rational explanations for the multifarious effects that ADEPs exerts on ClpP. ADEPs occupy a binding site at the apical rim of the ClpP barrel that is also important for Clp-ATPase binding. By establishing contacts with two subunits, ADEPs stabilize the proteolytically active ClpP tetradecamer. In addition, they induce a conformational change that extends towards the central substrate entrance pore. The structures show the closed- to open-gate transition of the ClpP N-terminal segments upon activation and conformational changes restricted to the upper portion of ClpP. The direction of conformational movement and hydrophobic clustering for stabilizing the closed structure is markedly different from other ATP-dependent proteases, providing unprecedented insights into the activation of ClpP.

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[2] Hinzen *et. al.* 2006, Chem. Med. Chem. 1: 689-693
[3] Kirstein *et al.* 2009, EMBO Mol. Med. 1: 37-49

SPV15
Oncogenic mechanism of *Helicobacter pylori* CagA

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Infection with *Helicobacter pylori cagA*-positive strains is the strongest risk factor for the development of gastric carcinoma. The *cagA* gene product CagA is delivered into gastric epithelial cells via the bacterial type IV secretion system and, upon tyrosine phosphorylation by Src kinases, specifically binds to and aberrantly activates SHP-2 tyrosine phosphatase, a bona-fide oncoprotein gain-of-function mutations of which are associated with a variety of human malignancies. CagA-activated SHP-2 deregulates Erk MAP kinase signaling and also induces an extremely elongated cell-shape known as the hummingbird phenotype. CagA also binds to partitioning-defective 1 (PAR1)/microtubule affinity-regulating kinase (MARK) independently of CagA tyrosine phosphorylation and inhibits the kinase activity, thereby causing junctional and polarity defects in polarized epithelial cells. PAR1 regulates cell polarity through controlling stability of microtubules. Because microtubules are essential components of mitotic spindles, CagA-mediated PAR1 inhibition also influences mitotic spindle function. Indeed, cells expressing CagA display a delay in the transition from prophase to metaphase, which is associated with chromosomal misalignment at the onset of anaphase. Consequently, chronic exposure of cells to CagA induces chromosomal instability. These results indicate that, on one hand, CagA stimulates uncontrolled cell proliferation through deregulation of SHP-2 and, on the other hand, it induces chromosomal instability by impairing PAR1 function. These CagA activities may collectively contribute to the progression of multistep gastric carcinogenesis.
Based on the structural polymorphism, *H. pylori* CagA can be subdivided into two major isoforms - Western CagA and East Asian CagA. To investigate oncogenic potential of CagA *in vivo,* we generated transgenic mice that systemically express East Asian CagA. The established CagA transgenic mice showed gastric epithelial hyperplasia and some of them developed adenocarcinomas of the stomach and small intestine. The mice also showed leukocytosis with IL-3/GM-CSF hypersensitivity and some mice developed myeloid leukemias and B-cell lymphomas. In marked contrast, transgenic mice expressing phosphorylation-resistant CagA did not show any pathological abnormalities. These results provide direct evidence for the oncogenic potential of CagA and indicate the importance of the tyrosine-phosphorylated form of CagA for *in vivo* tumorigenesis. More recently, we generated transgenic mice that express Western CagA. Comparison of Western CagA transgenic mice with those expressing East Asian CagA revealed that East Asian CagA is qualitatively more oncogenic than Western CagA *in vivo*. Differential oncogenic potential of geographically distinct CagA isoforms may underlie the higher incidence of gastric carcinoma in East Asian countries compared to the incidence of that in Western countries

SPV16
Ecological based strategies to exploit interactions between fungi and bacteria

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Whereas bacteria are the key microorganisms responsible for degradation of aquatic organic matter, they have to share this position with fungi with respect to the decomposition of terrestrial organic matter. The prominent position of fungi in terrestrial ecosystem functioning is linked to the hyphal growth form which is well equipped to explore the soil matrix and to penetrate organic solids. The importance of fungi in terrestrial ecosystems must have had a strong impact on the evolution and ecology of terrestrial bacteria. This impact can be revealed by studying interactions between fungi and bacteria. In the current paper I will address several examples of such studies. The focus will not only be on the basic ecological aspects of these studies but also on the possibilities to exploit fungal-bacterial interactions for application purposes.

SPV17
The role of quorum sensing in the pathogenesis of *Burkholderia cenocepacia* in multiple infection hosts

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Over the past few decades, strains of the *Burkholderia cepacia* complex (Bcc) have emerged as important pathogens for patients suffering from cystic fibrosis (CF). Identification of virulence factors and assessment of the pathogenic potential of Bcc strains have increased the need for appropriate infection models. In previous studies different infection hosts, including mammals, nematodes, insects and plants, have been used. At present, however, it is not known to which extent the virulence factors required to infect different hosts overlap. In this study we have analyzed the importance of the CepIR quorum sensing system of two closely related *Burkholderia cenocepacia* strains, H111 and the epidemic strain K56-2, for virulence in a multihost pathogenesis system using four different model organisms: *Caenorhabditis elegans, Galleria mellonella*, the plant alfalfa and mice or rats. We also demonstrate that many of the previously identified virulence factors of *B. cenocepacia*are specific for only or a few of the infection models. The fact that quorum sensing plays an essential role in pathogenesis in various hosts makes it a promising target for the development of novel antiinfectives.

SPV18
Phenotypic heterogeneity of a *Vibrio harveyi* population under quorum sensing conditions

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Quorum sensing (QS) plays an important role in regulating gene expression for a variety of functions in bacterial populations. This intercellular communication through small diffusible molecules, called autoinducers (AIs), enables single cells to coordinate their behaviour within a population. In *Vibrio harveyi* QS regulates type III secretion, siderophore production, biofilm formation, exoprotease activity and bioluminescence. This bacterium responds to three different AIs, HAI-1, an N-(3-hydroxybutyryl)-D-homoserine lactone, AI-2, a furanosylborate diester and CAI-1, a 3-hydroxytridecan-4-one. In order to understand how single cells behave within a QS-activated community, QS-induced processes in *V. harveyi* were investigated in a homogeneous environment over time. Analysis of wild-type single cells with respect to QS-induced bioluminescence revealed that even at high cell densities only 69% of the cells of the population produced bioluminescence, 25% were non-luminescent, and 6% were dead.[1] A similar fractionation of the population was found for the induction of an exoprotease which was monitored in a mutant containing a transcriptional *pap6*::*gfp* fusion. These results indicated phenotypic heterogeneity of a genetic homogeneous population. An artificial increase of the AI concentration in the wild-type resulted in an all-bright cell population that was also found for a Δ*luxO* mutant. Both wild-type and mutant switched to biofilm formation at high cell density. However, the capability of the Δ*luxO* mutant to produce biofilm was significantly reduced in comparison to wild-type. Our data suggest that a high-dense population of the non-differentiating bacterium *V. harveyi* takes advantage of division of work.

[1]Anetzberger, C., Pirch, T., und Jung, K. (2009) Heterogeneity in quorum sensing regulated bioluminescence of *Vibrio harveyi*, *Mol. Microbiol.* 73, 267–277.

SPV19
Efficient down-regulation of enzyme activities in *Corynebacterium glutamicum* and beneficial application towards improved L-lysine production

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In the recent years, metabolic engineering became more and more popular in industrial biotechnology to create superior strains for a great variety of fermentation products. L-Lysine production by *Corynebacterium glutamicum* with a current world market of 850.000 tons per annum was, for instance, successfully improved by rational engineering of the NADPH metabolism (Becker *et al*.,2007) or engineering of the precursor supply (Wittmann and Becker, 2007). In this regard, modulation of enzyme activities within the cell is crucial to increase or reduce carbon conversion by selected metabolic pathways. Plasmid-related over expression, over expression by promoter exchange or gene deletion are the most common strategies to achieve modified enzyme activities. Gene deletion, however, always results in a complete lack of the corresponding enzyme activity and is not suitable to down-regulate specific metabolic pathways.
In this work, we applied a novel strategy to reduce the specific activity of selected enzymes of the central metabolism in *C. glutamicum*. We substituted the common start codon ATG by the rare start codon GTG and investigated the effect on specific enzyme activity. Introduction of this nucleotide exchange into the sequences of, isocitrate dehydrogenase (ICD) and pyruvate dehydrogenase significantly reduced the specific activity of these enzymes by 70 % and 60 %, respectively. Implementation of this modification into the isocitrate dehydrogenase sequence of the L-lysine producing strain *C. glutamicum* BS87 resulted in an improved lysine production by more than 40 % (Becker *et al*., 2009). This was due to an efficient down-regulation of the carbon flux through the tricarboxylic acid cycle (TCA cycle) by decreasing the intracellular ICD-availability. In *C. glutamicum*, more than 80 % of the genes encoding enzymes of the central metabolism use ATG as a start codon. This high frequency reflects the great potential of the applied strategy for metabolic engineering to effectively reduce selected enzyme activities within the cell.

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[2] Becker J, Klopprogge C, Schröder H, Wittmann C (2009) Appl Environ Microbiol, 75, 7866-7869
[3]Wittmann C, Becker J (2007) In: Wendisch VF (Ed) Amino Acid Biosynthesis – Pathways, Regulation and Metabolic Engineering. Springer, pp. 39-70

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SPV20
Novel metabolic properties of foodborne pathogens

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Little attention has been given so far to the metabolic requirements and the metabolic flexibility of foodborne pathogens that are often capable to grow under varying conditions encountered in the environment, the food chain, or during infection. Therefore, our knowledge about substrates and metabolic pathways used by pathogens such as Salmonella enterica is still fragmentary. Key metabolic enzymes, however, that are specifically required during growth within food samples constitute a set of possible targets for food formulas that suppress *Salmonella* growth.
Here, we present two degradation pathways that contribute to *S. enterica* serovar Typhimurium proliferation in varying compartments. (i) *S. enterica* serovar Typhimurium, albeit with an extraordinarily long lag phase, shows wild type-like growth in the presence of MI. A novel genomic island harbouring the genes for myo-inositol (MI) uptake and utilization was characterized. Upon nonpolar deletion, the genes involved in six enzymatic steps of the MI pathway were identified, as well as the main repressor IolR and the major MI facilitator. (ii) Propanediol utilization is another metabolic adaptation that plays a role in the virulence of salmonellae. Propanediol is an abundant compound in the human gut and in processed food, and can be utilized in a coenzyme B12 (cobalamine) dependent manner as the main source of carbon and energy under anaerobic conditions. Interestingly, only two other hazardous food-borne pathogens, namely *Listeria monocytogenes* and *Clostridium perfringens*, carry the conserved gene cluster for cobalamine synthesis and propanediol degradation. A proteomic approach revealed a high abundance of pdu operon products during intracellular replication of *S. Typhimurium* strain 14028. Propanediol is derived from fucose, a common constituent of the glycoconjugates of eukaryotic cells. The propanediol utilization pathway appears to constitute a crucial determinant for pathogens associated with food poisoning.

SPV21
Assessment of probiotics against *Salmonella* infection using combined *in vitro* models of colonic fermentation and human epithelial cell layers

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Salmonella infections are frequently encountered in children less than 5 years of age and may lead to severe complications. Antibiotic therapy can then be life-saving but the incidence of treatment failures due to resistant strains has increased significantly. In this context, probiotic bacteria are potential alternatives to antibiotics since they enhance host defense mechanisms against various enteropathogens. We developed novel *in vitro* intestinal fermentation and cellular models to study enteric *Salmonella typhimurium* infection which were combined to better assess the potential of two probiotic strains producing antimicrobial peptides active against *Salmonella, Bifidobacterium thermophilum* RBL67 (isolated from newborn faeces) and *E. col*i L100 (microcin B17). The preventive and therapeutic effects of *B. thermophilum* RBL67 and *E. coli* L1000 were tested in a continuous *in vitro* colonic fermentation model of *Salmonella* infection (one and three stages) inoculated with immobilized child fecal microbiota and operated for 43-54 days, and compared to classical antibiotic therapy (amoxicillin/clavulanic acid at two levels). The mucus-secreting intestinal cell line HT29-MTX was used to test probiotic effects on adhesion-invasion and competitive-exclusion of *Salmonella* and protection of cell junction integrity (transepithelial electrical resistance) in defined cultures and complex fermentation effluent containing a complete child gut microbiota.
We showed that antibiotic therapy produced only a transient decrease of *Salmonella* concentrations and massively disrupted human microbiota composition (FISH-Flow Cytometry) and its metabolic activity. In contrast, the addition of *B. thermophilum* RBL67 before (preventive effect) or after infection (therapeutic effect) induced a constant decrease of the pathogen, preventing its propagation without effecting the gut microbiota composition and activity. On the other hand although *E. coli* L1000 was very effective in inhibiting a panel of 68 infectious *Salmonella* strains, addition of this strain in the infection gut model unexpectedly stimulated *Salmonella* growth. *B. thermophilum* RBL67 in the colonic effluent reduced 5-fold *S. typhimurium* N-15 invasion into mucus-secreting HT29-MTX epithelial cells and completely protected cell integrity measured with TEER. In contrast *E. coli* L1000 increased invasion of *S. typhimurium* N-15 2-fold and showed no protection effects on epithelium integrity which was largely decreased following *Salmonella* infection.
Our data showed that the combined colonic fermentation-cell model is a powerful tool to investigate *Salmonella* and probiotics interaction in a gut ecosystem. Different modes of action against *Salmonella* were shown for the probiotics tested in conditions akin to the gastrointestinal tract, providing important data for probiosis assessment for preclinical development. Our study highlighted the high potential of *B. thermophilum* RBL67 for prevention and treatment of *Salmonella* infection in the gut.

SPV22
Biosecurity als komplementäres System zu Biosafety

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Die Begriffe Biosafety und Biosecurity werden in der deutschen Sprache mit Biologische Sicherheit übersetzt. Je nach Verwendungskontext werden dabei nicht nur der Begriff Biologische Sicherheit sondern auch die Begriffe Biosafety und Biosecurity unterschiedlich definiert.
Ausgehend von den Definitionen der WHO versteht man Biosecurity als ein zu Biosafety komplementäres Konzept, das beim Umgang mit Mikroorganismen und Toxinen den Schutz biologischer Materialien vor unberechtigtem Zugriff sicherstellen soll. Die in den USA seit 2001 nach den Terroranschlägen des 11. September und den Anthrax-Briefen ergriffenen Maßnahmen haben weltweit die Diskussion um Biosecurity stimuliert. In den Entwicklungsländern ist sie vorwiegend der Auslöser für die Etablierung von Biosafety-Maßnahmen. In Ländern mit entwickelter aboratoriumsinfrastruktur liegt der Schwerpunkt auf der Implementierung von Maßnahmen zum Schutz risikobehafteter biologischer Materialien.
Am 30. November 2009 hat die EU ein Maßnahmenpaket zur Stärkung der chemischen, biologischen, radiologischen und nuklearen (CBRN) Sicherheit verabschiedet, das u.a. die Verbesserung der Sicherheit für biologische Agenzien und Toxine vorsieht. Der Aktionsplan des CBRN-Pakets listet zahlreiche präventive Maßnahmen für den Bereich Biologie auf, die in Deutschland zum Teil bereits umgesetzt sind. Er spricht darüber hinaus auch Maßnahmen zur Selbstkontrolle in Form von Verhaltenskodizes an. Einer der Kernpunkte des Plans ist die Erstellung einer EU Liste risikobehafteter biologischer Stoffe, an der alle vorgeschlagenen Maßnahmen aufgehängt werden. Zu den EU Vorschlägen gehört u.a., dass Einrichtungen, die mit gelisteten biologischen Agenzien und Toxinen arbeiten, die Implementierung des CEN Workshop Agreements (CWA) 15793 *Laboratory biorisk management standard* in Betracht ziehen.

SPV23
„Biosafety"-Regelungen in Deutschland – besteht weiterer Regelungsbedarf? Eine kritische Bestandsaufnahme

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Vor dem Hintergrund neuer Entwicklungen wie dem CEN Workshop Agreements (CWA) 15793 *Laboratory biorisk management standard*, dem neuen geplante CEN Workshop Agreements "Biological Safety Professional", sowie dem EU Aktionsplan zur Stärkung der chemischen, biologischen, radiologischen und nuklearen Sicherheit (CBRN) sollen die derzeit bestehenden Regelungen zur "Biosafety" im Deutschen Rechtsbereich nochmals vorgestellt werden.
Erstaunlicherweise wird den genannten CWAs derzeit mehr Aufmerksamkeit geschenkt, als den bereits lange bestehenden Regelungen der Biostoffverordnung und den nachgeschalteten Technischen Regeln. Details zu diesem Technischen Regelwerk und Antworten auf häufig gestellte Fragen zur Biosecurity-Problematik können auf der Homepage der Bundesanstalt für Arbeitschutz und Arbeitsmedizin (www.baua.de) abgerufen werden. Der Ausschuss für Biologische Arbeitsstoffe (ABAS) beschäftigt sich derzeit mit notwendigen Aktualisierungen und der Fortschreibung bestehender technischer Regeln (TRBAn).
Neue TRBAn zu den Bereichen der "Biotechnologie" und zu "Zellkulturen" sind geplant. Auch die Bewertung und Einstufung von Mikroorganismen und die Abfassung von Stellungnahmen für die ZKBS (gemäß § 5 GenTG), als Daueraufgaben des ABAS soll im engen Austausch mit der ZKBS erfolgen; darüber hinaus ist jedoch auch eine Kontinuität und Verstärkung der Zusammenarbeit mit weiteren Gremien und Institutionen vorgesehen.
Eine kritische Bestandsaufnahme durch den Unterausschuss I (Grundsatzfragen) des ABAS kam zu der Schlussfolgerung, dass fast alle der im CWA 15793 *Laboratory biorisk management standard*, geforderten Vorgaben zur "Biosafety" durch nationale Regelungen bereits abgedeckt werden. Dennoch wird es unumgänglich sein, im Bereich der Normung internationale Aktivitäten aktiv mitzugestalten und national umzusetzen.

SPV24
Getting the fuller picture or prokaryote genome evolution with networks instead of trees

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Lateral gene transfer (LGT) is an important mechanism of natural variation among prokaryotes, but the extent of genomic exchange among different species and possible barriers to it are still debated. Phylogenetic networks can capture both vertical inheritance and recent lateral gene transfer among prokaryote genomes, as example using hundreds of sequenced genomes illustrate. Networks can also help to uncover the mechanisms involver in specific gene transfer events.

SPV-MRSA01
SWAB, real-life antibiotic stewardship in the Netherlands

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The aim of the Dutch Working Party on Antibiotic Policy (Dutch acronym is SWAB) is "to contribute to the containment of the development of antimicrobial resistance and of the expanding costs of the use of antibiotics. This is achieved by optimizing the use of antibiotics by means of guideline development, education and antibiotic resistance surveillance".

At present 12 guidelines(such as on pneumoniae, sepsis, fungal infections, MRSA eradication) have been developed for the adult patient mainly in the hospital setting and are up-dated on a regular base. The guidelines are published primarily in Dutch, some of which are also translated in English. Yearly in Nethmap data on antibiotic resistance surveillance both intra- and extramural as well as data on antibiotic use are published. Furthermore, the national antibiotic formulary became available with general guidelines as to the antibiotic use in the hospital setting. These general guidelines can be adapted to specific choices based on the local situation in each hospital. Also the University hospital Maastricht used the national SWAB antibiotic formulary and adapted the choices where appropriate to the local situation.

The university hospital Maastricht is located near the borders between Belgium and Germany. Due to this unique geographical situation we experience a lot of cross border traffic, both of patients as well as health care workers with influences the choice of the agents used in our formulary.

Cross border traffic is also the main focus of a recently started project "EU prevent" on patient safety in the Euregion Meuse Rhine. The aim of the project is among others to collect data as to the prevalence on antibiotic resistance in the Euregion, both inside and outside hospitals and finally to harmonize the different local protocols aiming at improvement and facilitation of cross border health care

SPV-MTA01
Chancen und Probleme des *C. trachomatis*-Screening

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Die Häufigkeit und Zunahme genitaler *Chlamydia trachomatis*-Infektionen und Erkrankungen hat zur Implementierung diverser Kontrollaktivitäten geführt. Als höchste Stufe der Infektionskontrolle wird das Chlamydien-Screening angesehen, das in verschiedenen europäischen Ländern (u.a. Schweden, Dänemark, Norwegen, Deutschland, Niederlande und Großbritannien) und in den USA durchgeführt wird. Meistens handelt es sich dabei um ein opportunistisches Screening bestimmter Populationen. Staatlich organisierte Screening-Programme existieren nur in den Niederlanden und in Großbritannien. Ziel des Screenings ist die Reduktion der Häufigkeit der Infektion und der Folgeerkrankungen durch die frühzeitige Identifizierung infizierter Personen und die antibiotische Behandlung der positiv getesteten Personen und ihrer Partner.

Mit einer jährlichen Inzidenz von ca. 100 Millionen verursacht *Chlamydia trachomatis* weltweit die meisten bakteriellen sexuell übertragenen Infektionen. In Deutschland wird die Zahl der Neuinfektionen vom Robert-Koch-Institut auf ca. 300.000 pro Jahr geschätzt; die Gesamtzahl der Infizierten soll bei 1.1 Millionen liegen. Die Infektion tritt vor allem bei Jugendlichen und jungen Erwachsenen auf und verläuft in der Mehrzahl der Fälle klinisch inapparent. Die symptomatische akute Infektion äußert sich meist als Urethritis oder Zervizitis.

Sowohl aus symptomatischen als auch aus asymptomatischen Infektionen können chronisch-persistierende Infektionen hervorgehen, die vor allem bei Frauen Folgeerkrankungen verursachen, wie Endometritis, Salpingitis, Peritonitis, Periappendizitis oder Perihepatitis. Diese Erkrankungen werden zusammenfassend auch als PID (pelvic inflammatory diseases) bezeichnet. Bei einem entzündungsbedingten Verschluss der Eileiter besteht die Gefahr einer ektopen Schwangerschaft und Infertilität. Laut eines Berichts der DAHTA (Deutsche Agentur für Health Technology Assessment) sind mindestens 6% der Paare in Deutschland (entspricht ca. 1 Millionen) ungewollt kinderlos. Die Ursache ist in ca. 20% ein Tubenverschluss, der in der Mehrzahl der Fälle auf einer *C. trachomatis* Infektionen beruht. Über 100.000 Frauen wären daher als Folge einer Chlamydien-Infektion steril. Diese relativ hohe Komplikationsrate soll durch das Chlamydien-Screening reduziert werden.

Bereits 1995 wurde das Chlamydien-Screening in das Vorsorgeprogramm der Mutterschaftsrichtlinien aufgenommen, mit dem Ziel die Übertragung auf das Neugeborene zu verhindern und die Häufigkeit von Frühgeburten und sekundärer Sterilität zu reduzieren. Die Effektivität des Schwangeren-Screenings ist allerdings begrenzt, da lange Zeit nur Antigen-Tests zugelassen waren, die eine geringere Sensitivität besitzen als Nukleinsäure-Amplifikationstests (NATs), und die Untersuchung gewöhnlich nur einmal in der Frühphase der Schwangerschaft erfolgte. Zudem beträgt das Durchschnittsalter bei der ersten Geburt inzwischen fast 30 Jahre, einem Alter in dem *C. trachomatis* Infektion des unteren Genitaltrakts relativ selten sind (die Prävalenz beträgt in dieser Altersgruppe nur ca. 1%). Die Identifizierung und Behandlung positiver Patienten hat daher kaum einen Einfluss auf die Infektionshäufigkeit der gesamten Bevölkerung.

Am 1.1.2008 ist das Chlamydien-Screening auf die Allgemeinbevölkerung ausgedehnt worden. Danach besteht für alle sexuell aktiven Frauen unter 25 Jahre und weiterhin auch für Schwangere, unabhängig vom Alter, die Möglichkeit sich einmal pro Jahr auf Chlamydien untersuchen zu lassen. Der Nachweis der Infektion soll durch die Analyse von Urinproben mittels NAT erfolgen. Für Labore besteht die Möglichkeit bis zu 5 Urinproben gemeinsam zu analysieren (pooling). Bei negativem Ergebnis des Pools können alle Einzelproben als negativ bewertet werden, bei positivem Ergebnis oder Inhibition (invalides Ergebnis) müssen jedoch alle Proben einzeln nachgetestet werden.

Zurzeit wird das Chlamydien-Screening in Deutschland nur in geringem Maße wahrgenommen. Die Hauptursachen sind der begrenzte Kenntnisstand in der Bevölkerung und auch die geringe Kostenerstattung. Zudem ist die Eingrenzung des Untersuchungsmaterials auf Urinproben umstritten. Wenn die Diagnostik genitaler Chlamydien Infektionen durch die Analyse von Urinproben erfolgt, ist es wichtig die erste Portion beim Wasserlassen zu testen (sog. Erststrahlurin), da die Erregerkonzentration mit zunehmendem Harnvolumen stark abnimmt. Das Volumen von Erststrahlurin ist aber nicht exakt definiert und die erste Portion als solche auch nicht optisch zu identifizieren. Bei Frauen konnte gezeigt werden, dass die Chlamydienkonzentration im Erststrahlurin niedriger ist, als in parallel gewonnenen zervikalen und vaginalen Abstrichproben. Des Weiteren ist zu bedenken, dass Urin häufiger inhibitorische Substanzen enthält als Abstrichmaterial.

Die Möglichkeit der Testung gepoolter Urinproben wird ebenfalls kritisch gesehen. Die Kosten für Testreagenzien können dadurch zwar reduziert werden, Pooling verursacht aber einen Verlust an Sensitivität und repräsentiert zudem ein erhöhtes Risiko für Probenverwechslungen und Kontaminationen.

Im Hinblick auf ein effizienteres Chlamydien-Screenings liegt das größte Verbesserungspotential in der Rekrutierung der Zielpopulation, da die Screening-Untersuchung zurzeit nur von einem kleinen Teil der Frauen unter 25 Jahre in Anspruch genommen wird. Wirksame Maßnahmen beinhalten daher vor allem die Verbesserung des Kenntnisstands in der allgemeinen Bevölkerung bezüglich der Bedeutung von Chlamydien-Infektionen und der Möglichkeit zur Vorsorge durch das Chlamydien-Screening.

SPV-MTA02
PCR-gestützte *Clostridium difficile* Diagnostik: was ist möglich und was ist sinnvoll

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Clostridium difficile-Infektionen (CDI) sind aufgrund zunehmender Inzidenz und damit steigender Kosten für das Gesundheitssystem von hoher Relevanz. Das Auftreten von Stämmen mit spezifischen virulenten Eigenschaften, die u.a. schwere klinische Verläufe verursachen, hat *C. difficile* wieder stärker in den Fokus von wissenschaftlicher Untersuchungen gerückt. Die Anforderungen an diagnostische Systeme zur Detektion von CDI umfassen eine zeitnahe Aussage zum Nachweis von toxinbildenden Stämmen im Stuhl von Patienten sowie die Anzucht des Erregers für die Typisierung und ggfs. antimikrobielle Empfindlichkeitsbestimmungen.

Verschiedene kommerziell verfügbare molekularbiologische Assays weisen innerhalb kurzer Zeit hochspezifisch und –sensitiv Genabschnitte von *C. difficile*, Toxingenen sowie Virulenzfaktoren nach. Verschiedene Testsysteme werden vorgestellt und ihre Anwendung im klinischen Alltag bzw. für epidemiologische und wissenschaftliche Fragestellungen diskutiert.

SPV-MTA03
Nukleinsäure-Amplifikationstechniken in der bakteriologischen Schnelldiagnostik

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Traditionell beruht der Nachweis von pathogenen Bakterien und Pilzen auf deren kultureller Vermehrung und anschließender Differenzierung. Mit der Verfügbarkeit von hochsensitiven Nukleinsäure-Amplifikationsverfahren und Detektiontechniken zur sequenzspezifischen Charakterisierung der Amplifikationsprodukte eröffnen sich in letzter Zeit jedoch neue diagnostische Möglichkeiten. In vielen Bereichen der modernen mikrobiologischen Diagnostik erweist sich der Einsatz dieser enorm sensitiven, spezifischen und zumeist auch sehr schnellen Testsysteme bereits als ideale Ergänzung zu konventionellen Untersuchungsverfahren wie Mikroskopie und Kultur. Durch die Verfügbarkeit aussagekräftiger "same day results" eröffnen sich aktuell neue Optionen zur frühzeitigen Einleitung gezielter Behandlungsmaßnahmen bzw. Isolationsmaßnahmen zum Schutz "negativer" Patienten.
Auch wenn die Festlegung von klinischen Indikationen für die Durchführung von erregerspezifischen NAT-Untersuchungen in vielen Bereichen noch umfangreicher Studien und Bemühungen zur Konsensfindung bedarf, sind für den gezielten Nachweis von nahezu allen bakteriellen, viralen, fungalen oder anderen eukaryonten Pathogenen bereits eine Reihe kommerzieller Testsysteme sowie mehr oder weniger gut geeignete selbst entwickelte (*in house*) Protokolle etabliert. Seit kurzem sind auch sog. real-time PCR Assays in vollautomatisierten Testkonzepten verfügbar, die sehr schnelle Ergebnisse liefern und z.T. auch ohne molekularbiologisch versiertes Personal oder umfangreiche apparative Ausstattung durchgeführt werden können. Mit der Entwicklung automatisierter molekularbiologischer Testsysteme zum kulturunabhängigen Erregernachweis stehen auch dem Routinelabor die vielfältigen Möglichkeiten der Nukleinsäurediagnostik zur Verfügung. An ausgewählten Beispielen der mikrobiologischen Praxis und der in diesem Zusammenhang nachgewiesenen bakteriellen bzw. fungalen Pathogene werden die jeweiligen Konzepte, potentielle Anwendungsmöglichkeiten aber auch einige der technischen und ökonomischen Limitationen von Nukleinsäure-gestützten Testsystemen dargestellt.

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Unfruchtbarkeit durch unerkannte Bakterieninfektionen

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Chlamydiose und Gonorrhö gehören zu den häufigsten Infektionskrankheiten des Urogenitaltraktes. Die Bakterien *Chlamydia trachomatis* und *Neisseria gonorrhoeae* werden sexuell übertragen und können chronische Bauchbeschwerden, pelvic inflammatory disease, extrauterine Schwangerschaften, Tubenverschluss oder Prostatitis verursachen. Die Infektion während der Schwangerschaft kann eine Fehlgeburt auslösen, oder beim Geburtsvorgang auf die Augen des Neugeborenen übertragen, zur Erblindung führen. Nicht selten verlaufen die Infektionen jedoch asymptomatisch; dann kann sowohl beim Mann als auch bei der Frau Sterilität die Spätfolge sein.
Die Diagnostik kann mikroskopisch, kulturell, mit monoklonalen Antikörpern oder durch den direkten Nachweis der Erreger-DNA erfolgen. Für einen sehr sensitiven und gleichzeitig spezifischen Nachweis ist der DNA-Test auf Basis der Polymerase-Kettenreaktion (PCR) die Technologie der Wahl. Mischinfektionen zwischen Chlamydien und Neisserien sind häufig; beide Erreger können mittels PCR gleichzeitig in einem einzigen Test nachgewiesen werden. Dies vermeidet die erneute Probennahme und reduziert den Laboraufwand.
Die neuen cobas® 4800 Teste von Roche Diagnostic zum Nachweis von *Chlamydia trachomatis* und *Neisseria gonorrhoeae* erfüllen alle Kriterien, die an eine innovative PCR heute gestellt werden: Sensitivität, Spezifität und Sicherheit.
Ein verlässliches Testergebnis gewährleisten hochkonservierte Zielregionen, Dual-Target-Detektion, verschiedene Kontrollreagenzien und die automatisierte Testdurchführung. Sie beginnt mit der Probenvorbereitung aus Primärröhrchen und endet mit der Übermittlung des Resultates an das Laborinformationssystems.