Poster Abstracts

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Antimicrobial compunds and therapies

[P001] ISOLATION AND CHARACTERIZATION OF NOVEL CLOSTRIDIUM PERFRINGENS BACTERIOPHAGE SUSFORTUNA

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The emergence of antibiotic-resistant bacterial pathogens has increased in recent years, which have created a renewed attention towards phage therapy. *Clostridium perfringens* is one of the most common causes of food-borne illness in humans. Furthermore, *C. perfringens* is commonly present in the intestine of pigs, where it is associated with chronic or acute enteritis in piglets. Here, the disease varies greatly in morbidity, from bloody diarrhea to sudden death. Several studies have demonstrated antibiotic resistance among fecal isolates of *C. perfringens* from piglets, emphasizing the importance of new therapeutic agents towards this pathogen.
Here we describe the isolation and characterization of a novel bacteriophage infectious to *C. perfringens* from filtrated pig fecal samples, representing a new genus of bacteriophages. The *C. perfringens* bacteriophage Susfortuna has a double-stranded DNA genome of 19046 bp with a G+C% content of 29.2%. The genome contains 28 predicted open reading frames (ORFs). However, due to very limited similarity to known sequences present in databases, 16/28 of the ORFs could not be assigned with a function. Furthermore, transmission electron microscopy revealed that Susfortuna belong to the *Podoviridae* family, and based on the small genome and capsid size, it likely belongs to the subfamily *Picovirinae*.

[P002] POTENTIAL OF A BACTERIOPHAGE COCKTAIL TO TREAT RAINBOW TROUT FRY SYNDROME (RTFS): COMPARISON OF DELIVERY METHODS

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Potential of a bacteriophage cocktail to treat Rainbow Trout Fry Syndrome (RTFS): comparison of delivery methods

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Bacteriophages, natural enemies of bacteria, represent a potential alternative to control the spread of *Flavobacterium psychrophilum*, a worldwide-known pathogen in salmonid aquaculture[1,2]. In our preliminary experiments, the administration of phages showed not to have any negative impact on fish welfare. However, a further challenge was to increase the concentration of phages on the feed, aiming to enhance their spread over the intestinal barrier to the inner organs through the circulatory system.

In this study, we investigated the efficiency of a bacteriophage–based prophylactic treatment of rainbow trout (*Oncorhynchus mykiss*). A bacteriophage cocktail was orally administered through the feed. Rainbow trout fry (1-2 g) were fed with phage-immobilized and phage-sprayed feed for 15 days before the exposure with *F. psychrophilum*. Controls fed with conventional feed as well as controls not infected with the bacterium were included in the study. The effects of the prophylactic treatment on fish survival, growth and welfare were quantified and samples from several fish organs were taken over time in order to assess the spread and density of phages.

Results will be presented and future perspectives outlined.

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[P003] ANTIMICROBIAL ACTIVITY ANALYSIS OF SILVER NANOPARTICLES FROM GINKO BILOBA

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The synthesis of metallic nanoparticles using plant extracts is an emerging field of scientific and global interest wherein the target is the reinforcement of synthesis of safer nanotechnology based products. The therapeutic effects of Ginko biloba can be increased efficiently with the help of nanotechnology. Ginko biloba is one of the most commonly herbal supplements used in worldwide. The dry extracts of Ginko biloba leaves widely used in treatment of Alzheimer's disease, cardiovascular disease, premenstrual syndrome, liver fibrosis, tinnitus etc. This plant contains various numbers of biologically active compounds which provide defence mechanism against insects, bacteria and fungi. In the present study, analysis of plant extract of Ginko biloba has been done for different photochemical constituents. After that silver nanoparticles (AgNPs) were synthesized from leaf extracts of Ginko biloba. The effect of quantity of leaves, concentration of Ag nitrate ($AgNO_3$), temperature and pH were studied to optimize the silver nanoparticles (AgNPs) synthesis. Furthermore, antimicrobial activity of plant mediated AgNPs was performed through inhibition zone method.

Keywords: *Ginko biloba, Silver nanoparticles (AgNPs), Plant extracts, Antimicrobial activity*

[P004] ANTIBACTERIAL CUE FROM ECOLOGICAL NEIGHBOR INCREASES HOLOMYCIN PRODUCTION IN PHOTOBACTERIUM GALATHEAE

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The antibiotic crisis has reinvigorated the search for new antibiotic compounds. Secondary metabolites produced by microorganisms have been the main source for therapeutic molecules. Recently, mining of microbial genomes has revealed an untapped potential for the discovery of new antibiotics from microorganisms. However, the lack of knowledge about the ecological function of antibiotics hampers drug discovery. This study aims to answer ecology relevant questions to elicit production of potential new compounds in marine bacteria.
Using a genetic reporter system in *Burkholderia thailandensis*, an initial screen identified andrimid as elicitor of an otherwise silent biosynthetic gene cluster (BGC). Andrimid is produced by the marine bacterium *Vibrio coralliilyticus* and has potent antibacterial properties. Andrimid was used in sub-MICs as potential inducer of the secondary metabolome of *Photobacterium galatheae*, an ecological neighbour of *V. coralliilyticus* and producer of the antibiotic holomycin. HPLC-DAD-HRMS analysis of andrimid exposed cultures showed a 2-3 fold increase in holomycin concentration compared to control cultures, a result that was backed up by RT-qPCR measurements, which also showed upregulation of other BGCs. Furthermore, it was found that holomycin production occurs in the transition from exponential to stationary growth phase, hinting on a stress response via competition sensing.
Stress-induced production of antibacterial compounds makes biological sense and could be a viable strategy for future drug discovery studies.

[P005] THE ROLE OF ELLAGIC TANNINS TO REDUCE ANTIBIOTICS TREATMENTS IN PIG HERDS FROM 300% TO 10%

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In 2015 Danish Crown introduced a standard for a certified production concept for pigs "Raised Without Antibiotics" (RWA). According to EU statistic Danish pig production has one of the lowest consumptions of antibiotics in Europe at about 20 Animal Daily Doses (ADD/pig/year). This will however still allow for all pigs to be treated with antibiotics 3 times of 1-4 days each before the pigs reach slaughter, corresponding to a treatment frequency of 300%.
Among the RWA pig herds that receive ellagic tannins about 10% of pigs receive 1 antibiotic treatment of 1-3 days duration – corresponding to a treatment frequency of 10%. The reduction has been achieved through several changes and interventions but foremost through the introduction of an extract from Sweet Chestnut (Castanea sativa Mill.) high in ellagic tannins and approved as a feed additive. Ellagic tannins have a strong astringent effect on the mucosa of the gastrointestinal tract (GIT) as it targets tight junctions, whereby they exerts an all cause anti-diarrhoeal effect. Ellagic tannin extract has also been documented to reduce the GIT load of ammonia and other pro-inflamatory waste metabolites from the microbial fermentation of amino acids.
The better GIT integrety has allowed a better analysis of other causes for GIT problems at different ages which has been an important basis for an improved vaccination program for sow and piglet immunity. Other changes include new management procedures, phasing out of routine group treatment, a balanced grinding fineness of the feed, and reductions of inclusion rates of copper and iron.
As much research is done with antibiotics at hand it is challenging to find scientific documentation that points forward to further reductions in antibiotics.

[P006] A MODIFIED ISOLATION CHIP FOR IN SITU CULTIVATION AND ISOLATION OF ANTIMICROBIAL DRUG-PRODUCING BACTERIA FROM SOCIAL SPIDER NESTS

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Antimicrobial resistance is an ever-increasing problem for human health, and with only a few novel antimicrobials discovered in recent decades an extraordinary effort is needed to circumvent this crisis. A promising and largely understudied source of antimicrobials is found among symbiotic microbes associated with their invertebrate hosts. Social spiders (*Stegodyphus dumicola*) are highly inbred communal nest spiders with the lowest estimates of population genetic diversity ever recorded in an animal. Low genetic diversity in combination with group-living imposes a potential threat for nest extinction by pathogens. However, this does not seem to be the case for *S. dumicola*, therefore we propose that microbial symbionts residing within the nests facilitate a "first line of defense" against pathogens. Here we use *in situ* cultivation in spider nests to isolate a large diversity of microbes for antimicrobial testing against human pathogens. We modified and miniaturized the previously described isolation chip (iChip) with a water reservoir to fit the arid environment of Namibia and for application inside spider nests. The diversity of iChip-reared isolates was much broader (25 families) and more representative of the nest microbiome compared to isolates retrieved from standard cultivation (16 families). Among the unique isolates retrieved from the iChip were species of *Streptomyces*, *Micromonospora*, *Pseudomonas*, and *Myxococcus,* i.e., genera known to produce antimicrobial compounds. Antimicrobial screening of iChip-reared isolates also resulted in the finding of eleven isolates with bactericidal activity against Gram-negative and Gram-positive human pathogens. Chemical analysis of secondary metabolites and genome mining for biosynthetic gene clusters are currently ongoing

[P007] ISOLATION AND CHARACTERIZATION OF BACTERIOPHAGES TARGETING ESBL-PRODUCING E. COLI IN DANISH PRIMARY PRODUCTION

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Resistance to beta-lactam antibiotics is an increasing problem in human and veterinary medicine. In particular, *Escherichia coli* produces Extended Spectrum Beta-Lactamase (ESBL) resistance by plasmid-encoded genes as well as by up-regulation of the chromosomal *ampC* gene. Unfortunately, ESBL-producing *E. coli* are commonly found in broilers and pigs and meat hereof, posing a risk of spreading to the human reservoir. Bacteriophages, viruses that target and kill bacteria, can be used as alternative antimicrobials thereby preventing further development of antibiotic resistance. Here we isolated bacteriophages targeting ESBL-producing *E. coli*.
Representatives of ESBL-producing *E. coli* strains (198 strains isolated from pigs, broilers and broiler meat) with different ST-types and carriers of different plasmids and beta-lactamase genes were chosen for isolation of the new phages. Using waste water from both a pig farm and a waste water treatment plant and feces from chickens, 85 new phages were isolated. The initial screening showed that all strains were sensitive to at least one phage. While many of the new phages showed a broad host range, across different ST-types of ESBL-producing *E. coli*, a few were more specific to only a few hosts. Based on the genome sequences, the majority of the phages belong to the *Myoviridae* family and have lytic properties with a potential for biocontrol purposes. Further characterization and host range analysis of these phage is ongoing.

[P008] COMBINATION OF CANNABIDIOL AND BACITRACIN AGAINST RESISTANT BACTERIA

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Emergence of antibiotic resistant bacteria has become a world-wide concern resulting in approximately 700.000 deaths annually and is thought to increase to 10 million by 2050 if we do nothing to stop this. Many pharmaceutical companies have chosen to turn their back on developing new antibiotics since the financial risk of this is too high compared to the possible gain. Therefor we need to turn to other directions such as use of helper compounds.
Helper compounds, also known as antibiotic adjuvant and resistance breakers are in general non-antimicrobial compounds normally found to be a former drug used in psychopharmacology. These compounds are able to potentiate an antibiotic towards bacteria that would otherwise be resistant towards the antibiotic. The mechanism for this can be through efflux pump inhibition, inactivation of antibiotic degrading enzyme, biofilm inhibition and so on.
In the research group of Janne Kudsk Klitgaard, we have found that a compound from the cannabis plant, Cannabidiol (CBD), is able to potentiate the effect of the cell wall active antibiotic bacitracin against resistant Gram-positive bacteria (Patent No. 17176612.4–1466) such as Methicillin-Resistant *Staphylococcus aureus* (MRSA), *Listeria monocytogenes*, and *Enterococcus faecalis*. During our search to reveal the mechanism behind the synergy between CBD and Bacitracin we found that the combination is able to cause various changes in *S. aureus* such as cell division complication visualized by several septum formation and initiation of septum as well as membrane irregularities.
Furthermore, we have created CBD resistant strains by growing *S. aureus* in daily increasing concentration of CBD followed by whole-genome sequencing of the strains. The sequence data revealed mutations in wall teichoic acid synthesis as well as mutations in a gene encoding an efflux pump and its regulator.
Our future plan is to create a crème containing CBD and Bacitracin for treatment of skin infections with Gram-positive bacteria such as *Staphylococcus aureus*.

[P009] GENETIC POTENTIAL FOR SECONDARY METABOLITE BIOSYNTHESIS IN THE NEST MICROBIOME OF SOCIAL SPIDERS

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The social spider *Stegodyphus dumicola* shows extremely low species-wide genetic diversity and lives in communal nests with hundreds of individuals. Such high population density combined with a low immune gene diversity should make the spiders vulnerable to pathogens. We, therefore, hypothesized that microbial symbionts provide a defense against pathogens by producing antimicrobial compounds. Many of these microbial secondary metabolites are nonribosomal peptides (NRPs) and polyketides (PKs). The multimodular enzymes responsible for the synthesis of the NRPs and PKs are composed of linear modules, that each comprises three conserved domains. In this project, the genes encoding the adenylation domains (ADs) of nonribosomal peptide synthases (NRPSs) and the ketosynthases (KSs) of polyketide synthases (PKSs) were analyzed by targeted amplification and sequencing of DNA from 13 spider nests of three distinct spider populations collected in Namibia. Up to 700 AD amplicon sequence variants (ASVs) and 200 KS ASVs were identified per nest, and AD and KS compositions were similar in samples from the same population, whilst differing between the populations. Based on the alignment of the ASVs against NCBI's non-redundant protein database, our preliminary results indicate that the majority of the AD and KS ASVs belongs to the class Actinobacteria and the fungal class Dothideomycetes, respectively. These results will be used as an initial screening to select samples for metagenomic sequencing in our search for novel secondary metabolites.

[P010] REMOVAL OF ANTIBIOTICS AND ANTIBIOTIC RESISTANCE-PROMOTING PHARMACEUTICALS IN A COLUMN STUDY SIMULATING MANAGED AQUIFER RECHARGE (MAR)

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Due to challenges of ground- and drinking water deficits in many countries, development of sustainable methods ensuring safe drinking water are urgent. Managed aquifer recharge (MAR) is a technology used to replenish groundwater reservoirs with e.g. treated wastewater. Here, we assessed the fate of organic micropollutants (OMPs), including antibiotics, in a MAR system. Although occurring at very low concentrations (ng-µg/L) presence of OMPs in ground- and drinking water may compromise ground- and drinking water quality.
We assessed removal of OMPs using laboratory columns simulating top layer in a MAR system, evaluating impact of compost amendment into sand, and inoculation with activated sludge. During 16 weeks of operation, the columns were fed with synthetic wastewater containing OMPs (1 µg/L each). The OMPs included antibiotics: sulfamethoxazole (SMX) and sulfadiazine (SDZ), and pharmaceuticals: carbamazepine and paracetamol (acetaminophen), which has been reported to promote antimicrobial resistance. The degradation and sorption of the OMPs was quantified by SPE-LC-MS/MS. The microbial community structure was elucidated by 16S rDNA sequencing and qPCR.
Despite low C and N influx, oxygen was completely depleted within the top few cm of the column. Addition of compost and inoculation both enhanced removal of the OMPs, yet dissipation of the antibiotics varied. SMX showed significant removal only in columns with high amount of compost (reaching 80%, otherwise <25%) under denitrifying conditions. SDZ removal was limited (<30%); a parallel mineralization experiment showed no mineralization of SDZ under aerobic conditions. Among non-antibiotic OMPs, carbamazepine displayed retarded transport (sorption), but no degradation. In contrast, paracetamol was removed in all columns with increasing efficacy with time and clear beneficial effect of both compost and inoculation. Microbial community composition was affected mostly by presence of compost, and to less degree by time, but with marginal effect of depth and, surprisingly, inoculation.
Overall, certain OMPs were degraded in the system, in some cases stimulated by compost and inoculation. However, substantial fractions of the antibiotics and carbamazepine leached and thus might end up in drinking water. The presence of sub-inhibitory concentrations of antibiotics and resistant bacteria originating from wastewater might pose a risk for the spread of antibiotic resistance in MAR systems.

[P011] ISOLATION OF NOVEL PHAGES TARGETING PSEUDOMONAS SYRINGAE

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Strains of Pseudomonas syringae are ubiquitous epiphytic plant pathogens. They can infect a wide range of important agricultural plant species, causing diseases such as bacterial canker of wild cherries and kiwifruit, bacterial speck of tomato and leaf blight in wheat. Biocides, such as copper and antibiotics, are currently in use against P. syringae. However, many of these current methods have a negative environmental impact and can lead to bacterial resistance. Bacteriophages provide a promising alternative for biocontrol of plant-pathogens without adverse effects on the environment. Previously, we at the EMG lab have isolated phages infecting most of the known diversity within the P. syringae species complex. However, five of the tested species were not infected by the initial phage library. To complete the picture of the interactions between the phages and the different strains of P. syringae, we isolated phages against the remaining hosts. Here we present the isolated phages and their genomes characterized together with an assessment of biological qualities, such as host range. As such, we aim to build an extensive phage library and gain improved understanding of the phages targeting P. syringae. This will be essential in selecting relevant virulent phages for potential use as biocontrol agents. Moreover, a phage library targeting most of the P. syringae species complex also allows for a future evaluation of phage-host interactions in a broader ecological sense.

[P012] EXTENDED MIC VALUES: DETERMINING TENTATIVE EPIDEMIOLOGICAL CUT-OFF VALUES FOR ANTIBIOTICS IN BACTERIAL MINK PATHOGENS

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Antibiotic treatment in mink has been empirical and based on recommendations for other animal species. Hence, there is only one antibiotic registered for used in mink and there are no antibiotic treatment guidelines.
The aim of this study was to provide tentative epidemiological cut-off values for clinically relevant antibiotics in bacterial mink pathogens.
Clinical bacterial pathogens were collected from Denmark, Spain, the Netherlands, Iceland, and Finland. The following species were tested: *E. coli* (n=149)*, Staphylococcus delphini* (n=52)*, Streptococcus canis* (n=26), and *Pseudomonas aeruginosa* (n=46). The bacterial pathogens were susceptibility tested by using a semiautomatic broth microdilution system (SensiTitre, ThermoFischer), with custom made panels providing extended two-fold dilution rows with antibiotics typically used in mink.
For some drug/bug combinations, the epidemiological cut-off values suggest that earlier applied breakpoints could be reconsidered, or at least this new information could be taken into account when considering future treatment regimens.
This study serves as essential background information in facilitating optimal treatment along with prudent use of antibiotics in mink industry. Thus, the epidemiological cut-off values will serve as pharmacodynamics information in an antibiotic treatment guideline for mink.

Microbial - omics

[P013] SMOKE ON THE WATER: DRAFTING THE GENOME OF THE PIGMENTED ALGEA MESOTAENIUM BERGGRENII THAT DARKENS THE GREENLAND ICE SHEET

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Glacier surfaces are habitat to a plethora of prokaryotic and eukaryotic microorganisms. Among the latter, microalgae dominate the Greenland ice sheet and cryoconite holes, especially during the warm months when the simultaneous presence of liquid water and sunlight allows photosynthetic organisms to thrive. The growths of these organisms darkens the ice sheet's surface: the deep purple intracellular pigments absorb more sunlight and lower the albedo, thus raising the temperature of their microenvironment, which subsequently leads to increased melting of the ice. The purple spots are conglomerates of microalgae (primarily *Mesotaenium berggrenii*) that produce a dark purple pigment, purpurogallin, which absorbs UV and excessive VIS radiation. The pathway behind production of this pigment in *Mesotaenium* remains unknown primarily due to lack of genome information. Thus, we aim to draft the first genome of this species. Cultures of *M. berggenii* were grown under lab conditions (where they lost their orginal pigmentationwith purpurogallin) and DNA was extracted. Taxonomical identification of the algae was confirmed using microscopy and Sanger sequencing of the ITS region using *Mesotaenium*-specific primers. High-throughput sequencing on the Illumina NextSeq 500 platform, using the 2x150bp pair end chemistry, yielded $72*10^6$ reads, which were assembled using MEGAHIT. The assembly resulted in $35*10^3$ contigs, which are still analyzed and annotated. In order to produce a useful draft genome, which is estimated to be around 140 Mb long, Nanopore sequencing will also be employed. The resulting genome will be of great value to study the regulation of purpurogallin production under different environmental conditions and linking it the melting of the ice. Having the first draft genome of *Mesotaenium* will also answer lingering questions regarding the evolution of these algae and will open the possibility of comparing populations from different glaciers around the globe.

[P014] UNIVERSAL DERMAL MICROBIOME IN HUMAN SKIN

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Introduction: Human skin microbiota protects against pathogens, helps maintain healthy skin and is thought to act as an individual´s unique 'microbial fingerprint'. Current understanding of the cutaneous microbiota is based on sampling of the outermost layers of the epidermis. However, the remaining skin layers have not yet been fully characterized.
Hypothesis and aims: Physiological conditions vary drastically between the cutaneous compartments, and we hypothesize that this gives rise to unique communities. The aim was to examine differences in the microbiota within skin compartments in two body locations belonging to the same skin habitat.
Methodology: This study investigates the dermal and epidermal microbiota in compartment-separated skin biopsies from hip- and knee of 44 healthy individuals. Metabarcoding of the V3-V4 16S region was performed on the extracted bacterial DNA and a 250 base pair paired-end high throughput sequencing was performed on the Illumina MiSeq platform.
Results: We demonstrate that the dermal microbiota is surprisingly similar among individuals and contains a specific subset of the epidermal microbiota. Variability in bacterial community composition decreased significantly from the epidermal to the dermal compartment, but was similar among the anatomic locations (hip and knee). Epidermal composition, as consistent with other published studies, was strongly affected by environmental factors, while the dermal community was unaffected by such external stresses but showed potential functional differences relative to the epidermal microbiota.
Conclusion: Our results indicate a well-conserved, functionally distinct dermal community; challenging the hypothesis, that human skin microbiota is highly individual. Future studies in cutaneous disorders and chronic infections may advantageously remove this environmental variation by focusing on the dermal microbiota.

[P015] POST TRANSCRIPTONAL CLEAVAGE OF CELL-CYCLE RGEGULATOR CTRA BY TOXIN-ANTITOXIN SYSTEM HIGBA IN CAULOBACTER CRECENTUS

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Bacterial toxin-antitoxin (TA) systems are chromosomally or plasmid encoded gene pairs found in free-living bacteria that often aid in survival during environmental and chemical stress [1]. There are many TA systems found to date, but currently 7 families or types are well known [2] Type II TA operons encode both a small antitoxin and toxin protein that under normal conditions form a tight, nontoxic complex with their cognate antitoxin [3]. Many toxins of this family are mRNA cleaving enzymes (particularly those of the RelE and HigB classes), while their antitoxins are both direct inhibitors of toxin activity through protein-protein interactions with the cognate toxin, and transcription factors that repress their own promoter [4]. HigBA is an exception to the other TA systems of the superfamily relBE, because its gene order is reversed (toxin before antitoxin, rather than the other way around). In Caulobacter crescentus higBA is a DNA – damage inducible TA system where the toxin HigB is tightly regulated by SOS regulator LexA along with its antitoxin HigA. *Caulobacter crescentus* undergoes asymmetric cell division giving rise to daughter cells with different morphologies and replicative potentials [5]. It was found out that this replicative asymmetry in Caulobacter daughter cells is predominantly governed by CtrA. CtrA is a transcription factor that regulates DNA replication by binding to the origin of replication and silencing it, as well as regulating (positively and negatively) transcription of a large number of other genes. CtrA is active when phosphorylated and is abundant in swarmer cells, keeping these cells in G1 phase, whereas it is degraded and dephosphorylated in stalked cells to permit onset of chromosome replication and cell division. It was also found in previous work that HigB cleaves the ctrA mRNA and thus could potentially influence the cell cycle during DNA damage conditions.

From previous work by Clare Kirkpatrick et.al [6] we know that activation of higB promotes growth through specific cleavage of one of the toxin targets (i.e. a toxic efflux pump acrB2). It was also found that when higB was deleted from ΔlexA (which has the SOS response constitutively induced, including other TA systems) it leads to improvement in the survival against DNA damage when tested on different antibiotics.

It was not unexpected that HigBA should be regulated by LexA because this has been observed for other TA systems, both in *Caulobacter crescentus* [4] and also in E. coli [7]. However, this was the first discovered example of a TA system where regulation by another transcription factor was more important than autoregulation by the antitoxin, enough to permit loss of the antitoxin without this being lethal. In *Pseudomonas aeruginosa* it has also been shown that a ΔhigA antitoxin mutant was viable and had HigB-mediated effects on motility and biofilm formation [8] [9], but the reason for this was not explored further in this study. The *Caulobacter crescentus* HigBA system is therefore the best choice of model to study the impact of an exclusively DNA damage-induced TA system on the SOS response and (through its apparent effect on ctrA) on the bacterial cell cycle.

[P016] INVESTIGATING FUNGAL ADAPTATIONS TO EXTREME ENVIRONMENTS USING HERBARIUM SPECIMENS

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The Basidiomycete fungal genus *Podaxis* lives in extreme (and extremely different) environments. Two basal species are found in Sub-Saharan Africa while another is found in North-Eastern Australia. These three species live exclusively in the chemically-defended mounds of grass-harvesting termites (Nausutitermitinae), where no other macrofungi or plants have been reported. More derived species, by contrast, can be exclusively free-living in arid environments, with three separate species found in the Namib (Southern Africa), Sonoran (North America) and Simpson (Australia) deserts. The presence of several species exhibiting two very different lifestyles within one globally-distributed genus makes *Podaxis* a promising model for investigating adaptations to extreme environments. Investigations which are aided by the successful germination of spores from centuries-old herbarium specimens, including two type specimens described by Linnaeus. We've generated whole genome sequences for one specimen per *Podaxis* species to identify shared genomic elements associated with desert and termite-living life histories, respectively. We then compared responses to stressors for *Podaxis* strains with desert- and termite-living lifestyles using *in vitro* growth assays on media varying in pH and H_2O_2 concentrations. Metabolites extracted from these cultures were analysed using LCMS. This allowed us to compare *Podaxis* species' abilities to survive extreme conditions and the metabolites putatively produced under these conditions. By elucidating functional ecological links between genomes, phenotypes and life history strategies, our results provide valuable insights into the basis for adaptations to extreme environments.

[P017] PRENATAL DIETARY SUPPLEMENTS INFLUENCE THE INFANT AIRWAY MICROBIOTA IN A RANDOMIZED FACTORIAL CLINICAL TRIAL

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Maternal dietary interventions during pregnancy with fish oil and high dose vitamin D have been shown to reduce the incidence of asthma and wheeze in offspring, potentially through microbial effects in pregnancy or early childhood. Here we analyze the bacterial compositions in longitudinal samples from 695 pregnant women and their children according to intervention group in a nested, factorial, double-blind, placebo-controlled, randomized trial of n-3 long-chain fatty acids (LCPUFA) and vitamin D supplementation. The dietary interventions affected the infant airways, but not the infant fecal or maternal vaginal microbiota. Changes in overall beta diversity were observed, which in turn associated with a change in immune mediator profile. In addition, the relative abundance of specific bacterial genera and species, as well as microbial maturation, were altered. Furthermore, mediation analysis revealed the changed airway microbiota to be a minor and non-significant mediator of the dietary interventions clinical effect on risk of asthma. Our results demonstrate the potential of prenatal dietary supplements as manipulators of the early airway bacterial colonization.

[P018] ASSESSING VERTICAL TRANSMISSION AND CONFLICT AMONGST GUT BACTERIAL SYMBIONTS IN TERMITES

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Vertical transmission aligns the reproductive interests of hosts and symbionts; this is intensified if vertical transmission is obligate. When both parents host similar symbionts, uniparental vertical transmission prevents the mixing of symbiont lineages that may compete at a potential cost to host offspring. New nests of higher termites are founded by a pair of unrelated queens and kings, both of which carry complex communities of microbial gut symbionts from their natal nests. While it is currently believed that a portion of these gut symbionts are vertically transmitted to the first worker cohort, our knowledge on this is sparse and no work has addressed the potential conflict over symbiont mixing if uniparental transmission is not the rule. To address this, we use metagenomic shotgun sequencing on four parent-offspring colony trios of the fungus-growing termite *Macrotermes natalensis*, sampled over three years during and after colony foundation, to elucidate patterns of vertical transmission and microbial community persistence within colonies. Using population genetic methods, we determine vertical transmission, follow strains through colony development, and assess microbial heritability at the strain and community levels. We also test for microbial traits associated with vertical transmission and the potential for host control over mixing through uniparental transmission.

[P019] FACETS OF DIAZOTROPHY IN THE OMZ OFF PERU REVISITED: WHAT WE COULD NOT SEE FROM A SINGLE MARKER GENE APPROACH

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Biological dinitrogen (N_2) fixation is the pathway making the large pool of atmospheric N_2 available to marine life. Besides direct rate measurements, a common approach to explore the potential for N_2 fixation in the ocean is mining based on molecular genetic methods targeting the key functional gene *nifH*. As sequencing and single-cell techniques improved, our knowledge on the diversity of marine N_2 fixers grew exponentially. One aspect of N_2 fixation in the ocean is commonly left aside. This is the existence of two alternative nitrogenases, the Anf and the Vnf. We explored Nif, Vnf and Anf to determine the ecological and evolutionary history of those. We screened a set of six metagenomes and -transcriptomes from a sulfidic water patch from the oxygen minimum zone off Peru for genes involved in N2 fixation. We found genes related to all three nitrogenases and an increased diversity as compared to our previous nifH- based study from the same waters. Yet, we could not confirm the expression of the alternative nitrogenases from our transcriptomes. We suggest that alternative nitrogenases may not be used under conditions present in those waters, however, depending on trace metal limitation in the future they may become active.

[P020] PARALLEL EVOLUTIONARY PATHS TO PRODUCE MORE THAN ONE PSEUDOMONAS AERUGINOSA BIOFILM PHENOTYPE

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Studying parallel evolution of similar traits in independent within-species lineages provides an opportunity to address evolutionary predictability of molecular changes underlying adaptation. In this study, we monitored biofilm forming capabilities, motility, and virulence phenotypes of a plethora of phylogenetically diverse clinical isolates of the opportunistic pathogen *Pseudomonas aeruginosa.* We also recorded biofilm-specific and planktonic transcriptional responses. We found that *P. aeruginosa* isolates could be stratified based on the production of distinct organismal traits. Three major biofilm phenotypes, which shared motility and virulence phenotypes, were produced repeatedly in several isolates, indicating that the phenotypes evolved via parallel or convergent evolution. Of note, while we found only a restricted general response to the biofilm environment, the individual groups of biofilm phenotypes reproduced biofilm transcriptional profiles that included the expression of well-known biofilm features, such as surface adhesive structures and extracellular matrix components. Our results indicate that there is more than one way to make a biofilm and that genetic adaptations can modulate multiple pathways for biofilm development. Uncovering core regulatory pathways that drive biofilm-associated growth and tolerance towards environmental stressors promises to give clues to host and environmental interactions and could provide useful targets for new clinical interventions.

[P021] METAGENOMICS PROFILING UNCOVERS DIVERSITY, ECOLOGICAL SUCCESS AND HABITAT PREFERENCE OF COMAMMOX NITROSPIRA

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The discovery of microorganisms capable of complete ammonia to nitrate oxidation (comammox) prompted a paradigm shift in our understanding of nitrification, an essential process in N cycling, hitherto considered to require both ammonia oxidizing and nitrite oxidizing microorganisms. This intriguing metabolism is unique to the genus *Nitrospira*, a diverse taxon previously known to only contain canonical nitrite oxidizers. Yet, a global insight into the abundance, niche preference, and genomic diversity of *Nitrospira* is missing. Here, we established the largest *Nitrospira* genome database to date, revealing 68 putative species, most without cultivated representatives. We performed read recruitment analysis with metagenomes from various environments across the globe to investigate how environment and spatial distance affect *Nitrospira* distribution, and to elucidate to what degree *Nitrospira* phylogeny associates with ecology. Our analysis suggests that environmental filtering structures species distribution, without large-scale biogeographical signal. Comammox *Nitrospira* ecological success is evident as they outnumber and are more diverse than canonical *Nitrospira* in all environments but wastewater treatment plants. Of the studied habitats, comammox *Nitrospira* is especially diverse and abundant in drinking water treatment systems.
In addition, we assessed which feature better explained comammox *Nitrospira* habitat preference by comparing, for pairs of species, similarity in their environmental distribution with overall genomic similarity, as well as, sequence similarity of the key nitrification enzymes ammonia monooxygenase (AMO), hydroxylamine dehydrogenase (HAO) and nitrite oxidoreductase (NXR). The similarity of AMO and NXR sequences poorly predict niche preference of comammox *Nitrospira*. Instead, HAO sequence similarity strongly correlates with comammox *Nitrospira* habitat preference. These results suggest a complex comammox *Nitrospira* eco-evolutionary history with subclades achieving rapid niche divergence putatively related with horizontal transfer of genes, including that encoding the hydroxylamine oxidoreductase.

[P022] THE INFLUENCE OF THE ENVIRONMENT ON THE POTENTIAL FOR NATURAL PRODUCT BIOSYNTHESIS

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Frequent use of antibiotics has led to a rising number of antibiotic resistant bacteria.[1] In contrast, the number of novel compounds has stagnated since the 1960's, mainly because of high rediscovery rates.[2] The majority of bioactive compounds has been isolated from environmental microbes. Many of these natural products (NPs) are produced by nonribosomal peptide synthetases (NRPS) and polyketide synthases (PKS).[3] However, efforts in screening more microorganisms for NPs have been hampered by our inability to cultivate a large fraction of the microbes found in nature.[4] The purpose of the project is to overcome these limitations by using cultivation-independent techniques. We will aim to establish a high-resolution map identifying the genetic potential for NP biosynthesis in different natural microbiomes. We will focus on environments with small-scale gradients such as pH, salinity and redox potential, in order to make general predictions about environmental parameters influencing the NP biosynthetic potential. In the process, we will link general features of natural microbial communities to their biosynthetic potential in order to identify universal interrelations between taxonomic and biosynthetic diversity. For this purpose, we will use amplicon sequencing of the 16S rRNA gene and of conserved regions in NRPS and PKS genes. This will facilitate focused efforts targeting chemical novelty. Based on these results, other cultivation-independent techniques like single cell sequencing may be used to further explore the biosynthetic potential of promising environments.

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[P023] A NOVEL AND DIRECT MOBILOME APPROACH IMPROVES THE DETECTION OF LARGER-SIZED CIRCULAR ELEMENTS ACROSS KINGDOMS

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Mobile genetic elements (MGEs) include DNA elements such as plasmids, transposons and bacteriophages (phages), as well as Insertion Sequences (IS-elements). MGEs represent a vast communal gene pool, allowing for genome plasticity and aiding microbes in adapting to abrupt changes in their environment. Given that they can add new traits and generate multidrug resistant or highly virulent pathogens, these mobile DNA elements represent an urgent human health risk. Building sequencing libraries encompassing circular MGEs, referred to as mobilomes, will allow for expansion of our current understanding of the mechanisms behind the mobility, prevalence and content of these elements. The current knowledge gap in mobilomics is massive, partly because of methodological biases arising from multiple displacement amplification (MDA). Most MGEs are extracted from sheared chromosomal DNA by their circular topology with an exonuclease, which removes all linear MGEs too.
We here show that MDA is detrimental for detection of larger-sized plasmids, in cases where smaller plasmids are present. We accomplished this by comparing the abundances of reads mapping to natural and spiked plasmids from a mock community in a wastewater sample, both with and without MDA. Clear evidence showed that by omitting MDA in sample preparation, the proportion of larger-sized circular elements in natural samples increased, making it possible to produce samples consisting almost exclusively of larger, more complete, circular MGEs from complex samples without MDA. The presented direct mobilome approach can significantly improve the quality of mobilomics data, together with advances in long read sequencing and bioinformatics tools.

[P024] SPECIES DETERMINATION OF NONTUBERCULOUS MYCOBACTERIA (NTM) BASED ON CORE SEQUENCES OF THREE GENES IN A MULTIPLEX PCR

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An increasing incidence of infections caused by NTM has been reported over the last decades. This development highlights the challenge of species identification in NTM, which is necessary to inform clinicians for correct treatment of these infections.
Currently, the sequence of the 16S rRNA gene is used as the gold standard for identification of bacterial species, including NTM. However, different species belonging to the genus *Mycobacterium* have identical 16S rRNA sequences.
Therefore, we have investigated the possibility of using additional bacterial housekeeping genes to allow distinction of all present mycobacteria in isolates originating from patients in Denmark. We sequenced the 16S rRNA, ITS, *hsp65*, *rnpB*, *rpoB* and *secA1* genes of 23 reference strains and 46 clinical specimens.
By comparing the results of various combinations with 3, 4, 5 or 6 genes we are confident that using the genes 16S rRNA, *hsp65* and *secA1* is the optimal combination to determine the correct species for the most common NTM in Denmark, and we confirmed generalization of these results using whole genome sequence data obtained from public repositories. In order to simplify the laboratory work, a multiplex PCR for the core sequences of the three genes has been designed.

[P025] THE GUT, ORAL, AND NASAL MICROBIOTA IN PEDIATRIC ALLOGENEIC HSCT AND PREDICTION OF ACUTE GVHD

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During the first month after allogeneic hematopoietic stem cell transplantation (allo-HSCT), patients' gut microbiota changes drastically. However, more information on long-term reconstitution of the microbiota is needed. Here, we have tracked gut, oral, and nasal microbial changes over a period of one year in 29 pediatric allo-HSCT patients. Sparse linear discriminant analyses showed that amplicon sequence variants (ASVs) affiliated with intestinal *Blautia* spp., and oral *Actinomycetacea* decreased after HSCT and returned to pre-transplant levels after three months, whereas intestinal *Enterococcus* spp. and *Lactobacillus* spp. exhibited the opposite pattern. Allo-HSCT is challenged by side effects, such as acute graft-versus-host disease (aGvHD). Here, we predicted high aGvHD severity from high pre-transplant abundances of intestinal *Parabacteroides distasonis,* and oral and nasal *Actinomyces* spp. in machine learning models. Our findings indicate that the microbiota reconstitutes 1-3 months after allo-HSCT. Early microbial predictive markers might in the future inform preventive management of aGvHD.

[P026] PHYLOGENY AND SECONDARY METABOLITE GENE CLUSTERS FROM THE TERMITE FUNGAL CROP TERMITOMYCES.

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The use of compounds produced by hosts or symbionts for defence against antagonistic organisms has been identified in many organisms, including in fungus-farming termites in the subfamily Macrotermitinae. Obligate mutualistic *Termitomyces* fungi play an essential role for the termites for plant biomass decomposition and food, but the fungus has recently also received attention as a possible contributor to symbiosis defence through the production of secondary metabolites. These have enormously diverse functions in both prokaryotes and eukaryotes and antimicrobial secondary metabolites produced by *Termitomyces* would serve as an effective and important strategy in keeping termite fungus gardens disease free. Here we used a bioinformatics approach to analyse genomes of *Termitomyces* species associated with five genera of African Macrotermitinae colonies. The presence and exploration of putative secondary metabolites was performed by analysing the diversity and sequences of biosynthetic gene clusters. Through results obtained from fungiSMASH we tested variation in *Termitomyces* species and associations to termite host genera based on phylogenetic analysis of the antimicrobial gene cluster sequences. We evaluated the domain organisation of the gene clusters present among the fungal isolates and the challenges in obtaining complete fungi-derived gene clusters. Overall, this will provide insight into the evolution of fungus-growing termites defence strategies, together with the identification of putative defence compounds using a genome-guided approach.

[P027] ACTINOBACTERIA: GENOME SEQUENCING AND ASSEMBLY

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Actinobacteria have some of the largest and most repetitive genomes of any prokaryotes. They can have a size of 15M bases, a GC content of 75%, large linear plasmids, individual genes of over 30 kb inside 200kb gene clusters, and linear genomes with megabase size inverted repeat ends. Because the longest repeats exist in scientifically and commercially interesting biosynthetic clusters, it is crucial to completely assemble the genome rather than analyze genome fragments. The single molecule long read technologies PacBio and Oxford Nanopore have largely changed the genome assembly landscape by allowing researchers to generate contiguous genome assemblies, at the cost of an increase in the number of frameshift errors, which we show leads to wrong gene calling and wrong annotations in downstream applications like antiSMASH. We have sequenced and assembled over 50 actinobacteria strains and investigate the genome topology and core gene content of them, in addition to the basic measures such as GC%, total length, number of contigs, and closest database relatives. Notably, hybrid assemblies perform better than single technology assemblies, and short read-first assemblies are more accurate but generally less contiguous than polished long read-first assemblies. In the isolates, we have identified over 1,500 biosynthetic clusters, many of them encoding unknown compounds with potential antimicrobial or cytotoxic effects.

[P028] INVESTIGATION OF SMALL, CIRCULAR DNA ELEMENTS REVEAL TRANSPOSITION FREQUENCY AND MECHANISMS OF MOBILE GENETIC ELEMENTS AND THEIR POTENTIAL INVOLVEMENT IN RESISTANCE DEVELOPMENT

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The newly developed direct mobilome sequencing of small circular DNA elements, that appear separate from chromosomes and plasmids, was applied to 50 clinically relevant microbial strains. The complete genomes of the 50 strains were sequenced using the Illumina and Nanopore platforms to provide fully closed genomes. Direct mobilome sequencing, where total genomic DNA is digested using exonuclease to remove linearized chromosomes and large plasmids, was used to confirm plasmid content in the sequenced strains. Furthermore, mobilome sequencing revealed many regions across the 50 genomes that have circular intermediate states disparate from their genomic location. Most small circular DNA elements are mediated by transposase activity and some elements carry accessory genes, including antimicrobial resistance genes. Direct mobilome sequencing holds great potential in investigating the transposition mechanism and frequency of insertion sequence elements and transposons and provides a promising tool to study genome evolution.

[P029] GLOBAL GENOME-CENTRIC METATRANSCRIPTOMICS UNRAVELS FOOD WEBS IN COMPLEX MICROBIAL COMMUNITIES

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Advancements in high-throughput sequencing have led to an increasing amount of time series studies, investigating the temporal variation of microbial communities across multiple environments. However, the synthesis of this vast amount of data into quantitative functional aspects that can be rigidly tested in translational studies are lacking behind. There is a need to develop efficient holistic methods that can quantify and measure activity not related to growth in microbial communities. Here, we propose a genome-centric metatranscriptomics approach, utilizing recent advancements in sequencing technology and bioinformatic algorithms to characterize and quantify the temporal activity of a complex microbial system from sequencing data alone. We focused on a continuous stirred tank reactors (CSTRs), inoculated with biomass from a waste water treatment plant. After a starvation phase the bioreactors were stimulated with acetate and butyrate and monitored. Metagenomes and –transcriptomes were obtained from half an hour before stimulation and in regular intervals up to eight hours after stimulation. The multi-metagenome concept were used to assemble near-complete genomes and characterize the microbial community composition. The associated transcriptomic data were used to quantify activity response of individual community members. Preliminary results shows it is possible to obtain whole-genome activity measures from transcriptomics data and the up- or downregulation of certain organisms can be correlated with substrate stimulation. Ongoing work will include a higher time resolution to allow quantitative and functional analysis of intra- and inter organism variability correlated to associated biochemical data.

Microbial ecology

[P030] MICROBIAL DIVERSITY IN SUBTROPICAL BEACHROCK

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Beachrock is an environment consisting of sedimentary rock formations along a shoreline. The beachrock of subtropical Heron Island, Australia, is constantly prone to varying and extreme conditions, including temperatures up to 60 °C and cycles of complete dryness and moistness as the tide rises and falls. The formation process of beachrock is unknown but likely involves photosynthetic microbes because photosynthetic activity appears to promote the inorganic sedimentation process. Early studies suggested that cyanobacteria are abundant but a detailed analysis of the communities are yet to be completed. Our study is directed towards revealing the underlying mechanisms and composition of microbial organisms and communities involved in beachrock formation using molecular, biochemical, and physiological techniques. Alpha and beta diversities have been calculated for samples of the different beachrock environments, revealing an overview of the microbial composition which is further illustrated by Krona plots produced by using the 16S bacterial rRNA sequences and specific taxonomic OTU data. Pigment analyses suggested that cyanobacteria capable of far-red-light photosynthesis using chlorophyll *f* are abundant and that eukaryotic phototrophs (microalgae) are scarce. Overall, the microbial composition was dominated by cyanobacteria and nonphotosynthetic chloroflexi that seem to constitute a low-diversity and highly specialized community.

[P031] WHAT MAKES A "CULT-WINE"? PRODUCTION-CHAIN MICROBIAL DIVERGENCES IN VINEYARDS AND WINES FROM RIBERA DEL DUERO

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What makes a "cult-wine"? Production-chain microbial divergences in vineyards and wines from Ribera del Duero
"Cult-wines" are expensive, iconic and should represent at the very best the characteristics of the place where they come from. In our case, the vineyards are located in la Horra, Ribera del Duero, Spain. A place known for the warm and dry climate in summer, where the vines of Tempranillo have been planted and grown for decades, following the traditional "goblet" canopy system, in vineyards subjected to biodynamic management. In this scenario, the same winery produces two different wines through spontaneous fermentation, one of which considered being a masterpiece of oenological production. Given the importance attributable to microbes, in all different stages of wine-production, we will focus on their role in differentiating the finished products. Therefore, we will highlight the microbial differences occurring along the wine production-chain, from vineyards to finished wine, over three consecutive vintages, combining amplicon-based NGS and metabolomics profiling of the fermentation samples. We expect to see whether the microbial communities, living in two nearby vineyards, are different at harvest time, or if the communities, initially similar, diverge during the process, generating a measurable effect during the fermentation. Until now, differences within the microbial communities of soils and musts, during fermentations, are partially revealed.

[P032] RESCUING ROLE OF STAPHYLOXANTHIN IN MIXED MICROBIAL COMMUNITIES

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Microbes seldom exist in isolation and are faced with oxidative stress during infection. Therefore, it is important to understand how a mixed bacterial community copes with reactive oxygen species (ROS) as a whole. The antioxidant staphyloxanthin (SX) is a carotenoid pigment responsible for *Staphylococcus aureus'* characteristic golden color. *S. aureus* strains expressing SX are able to withstand high levels of ROS present both in the environment and those produced by immune cells, leading to the question if SX provides a benefit to other neighboring community members that do not invest in producing SX themselves during co-culture. Here we use the community acquired methicillin-resistant *S. aureus* USA300 that produces SX, an isogenic Δ*crtM* mutant that does not produce SX, and *Staphylococcus epidermidis* to determine the role of SX from a social evolution perspective in mixed-strain/species communities and how its radical scavenging effect modulates spatial location of non-producers under oxidative stress.

[P033] THE POTENTIAL OF SOIL-BORNE BIOCONTROL AGENTS: ELUCIDATING THE FUNCTION, VARIATION AND BIOCONTROL POTENTIAL OF SECONDARY METABOLITES BY BACILLUS SUBTILIS ISOLATES

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Bacillus subtilis is well known for its ability to produce various secondary metabolites and to form biofilm in the rhizosphere, the traits that are important for biocontrol. To inspect the abundance, the biofilm formation ability and the biocontrol properties of *B. subtilis* isolates, a comparative strain analysis of diverse soil ecosystems was performed. The genetically confirmed *B. subtilis* strains were isolated from diverse ecosystems in Thuringia, Germany, while soil of grasslands with a non-agricultural background as well as from close proximity of different mushrooms were targeted in Denmark. Importantly, the abundance of *B. subtilis* depended on the soil ecosystem used for sampling. The isolated *B. subtilis* strains showed robust biofilm development both *in vitro* and on the roots of *Arabidopsis thaliana*. In addition, secondary metabolite production was tested against various phytopathogenic fungi. Both targeted gene knockouts and LC-MS analysis for secondary metabolites of the strains highlighted that anti-fungal properties depend primary on plipastatin and either plipastatin or surfactin, depending on the tested fungus or the tested bacterial soil isolate. Interestingly, some isolates show different production of certain secondary metabolites, even though they were isolated from the same soil sample. These differences effect especially their anti-fungal properties, since their plipastatin production is absent. We propose that *B. subtilis* isolates from soil ecosystems are promising targets to use them as biological control agents against plant pathogens.

The project is connected to the Center for Microbial Secondary Metabolites that is supported by the Danish National Research Foundation (DNRF137).

[P034] BACILLUS SUBTILIS BIOFILM FORMATION AND EVOLUTION ON PLANT ROOTS

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The soil-dwelling bacterium *B. subtilis* is naturally found in soil and known to promote plant growth and protect plants against disease. This bacterium therefore represents a great potential for controlling plant diseases within agriculture, thereby enhancing the yield and reducing the application of pesticides. To harvest this potential, we need a better understanding of the complex interplay between *B. subtilis* and plants. In this project, *B. subtilis* root colonization is studied through experimental evolution of the bacterium on *Arabidopsis thaliana* roots. At the end of the evolution, evolved strains will be isolated and re-sequenced to identify the genetic alterations responsible for the improved properties. This project will thereby allow identification of genes involved in root colonization by *B. subtilis* as well as provide novel knowledge about the adaptation potential of *B. subtilis* to plants.

[P035] STUDYING B. SUBTILIS IN ROOT COLONIZATION OF DIFFERENT PLANT SPECIES

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The soil bacterium *B. subtilis* is known to supress pathogens as well as promote plant growth. It therefore has a huge potential as natural fertilizer, replacing chemicals traditionally used in agriculture, which are known to cause environmental threats. However, in order to fully exploit this potential of *B. subtilis* we need a better understanding of the interactions between *B. subtilis* and plants. In this study, *B. subtilis* will be examined for root colonization through experimental evolution of the bacterium on different plant species. The evolved strains will be studied for improved root colonization and re-colonization, as well as be re-sequenced to identify genetic alterations. This will allow identification of important genes involved in root colonization of and reveal how *B. subtilis* adapt to different plant species. Furthermore, the importance of chemotaxis and motility in root (re-)colonization will be examined by testing corresponding *B. subtilis* mutants on roots using fluorescence microscopy.

[P036] INCREASING THE PHYLOGENETIC RESOLUTION OF PSEUDOMONAS IN SOIL MICROBIOMES BY RPOD-SPECIFIC PRIMERS.

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Species of *Pseudomonas* coexist in different environments such as the plant rhizosphere, oceans and in animal bodies. Several *Pseudomonas* species have either beneficial (plant protection and bioremediation) or detrimental (pathogenic) effects. To understand and exploit the beneficial species and their mechanisms for microbiome engineering, we need to differentiate *Pseudomonas* species directly in the environment. Therefore, it is crucial to have high-throughput methods with high resolution to couple the population changes to environmental changes. The common way is amplicon sequencing targeting the 16S rRNA gene. However, previous studies showed this gene does not provide a species-level resolution. In our approach, we used the *rpoD* gene as target for amplicon sequencing rather than the 16S rRNA gene along with a custom bioinformatics pipeline. As of now, we show species-level resolution in a high throughput setting. The methodology can distinguish between species in an artificial DNA mixture in equivalent relative amounts. A preliminary investigation of soil showed the methodology could identify *Pseudomonas* species in same relative abundances to cultivation-based methods with *P. jessenii*, and *P. lini* as the most abundant. In comparison, the V3V4-based could not achieve the same resolution and identified the artificial mixture as primarily *P. fluorescens,* and the soil as solely *P. aeruginosa.* Current work focuses on the combination of 16S rRNA (the V3V4 region) and *rpoD* primers on a large set of root-associated soil samples from multiple plants to profile the ecological niches of *Pseudomonas* species.

[P037] PIG PRODUCTION WITHOUT THE USE OF ANTIBIOTICS - IMPACT ON THE PIG RESISTOME AND MICROBIOME

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Antibiotics are widely used in pig farming and the concern of its impact on human health through resistant bacteria has initiated an interest in pigs Raised Without Antibiotics (RWA) as an alternative to conventional farming in Denmark.
This issue of resistance has been addressed by nationwide associations of antibiotic usage and resistance genes, but not on a detailed longitudinal per-pig basis in a natural setting. In this study, we used a case/control setup of 200 pigs followed for 5 months from birth until slaughter sampled at 10 time points. This allows us to answer some fundamental questions: Does the microbiome and antibiotic resistance pattern change in pigs treated with antibiotics compared to untreated pen mates? If so, what is the effect over time?
In this project, we studied the effect of antibiotics usage on the resistance patterns as well as on the composition of the gut microbiome. These RWA pig farms give us a unique opportunity to study pigs in real life farming conditions with treated and untreated pigs having the same environmental and genetic background. We hypothesize that untreated pigs have a more diverse microbiome and fewer resistant bacteria. We are using four methods: 1) 16S rRNA gene sequencing to study the composition of their microbiomes, 2) high-throughput qPCR of 90 resistance genes, 3) a subset are analyzed by metagenomic sequencing, 4) the same subset is subjected to a Hi-C analysis to assign the resistance genes to the host species even if encoded on mobile genetic element (e.g. plasmid).

[P038] A BROAD-HOST RANGE, READILY PROGRAMMABLE CAS9 NUCLEASE DELIVERY VECTOR FOR RAPID AND PREDICTABLE PLASMID-CURING OF NATURAL ISOLATES

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Plasmids are a key player in microbial ecology and evolution, act as vehicles of laterally exchangeable genetic material, and give their hosts access to vast reservoirs of accessory gene functions. Yet, possibilities for studies unveiling direct plasmid-host interaction, the impact of plasmids on their host's phenotype, fitness, or transcriptomics, and the causation of horizontal gene transfer (HGT), remain to be improved. Such studies are, upon today, mainly carried out by the introduction of non-native plasmids into a well characterized host. This may not illuminate the true magnitude and sophistication of a plasmids influence on its natural host, with which co-evolution has likely occurred. We developed a CRISPR-Cas9 based plasmid curing tool, comprising an addition to the molecular toolbox for plasmid-host interrelation studies and investigations into HGT. CRISPR-Cas based plasmid removal from natural isolates avoids prolonged incubation times and stressful growth conditions, thereby keeping the risk for mutational biases minimal and enables the purposeful removal of exclusively the plasmid of interest. By encoding a readily programmable Cas9 nuclease on a mobilizable, broad host-range, suicide vector, we enable the removal of a plasmid of desire with a known, or partially known sequence from a host of choice. Predictable and consistent curing efficiencies from 97.1% at minimum could be obtained for the removal of different natural plasmids, by targeting varying plasmid-encoded protospacer sequences in deviating hosts, ultimately resulting in plasmid-free bacteria. This stresses the relevance of the created tool and indicates promising application possibilities in a wide range of studies.

[P039] THE ROLE OF MOBILE GENETIC ELEMENTS AND BACTERIOPHAGES IN PORCINE ENTEROTOXIGENIC ESCHERICHIA COLI (ETEC) VIRULENCE AND ECOLOGY.

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Enterotoxigenic *Escherichia coli* (ETEC) are the main cause of diarrhea in piglets after weaning. They are characterized and further classified according to the presence of two virulence factors: the fimbriae and the enterotoxins, usually encoded by plasmids or prophages. Despite the importance of mobile genetic elements (MGE) in ETEC virulence, no report explains the role of the chromosomal background in accepting MGE, nor the contribution of MGE in ETEC diversity and sensitivity to their natural predators, the bacteriophages.
To fill this gap, we gathered a collection of 110 ETEC strains isolated from piglets in Europe, affected by post-weaning diarrhea. We characterized the fimbriae and toxin types and investigated their genetic diversity by Pulse Field Genetic Electrophoresis. We hypothesize that a high number of prophages in the ETEC genomes could be responsible for the immunity against other bacteriophages, since it has been much easier to isolate temperate than lytic phages against these ETEC strains. Interestingly, the genome analysis of a lytic T7-like phage revealed a known anti-resistance mechanism against restriction-modification enzymes that could explain its high infectivity against ETEC. The presence of MGE will be explored by plasmid profile analysis and mitomycin induction of prophages.
The collected data will contribute to unveil the complexity of the interactions among ETEC strains, MGE and bacteriophages.

[P040] WASTEWATER BIOFILMS: HOW SOCIAL INTERACTIONS INFLUENCE BIOFILM FORMATION BY WASTEWATER BACTERIA

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Bacteria in Nature live mainly in multispecies biofilms, where they interact and alter the overall microbial community organization and function. In the engineered environment of wastewater treatment plants (WWTPs) bacteria thrive in suspended biofilms, which are fundamental in the biological treatment of our sewage. However, the influence of social interactions in the ability of the highly diverse species, which found each other in WWTP, to form biofilms is underexplored. Our study aims at evaluating the interactions between co-occurring wastewater bacteria in multi-species biofilms. For that, we have produced biofilms from combinations of 1 to 4 isolates from 7 different wastewater species, isolated from two different Danish municipal WWTP and identified by Sanger sequencing of 16S rRNA gene. Biofilm formation was assessed by Crystal Violet assay and we were able to choose the best consortium of 4 different species based on biomass production. The social interactions between each isolate were evaluated in Congo Red plates and by filter mating assay with an IncP1 plasmid. PCR primers were design to detect each strain in the mature biofilm. We can conclude there are differences in the production of biofilm related to the different isolates composing the consortium. This indicates that different niches within the wastewater biofilms might occur, where specific bacteria interact preferentially. Next, the transfer of antibiotic resistance genes by conjugation within this consortium will be study.

[P041] OPTIMIZING HOST SURVIVAL STRATEGY THROUGH RAPID PROPHAGE EVOLUTION

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Next to serving as killers or genetic parasites, phages can act as regulatory switches and modulate survival strategies of their bacterial hosts. For instance, prophage-like element *skin* and large SPβ prophage residing on *Bacillus subtilis* chromosome, excise prior sporulation, unblocking the expression of essential or accessory sporulation genes, respectively. Here we conducted experimental evolution of lysogenic *Bacillus subtilis* strains under sporulation selection regime. We discovered activation of a previously stable SPβ prophage (Martin et al., 2017). The evolved phage variants easily spread in the population, because they kill the ancestral lysogens and transduce the residual subpopulation of survivors. Surprisingly, *de novo* genome sequencing uncovered the diversity of newly evolved hybrid prophages, lytic phages and extrachromosomal phage elements. These assembled from indigenous SPβ and closely related phi3T, which seemed to 'hitchhike' in the ancestor in very low copy number. Bioinformatics analyses revealed that newly evolved phages carried potential regulators of *B.subtilis* sporulation process. In fact, when the ancestor strain was lysogenic for the 'new' phage variant, sporulation and germination timing were shifted, allowing higher number of survivors under sporulation selection regime. Our results suggest that reshuffling within large *Siphoviridae* phages is a common evolutionary scenario in natural populations, with crucial consequences for ecology of the species.

[P042] DIVERSITY, LOCALIZATION, AND MICROENVIRONMENTS OF THE SPECIFIC BACTERIAL SYMBIONTS OF SOCIAL SPIDERS (STEGODYPHUS DUMICOLA)

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Animals live in close association with microbes affecting them in many ways. The social spiders *Stegodyphus dumicola* live in large communal nests inhabited by hundreds of individuals, they spend their entire life in the nest and cooperate in many aspects of life including reproduction, brood care and foraging. Sociality leads to decreased genetic diversity and social spiders are highly inbred with extremely low genetic variation across populations. Hereby social spiders offer a standardized genetic background for studying animal-microbe symbiosis making it possible to separate the host and symbiont contribution to phenotype. The goal of this study was to identify specific symbionts of social spiders, and to determine, where and under which physical-chemical conditions these are present in the spider body.
Stegodyphus dumicola were sampled in Namibia, South-Western Africa. DNA was extracted from whole animals, and the microbiome analyzed by 16S rRNA gene amplicon sequencing. Putative symbionts were localized in 20 µm-thin cryo-sections by fluorescence in situ hybridization, and oxygen and pH was measured in whole animals and in dissected midguts with glass microsensors. The social spider microbiome showed low diversity and was dominated by a few spider-specific lineages related to the genera *Borrelia, Mycoplasma, Diplorickettsia*, and *Chlamydia*. These symbionts were detected in the entire, heavily branching gut system, which fills large parts of the abdomen and also extends into head and legs. Symbionts mostly colonized the gut epithelium and especially the cloaca was heavily populated. The spider body and midgut microenvironment was peripherally (micro-)oxic, but the oxygen concentration decreased to depletion towards the radial gut center, where a 1000 µM-wide anoxic zone was present. Thus, the spiders provide multiple niches for microaerophilic to anaerobe symbionts. The pH was near neutral in the outer gut tissue and decreased to 6.6 in the anoxic radial center, indicating fermentation activity inside the gut, consistent with a fermentative metabolism of the main symbionts.

[P043] UNCOVERING THE HIDDEN DIVERSITY OF ASGARD ARCHAEA

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In recent years, the discovery of the Asgard superphylum has shed new light on the link between prokaryotes and eukaryotes as the Asgard archaea have been demonstrated to be the nearest relatives to the eukaryotes in the tree of life. However, only a small fraction of the existing Asgard archaea diversity are believed to have been uncovered. In this study, we aimed at populating the tree of life with thousands of new full-length archaea 16S ribosomal RNA gene sequences around the Asgard clade. Primarily samples from various sediments of salt- and freshwater environments were collected and coupled with a novel method for generating high quality, full-length 16S ribosomal RNA gene sequences. In total, we were able to retrieve > 110,000 sequences belonging to the domain of archaea. Specifically relating to Asgard archaea, we uncovered more than 200 new species-level OTUs whereas a family-level cut-off revealed 21 novel Asgard families. With the results presented in this study, we have vastly expanded the current understanding of the diversity within the Asgard superphylum (as well as archaeal diversity in general) and potentially discovered a new sub-group of Thorarchaeota.

[P044] CLOACAL SWABS AND ALCOHOL BIRD SPECIMEN ARE GOOD PROXIES FOR COMPOSITIONAL ANALYSES OF GUT MICROBIAL COMMUNITIES OF WILD BIRDS

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Complications related to methods of sample acquisition and storage limit the numbers of microbial studies on wild animals, especially in birds. Non-invasive cloacal swab sampling for gut bacteria has previously been employed, and museum alcohol specimens may also be a promising resource to investigate wild bird microbiomes; however, the question remains, just how reliable these methods are to adequately describe the natural gut microbiota. To address this, we compared Great tit (*Parus major*) gut communities from 1) different digestive tract regions vs. cloacal swabs and 2) gut regions from freshly dissected specimens vs. specimens preserved in alcohol for two weeks or two months, respectively. We found no significant difference in alpha diversity between gut communities from different regions and cloacal swabs, and between fresh and alcohol preserved samples. However, we did find significant differences in community structure of cloacal swab samples compared to gut regions. Despite these quantitative differences, swabs captured the qualitative bacterial diversity of the entire gut better than any single compartment. Finally, we demonstrate that the bacterial community composition in alcohol preserved samples did not differ significantly from freshly dissected samples; however, some less-abundant bacteria were absent from alcohol specimens. To better understand the long-term effect of alcohol preservation on gut microbial communities, work should further investigate specimens stored for longer periods of time. The potential use of alcohol specimens to investigate animal microbiomes provides a promising avenue to utilize the extensive museum alcohol collections.

[P045] EXPERIMENTAL EVOLUTION ON PLANT ROOT SHAPES GENO- AND PHENOTYPIC ADAPTION OF BACILLUS THURINGIENSIS

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Bacillus thuringiensis has been wildly used as a commercialized bio-pesticide due to its entomopathogen properties. However, some strains of *B. thuringiensis* are reported to cause gastro intestinal disease in humans. The different pathogenic properties can be the results of adaptive strategies of *B. thuringiensis* to survive in various natural niches, such as soil and plants. Therefore, it is crucial to explore the relationship between bacterial lifestyle and pathogenic traits.
We devised an experimental evolution setup associating *B. thuringiensis* (Cry-) with the model plant, *Arabidopsis thaliana* to mimic the parallel evolution of the bacterium. After 40 transfers of laboratory evolution on the plant rhizosphere, all 5 bacterial lineages showed better root colonization ability compared with the ancestor. Intriguingly, single isolates from two of the evolved lineages tended to re-colonize a new root more easily compared with the other lineages. While these isolates demonstrated drastically reduced swimming ability, pellicle formation and submerged biofilm formation, they exhibited increased hemolytic activity, highlighting the possibility for increased toxin production. Genome re-sequencing of the plant adapted isolates from these two linages revealed key mutations in the transcription terminator Rho that might be responsible for the observed phenotypic changes.

[P046] IMAGING NEAR-INFRARED RADIATION DRIVEN PHOTOSYNTHESIS OF CHLOROPHYLL F-CONTAINING CYANOBACTERIA IN BEACHROCK BIOFILMS

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The notion that oxygenic photosynthesis is mainly driven by wavelengths between 400 – 700 nm (visible light) with the photopigment chlorophyll (Chl) *a* as major driver has been challenged repeatedly by the finding of cyanobacteria with red-shifted Chls *d* and *f*.[1] Specifically, Chl *f,* the most red-shifted Chl (absorption range between 700 – 760 nm) can be expressed in various types of cyanobacteria under far-red light-rich incubation, which enables the use of near-infrared radiation (NIR) for oxygenic photosynthesis.[1, 2] We previously showed the presence of Chl *f*-containing cyanobacteria below densely populated microbial biofilms on the surface of beachrock, a widespread sedimentary rock formation on (sub-)tropical, intertidal shorelines. Here cyanobacteria with Chl *f* are located in the endolithic space below the surface biofilm.[2] Similar low light, NIR-enriched habitats have been identified in microbial mats, forest canopies and caves. However, direct measurements of NIR-driven oxygenic photosynthesis in such habitats remain a challenge. We used a novel combination of hyperspectral imaging, confocal laser scanning microscopy and chemical O_2 imaging with sensor nanoparticles for high-resolution mapping of the distribution and photosynthetic activity of Chl *f*-containing endolithic cyanobacteria in beachrock. We found substantial rates of NIR-driven oxygenic photosynthesis, comparable to volume-specific photosynthesis rates of benthic microalgae and cyanobacteria using visible light. Cyanobacteria with Chl *f* may thus contribute significantly to primary production in shaded biofilm habitats.[3]

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[P047] ROLE OF THE SOLONAMIDES IN PHOTOBACTERIUM GALATHEAE S2753

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The marine environment is a potent source of novel chemical compounds due to its vastness as well as the many diverse ecological niches found there. During a global expedition in 2006, *Photobacterium galatheae* S2753 was isolated from a mussel in the Solomon Sea. The strain produces numerous secondary metabolites with bioactive potential, including the cyclodepsipeptides solonamide A and B. These depsipeptides interfere with the quorum sensing (QS) system of *Staphylococcus aureus* hereby inhibiting virulence gene expression. Whilst this activity potentially makes them pharmaceutically valuable it seems illogical that a marine bacterium targets the QS system of a bacterium not commonly found in the marine environment, prompting the question what the natural function of the solonamides is. However, to investigate this, it was first necessary to identify the gene(s) responsible for solonamide biosynthesis.
During this study a putative *sol* gene, encoding a non-ribosomal polypeptide synthase (NRPS), was identified through antiSMASH analysis. To experimentally confirm the biosynthetic gene, two mutants were constructed; a mutant with a complete deletion of the *sol* gene, and a mutant with a partial deletion of the *sol* gene lacking only the terminal module of the NRPS. Chemical analysis confirmed the absence of solonamide biosynthesis in both mutants.
The study successfully identified the gene responsible for solonamide biosynthesis and demonstrated that a partial deletion of a NRPS was sufficient for eliminating solonamide biosynthesis. This opens the door for further studies of the natural role of these compounds and facilitates the potential construction of a heterologous expression platform.

[P048] MUTATION ACCUMULATION EXPERIMENT SHOWS HIGH EVOLVABILITY IN P. AERUGINOSA.

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Mutation accumulation experiments are an invaluable tool for studying bacterial evolution. They are essential for the understanding of forces shaping genetic and phenotypic variation. We compared evolution of *P. aeruginosa* under weak and strong bottlenecking, otherwise in maximally similar, close to optimal conditions. Mismatch repair-deficient *P. aeruginosa* cultures were propagated for more than 800 generations under weak bottlenecking and for more than 950 generations under strong bottlenecking. The populations were passed through 45 single-cell or 100-cell bottlenecks. Evolution under strong bottlenecking led to decrease in fitness and evolution under weak bottlenecking led to increase in fitness, reflected in shorter lag phase, higher growth rate and higher maximal growth. Subsequently, we performed whole genome sequencing of starting cultures, small bottleneck (SBN) cultures and clones representing large bottleneck (LBN) cultures. It was demonstrated that all the experimental populations accumulated comparable amounts of mutations. It was not possible to reveal enrichment for particular mutations or even for mutations in particular gene categories in LBN clones, which suggested that different LBN clones acquired different beneficial mutations in different pathways. It also implied that *P. aeruginosa* can reach higher fitness via a multitude of genetic, metabolic and physiological adaptations. To gain an additional insight into the changes underlying fitness advantage of *P. aeruginosa* LBN clones, gene expression in three out of six clones for each LBN line was analyzed. RNA sequencing revealed that transcription changes were significantly enriched in gene categories related to signaling, motility, secretion and virulence factors. To study if the altered gene expression prompted the respective LBN mutants to change their life style, we analyzed swimming motility, virulence and biofilm formation in *P. aeruginosa* LBN clones with available transcriptome data. With a single exception, we were not able to correlate gene expression patterns and phenotype changes unambiguously. At the same time, we demonstrated that evolution of *P. aeruginosa* towards higher fitness was accompanied by increase in variability of motility, virulence and biofilm production, which are clinically and ecologically important phenotypes.

[P049] TWO NOVEL BACTERIOPHAGE GENERA DISCOVERED IN A GROUNDWATER RESERVOIR INDICATE LARGE PREDATOR-PREY DIVERSITY IN SUBSURFACE ENVIRONMENTS

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Despite metagenomics have shown groundwater reservoirs to harbor diverse and complex bacterial communities, closely associated with biogeochemical cycling, much remain unknown about the microbial ecology of groundwater reservoirs and other subsurface environments. In this context, bacteriophages are even less studied but considered to exercise fundamental impacts in shaping the bacterial communities, and consequently influencing biogeochemical cycling. While viruses (phages) constitute only 0.04% of earths biomass, they are widely regarded to outclass any other taxa regarding both numbers and diversity. Thus, a daunting task lies ahead to map out their taxonomy, distribution, and ecological role. Concerning groundwater systems, genome sequences of *isolated* phages are absent, despite that phage abundances and viromes in these systems have been described. Employing a collection of bacterial groundwater isolates as hosts, we isolated, sequenced and characterized two bacteriophages, native to a groundwater reservoir. Our results suggest that both bacteriophages represent new genera and thus emphasize groundwater reservoirs, and likely other subsurface environments, as underexplored biotopes regarding presence and ecology of bacteriophages. To our knowledge, this is the first report on groundwater phages to include their sequenced genome and phylogenomic affiliation.

[P050] BIOLOGICAL DRIVERS OF BACTERIAL COMMUNITIES IN THE ARCTIC WATER INFLOW REGION REVEALED THROUGH MANIPULATIONS OF MICROBIAL FOOD WEB INTERACTIONS

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In the Arctic, seasonal changes are substantial and as a result marine prokaryotic community composition and functions differ greatly between the dark winter and bright summer. Interplay between abiotic and biotic drivers governs the transition from a microbial community, dominated by chemolithotrophic Archaea in winter, to photosynthetic active phytoplankton in association with fast growing opportunistic carbon degrading bacteria in summer. We studied the effects of biological drivers on the microbial community composition during different times of the year at the inflow to the Arctic Ocean, by experimentally manipulating microbial food web interactions via the exclusion of grazers of different size classes. The most profound community changes were detected during the spring to summer transition, with mutualistic phytoplankton-β-Proteobacteria interactions in early spring and substrate dependent phytoplankton-Flavobacteria interactions during bloom conditions. Grazing pressure from bacterivorous microzooplankton had an overall limited effect on prokaryotic community composition, but is likely important in structuring the community composition subsequent to the increase of carbon degrading Bacteroidetes and thereby transferring carbon to higher trophic levels.

[P051] DICKEYA DADANTII PHAGE AMAETHON DEMONSTRATES A WIDESPREAD OCCURRENCE OF 5-METHYLCYTOSINE MODIFICATIONS IN PHAGE GENOMES

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While it is widely recognized that modified bases in the DNA of phages play an important role in phage-bacteria interactions, the prevalence and biological ramifications of these modifications remain poorly understood. At present, only *Xanthomonas* phage Xp12 and the archaeal *Halobacterium* virus ΦH have been found to contain 100% methylated cytosines in their DNA. Analysis of the genome of recently isolated phage Amaethon, infecting plant pathogen *Dickeya dadantii,* revealed that it is encoding a thymidylate synthase (TS) homolog. Based on the identity of Amaethon TS amino acids in positions homologous to residues of the *E. coli* TS that are known to govern substrate and product specificity, it was hypothesized that Amaethon TS utilizes dCMP as a substrate and produces m5dCMP. Thus, Amaethon genomic DNA should contain 5-methylcytosine fully substituting for cytidine. This prediction was verified through UHPLC analysis of enzymatic hydrolysates of Amaethon genomic DNA, showing an absence of cytidine, but containing m5dC in 1:1 stoichiometry with dG, similar to Xp12 phage. These results indicate that phages carrying TS homologs with identical key active-site residues should also contain m5dC substituted DNA. As such, preliminary data suggests that more than 50 phages, infecting a diverse selection of bacteria, are likely to similarly contain 100% modified cytosines in their genomic DNA, demonstrating a previously unknown widespread occurrence of 5-methylcytosine modified phages.

[P052] DISCOVERY OF NOVEL CLASS 1 AND CLASS 2 CRISPR-CAS INHIBITORS THAT CROSS SUB-TYPE BARRIERS

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Many prokaryotes employ CRISPR-Cas systems to protect themselves from invading mobile genetic elements (MGEs). In response, MGEs have evolved protein inhibitors to bypass this immunity, so-called Anti-CRISPR (Acr) proteins. Here, we describe the systematic analysis of gene neighbors of the recently described anti-CRISPR associated gene 5 (aca5) across different taxa. Aca5 is found in many microbes, including *Pseudomonas*, *Pectobacterium, Serratia*, and *Escherichia*, and serves as a marker for Acr discovery. Functional testing of gene candidates has revealed 10 new type I inhibitors, some of which display broad inhibitory properties and are widely distributed across diverse MGEs. Additionally, an Acr with robust V-A, I-C, and weak II-A inhibitory activity is presented, the first Acr described to exhibit strong cross-Class inhibitory activities. Attempts to uncouple the two primary inhibitory functions via truncation and site directed mutagenesis were unsuccessful, suggesting a potential common inhibitory mechanism of Class 1 and Class 2 CRISPR-Cas systems.

[P053] PLASMIDS PERSIST IN A MICROBIAL COMMUNITY BY PROVIDING FITNESS BENEFIT TO MULTIPLE PHYLOTYPES

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The current epidemic of antibiotic resistance has been facilitated by the wide and rapid horizontal dissemination of antibiotic resistance genes (ARGs) in microbial communities. Indeed, ARGs are often located on plasmids, which can efficiently shuttle genes across diverse taxa. While the existence conditions of plasmids have been extensively studied in a few model bacterial populations, their fate in complex bacterial communities is poorly understood. Here, we coupled plasmid transfer assays with serial growth experiments to investigate the persistence of the broad-host-range IncP-1 plasmid pKJK5 in microbial communities derived from a sewage treatment plant. The cultivation conditions combined different nutrient and oxygen levels and were non-selective and non-conducive for liquid-phase conjugal transfer. Following initial transfer, the plasmid persisted in almost all conditions during a 10-day serial growth experiment (equivalent to 60 generations), with a transient transconjugant incidence up to 30%. By combining cell enumeration and sorting with amplicon sequencing, we mapped plasmid fitness effects across taxa of the microbial community. Unexpected plasmid fitness benefits were observed in multiple phylotypes of *Aeromonas*, *Pseudomonas* and *Enterobacteriaceae*, which resulted in community-level plasmid persistence. We demonstrate, for the first time, that plasmid fitness effects across community members can be estimated in a high-throughput way without prior isolation. By gaining a fitness benefit when carrying plasmids, members within complex microbial communities might have a hitherto unrecognized potential to maintain plasmids for long-term community-wide access.

[P054] THE IMPACT OF PLASMID HOST RANGE ON THE DISSEMINATION OF ANTIBIOTIC RESISTANCE GENES TO THE URBAN WASTEWATER MICROBIOME

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Horizontal transfer of genetic material between different bacteria is a large contributor to the dissemination of antibiotic resistance genes (ARGs). In this way pathogenic bacteria can acquire resistance to multiple antibiotics. Specifically, the microbial community of urban wastewater treatment plants (WWTPs) is believed to be permissive to conjugational transfer of plasmids. This permissiveness of the microbial wastewater community, combined with a strong selective pressure towards drug tolerance and resistance in the WWTP, may result in an accelerated propagation of ARGs to pathogenic bacteria. Members of the Enterobacteriaceae family are well represented among those pathogens of the highest global concern. Dissemination of ARGs encoding such as extended spectrum beta lactamases and resistances to carbapenems are often found to be mediated by plasmids within this family. We aimed to elucidate the influence of plasmid host range on the dissemination of plasmid borne ARGs to the microbial community of influent in WWTP, and the potential secondary transfer from this community to potential pathogenic Enterobacteriaceae. To achieve this, we constructed a dual fluorescent reporter gene system coupled with fluorescence activated cell sorting, to track plasmid dissemination. We found that the microbial community of the influent from three Swedish municipal WWTPs were permissive towards the plasmids R27::*gfp*, R64::*gfp* and pB10::*gfp*. The broad host range plasmid pB10::*gfp*, were disseminated significantly more compared to the two Enterobacteriaceae specific plasmids R27::*gfp*, R64::*gfp*. Additionally, the permissive fraction of the wastewater community was revealed to be highly potent donors of R27::*gfp* and pB10::*gfp*, to models of potential pathogenic Enterobacteriaceae strains. By post sorting 16S rRNA gene sequencing analysis, we furthermore showed that though R27::*gfp* was preferentially transferred to members of Enterobacteriaceae, R27::*gfp* was additionally transferred to non-Enterobacteriaceae Gammaproteobacterial genera. Additionally, several Enterobacteriaceae genera, which includes clinically relevant pathogenic members, were found in the transconjugant pools. Even though the transconjugant pool of pB10::*gfp* was ~700 times larger, R27::*gfp* was significantly more, or as, transferred to two of these genera. Thus, both narrow and broad host range plasmids should be considered, when assessing the risk of ARG dissemination among and to pathogenic Enterobacteriaceae in the WTTPs.

[P055] GASTRIC CORE MICROBIOME OF THE CORAL GALAXEA FASCICULARIS

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Coral microbiomes play important roles for coral fitness and stress resilience, and microbes are distributed over several microhabitats within the coral holobiont, i.e., tissue, mucus secretions, and the gastric cavity ("stomach") of the coral animal as well as its porous calcium carbonate skeleton. While there is a strong focus on either bulk analyses or analyses of the coral tissue surface-associated microbiome, the microbiome of the gastric cavity of coral remains largely unexplored. This compartment has been shown to become anoxic during the night, but it is mostly viewed to be an open system regularly exposed to fluxes of ocean water. In this study, we explored whether the gastric cavity of the coral *Galaxea fascicularis* harbors a core microbiome, similar to the observation of core gut microbiomes in higher animals.
In order to investigate the gastric content, a modified fine scale sampling method was applied allowing for separation of gastric samples from the other compartments of the coral holobiont. Gastric content was extracted from *G. fascicularis* specimens kept in a closed aquarium system in Helsingør (DK) or in an open seawater system in Monaco. The microbial diversity in samples was characterized by 16s rRNA amplicon sequencing and subsequent bioinformatic and statistical analyses. The results showed a very similar gastric microbiome dominated by one taxon (<60% of OTU's) irrespective of geography and a widely different from the surrounding seawater. The analysis of the surrounding aquarium water at the two different holding facilities revealed the presence of a distinct bacterial community in the gastric samples independent from the microbial diversity found in the seawater. This disproves the assumption that coral stomachs are open systems while simultaneously indicating that the dominant taxa could potentially confer some essential functionality to the host, as the persistence of the association might suggest some level of selection for a core genome in the coral gastric cavity.

[P056] AN EASILY MODIFIABLE CONJUGATIVE PLASMID FOR STUDYING HORIZONTAL GENE TRANSFER

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Bacterial antimicrobial resistance is today one of the most complex human health problems, which is largely resulted from the extensive use of antibiotics. Therefore, it is of utmost importance to study how the bacteria interact with each other as social organisms, sharing their antibiotic resistance, and ultimately how they evolve their antibiotic resistance traits. Our aim of this study was to develop an efficient and convenient molecular tool that can aid investigations of bacterial resistance acquisition, maintenance and transmission. For that, we introduced a single *att*Tn7 site onto the conjugative broad-host-range IncP-1 plasmid pKJK5 in a non-disruptive manner. A non-conjugative version of pKJK5-*att*Tn7 has also been made. It has been suggested that IncP-1 plasmid-containing bacteria are key players in the horizontal transfer of antibiotic resistance in soil, water, and wastewater treatment plant. The advantage of having the *att*Tn7 sites is that any genes of interest, can be introduced in a single step with very high success rate, such as reporter proteins and resistance genes. In addition, this largely alleviate concerns regarding insert fragments size. Currently we have used these engineered plasmids to generate conjugative and non-conjugative plasmids with *gfp*-tags in addition to seven different beta-lactamase genes representing all know classes. These will be used to study the complex nature of the direct and indirect resistance that beta-lactamases provide and the relationship with horizontal gene transfer.

[P057] PREVALENCE OF HAEMOSPORIDIAN PARASITES IN BIRDS ALONG ABIOTIC GRADIENTS.

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Both biotic and abiotic factors can determine host and parasite associations and ultimately impact the community assemblies of all parties involved in this symbiosis. Through examining bird-parasite communities and the parasite prevalence along abiotic gradients (i.e. elevational, latitudinal, temperature and humidity), we may better understand how environmental conditions can affect this host-parasite symbiosis. Globally distributed haemosporidian parasites can cause avian malaria, and they offer a great model for studying the effect of abiotic factors on parasite prevalences in birds and parasite communities. Spatial patterns of haemosporidian parasites along gradients have been studied, yet results are somewhat contradictory, and a synthesis is lacking. Therefore, to provide a better overview on the ecological patterns that birds, and their malarial parasites follow, we conducted a literature survey focused on temperature, humidity, latitudinal and elevational gradient studies on avian malaria. Our results demonstrate that there is not one single general pattern in haemosporidian prevalences in birds along different gradients. However, three major genera of malaria parasites demonstrate genera specific distributions among gradients: 1) *Plasmodium* prevalence is positively correlated to temperature, and negatively with latitude, elevation and, possibly, humidity, 2) *Haemoproteus* prevalence correlates negatively with latitude and peaks at mid-elevations, and 3) *Leucocytozoon* prevalence might be linked to high elevations. Thus, our results suggest that observed differences in malaria prevalences in bird communities along gradients may be a result of community composition of different parasite genera.

[P058] IDENTIFICATION OF MICROBES INVOLVED IN ANAEROBIC OXIDATION OF METHANE IN FRESHWATER SEDIMENT

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Methane is a strong greenhouse gas, the atmospheric concentrations of which has increased over the past decades as a result of human activity (industry, agriculture) and enhanced emission from natural habitats. Anaerobic Oxidation of Methane (AOM) is potentially an important process for mitigating the emissions of methane from aquatic systems. The process has so far mainly been studied in marine environments, while studies in freshwater settings remain scarce. Archaea so far known to be involved in AOM are all grouped into ANME (ANaerobic MEthanotrophs) clusters in the Euryarchaeota. This multi-order group uses the reverse methanogenesis pathway to run methane oxidation. ANME are found in very diverse environments, from paddy soil to deep marine sediment, but whereas they play a major role in methane oxidation in marine sediments, their role in freshwater sediments remains mostly unknown.

As part of a wider effort to understand the role of AOM in attenuating methane emissions from freshwater systems, we aimed to identify which microorganisms are responsible for AOM, which electron acceptors are used for this metabolism, and whether AOM is carried out by a single group of organisms or by consortia, similar to those known in marine sediments.
Our study, conducted on sediments from Lake Ørn (central Jutland, DK) where AOM is active, confirmed the presence of a specific group of archaea, known as ANME-2D, related to '*Candidatus* Methanoperedens nitroreducens'. These organisms have been demonstrated to perform AOM with nitrate. However, geochemical analysis demonstrated overlapping depth distributions of sulfate and methane, suggesting a potential coupling of AOM to sulfate reduction, while nitrate and nitrite were only present at the sediment surface. Thus, we proceeded to identify possible interactions of ANME-2D members with other members of the microbial community. For this, we quantified functional and 16S rRNA genes of organisms of interest. This analysis revealed the co-occurrence of ANME-2D and specific groups of sulfate reducing bacteria, with peaks in the abundance of gene copy number in the AOM zone. To further link AOM to sulfate reduction, we will proceed to FISH analysis to observe the relation between ANME-2D organism and sulfate reducing bacteria.

[P059] INCN PLASMIDS ARE VECTORS DISSEMINATING COLISTIN RESISTANCE IN WASTEWATER MICROBIOTA

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Wastewater treatment plants (WWTPs) collect residual pharmaceutical compounds and massive amounts of human-gut associated bacteria, some of which are closely related to human pathogens. As high cell densities and recurrent antibiotic selective pressure favour plasmid-mediated dissemination occur in WWTPs, those are regarded as particularly active hot spots for the spread of plasmid-borne antibiotic resistance genes (ARGs). Plasmids belonging to the IncN incompatibility group are often associated with the dissemination of clinically relevant ARGs. Their presence in WWTPs and effluent waters together with their broad host range, high transfer rate and ability to replicate in Enterobacteriaceae, a group comprising several critical pathogens makes them a critical concern to public health. However, little is known about their transfer dynamics in urban water systems.

In this work, we describe the capture and characterization of conjugative plasmids harbouring clusters of resistance determinants from a municipal WWTP activated sludge sample (Odense, DK). Plasmids were recovered by exogenous isolation using an *Escherichia coli* recipient strain. Nanopore sequencing revealed the presence of a 73Kbp IncN plasmid carrying a complete set of functional conjugative genes and multiple ARGs. Antibiotic susceptibility testing showed that this plasmid can transfer resistance of colistin, a last-resort antibiotic, to a sensitive *Escherichia coli* recipient. Direct isolation from the WWTP community confirmed the presence of colistin-resistant strains in that environment. Most were identified as *Pseudochrobactrum* sp. and *Ochrobactrum* sp. using full-length 16S rRNA gene sequencing. Interestingly, a *Pseudochrobactrum* isolate hosted a similar-sized IncN plasmid also encoding complete conjugative transfer systems and multiple ARGs. Further filter mating experiments with a colistin-sensitive recipient strain confirmed the mobility of the IncN plasmid and its associated colistin resistance. However, the responsible determinant could not be readily identified by sequence homology as one of the already characterized mobilized colistin resistance (*mcr*) gene variants. The identification of a potentially novel determinant of colistin resistance transferable to *Escherichia coli* is of significant importance for surveillance purpose. Together, these results uncover the dynamic nature of self-transmissible IncN plasmids and their role in the dissemination of colistin resistance in the WWTP environment.

[P060] MECHANISMS OF MERCURY RESISTANCE IN HIGH ARCTIC SNOW AND FRESHWATER BACTERIA.

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Despite its pristine appearance, the Arctic is highly vulnerable to contaminants transported from lower latitudes. Among them, mercury can undergo rapid oxidation during atmospheric mercury depletion events leading to snow surface concentrations 400 fold higher than background values. The role of microbial communities in the cycling of this toxic element in the Arctic is largely unknown. We investigated mercury resistance in bacteria isolated from High Arctic snow and freshwater. One highly resistant (MIC of 50 µM) *Pseudomonas sp*. isolate harboured a mercuric reductase gene, *merA,* involved in $HgCl_2$ reduction, and reduced more than 90% of $HgCl_2$ within few hours. In comparison, *Rhodococcus qingshegii,* containing a truncated merA gene with the metal binding domain missing, presented a ten times lower minimal inhibitory concentration and a slow $HgCl_2$ reduction. Surprisingly, in two highly tolerant (MIC of 20µM) isolates belonging to *Pseudomonas sp*. and *Janthinobacterium sp*. no merA genes were detected. The use of transposon sequencing to further examine resistance mechanisms in these strains, such as extracellular sequestration or bioaccumulation, will be discussed here. The diversity of mercury resistance mechanisms in Arctic bacteria highlights the need to further understand the impact of microbes in mercury cycling via reemission to the atmosphere or bioaccumulation in the marine food chain.

[P061] PHAGE-MEDIATED MANIPULATION OF A MURINE GUT MICROBIOME DECREASE SYMPTOMS OF TYPE-2-DIABETES AND OBESITY

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Development of obesity and type-2-diabetes is associated with gut microbiota (GM) alterations. The gut viral community is predominated by bacteriophages (phages) which are viruses attacking bacteria in a host-specific manner. As a proof-of-concept we demonstrated the efficacy of faecal virome transplantation (FVT) for shifting the phenotype of obese mice into closer resemblance of lean mice. The FVT consisted of viromes extracted from cecum content from mice fed a low-fat (LF) diet. Subsequently 40 male C57BL/6NTac mice were divided into five groups: LF (as control), high-fat diet (HF), HF+Ampicillin (Amp), HF+Amp+FVT and HF+FVT. After 6 weeks on their respective diets, the HF+FVT and HF+Amp+FVT mice were treated with FVT. HF+FVT mice showed a significant decrease in weight gain compared to the HF group ($p = 0.0033$). Surprisingly, as the only group the HF+FVT showed a comparable glucose tolerance as determined by OGTT to the lean LF group ($p = 0.9648$). These observations were supported by significant shifts in GM composition ($p < 0.004$) and metabolome profile ($p < 0.05$) as well as expression levels of genes ($p < 0.05$) involved in metabolic syndrome. We hypothesise that bacteriophages within the FVT altered the abundance of bacterial species which, directly or indirectly, are involved in metabolic syndrome development. In conclusion, transfer of gut viral communities from a lean phenotype to an obese phenotype reduce weight gain and improve blood glucose parameters.

[P062] CHARACTERIZATION OF THE VAGINAL DNA VIROME IN HEALTH AND DYSBIOSIS: AN OPENING STUDY IN PATIENTS WITH NON-FEMALE FACTOR INFERTILITY

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Background. Bacterial vaginosis (BV) is characterised by a reduction in *Lactobacillus* spp. abundance and increased abundance of facultative anaerobes, like *Gardnerella vaginalis*. BV aetiology is not fully understood, but bacteriophages could play a pivotal role causing perturbation of the vaginal bacterial community. Here we investigate the vaginal viral community, including bacteriophages, and its association to the bacterial community and BV-status.
Methods. Vaginal samples from 48 patients undergoing IVF treatment for non-female factor infertility were subjected to metagenomic sequencing of purified virus-like particles. The vaginal viral community was characterized and correlated with BV-status, bacterial community structure and presence of key vaginal bacterial species.
Results. The majority of identified vaginal viruses belonged to the class of double-stranded DNA bacteriophages, with eukaryotic viruses constituting 4% of total reads. Clear links between viral community composition and BV ($q = 0.006$, $R = 0.26$) as well as presence of *L. crispatus* ($q = 0.001$, $R = 0.43$)*, L. iners, Gardnerella vaginalis* and *Atopobium vaginae* were found ($q < 0.002$, $R > 0.15$). Interestingly, also the eukaryotic viral community was correlated with BV-status ($q = 0.018$, $R = 0.20$).
Conclusions. The vaginal virome is clearly linked with bacterial community structure and BV-status.

[P063] MICROBIOME CHANGES IN CORALS RESPONDING TO DISTINCT FEEDING REGIMES

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Globally reef-building corals are in rapid decline due to climate change and a range of other anthropogenic stressors. A functioning microbiome in corals is often overlooked in aspects of coral health, although microbiomes have been shown essential for health and fitness in many other organisms. This study used 16S rRNA gene amplicon sequencing to describe the microbiome response (and change in coral health indices) under distinct feeding regimes of *Acropora tenuis* coral colonies. Overall, the microbial community was impacted by all treatments. The *in situ* population displayed a diverse microbiome, which was different from all experimental treatments and exhibited a lipid class and fatty acid profile indicative of surplus energy, higher than all treatments. Across all treatments, *Endozoicomonas*-affiliated bacteria dominated the coral microbiomes (between 77 to 98 % relative abundance of retrieved sequences). Members of this genus potentially perform diverse holobiont services such as modulating the native microbial community, facilitating nutrient cycling and enhancing metabolic pathways during times of stress. The relative abundance of *Endozoicomonas*-affiliated ASVs (exact sequence variants) revealed 11 dominant ecotypes displaying high variability in host association and responsiveness to treatments. The results indicate that culture conditions strongly impact the coral microbiome assemblage and are therefore of prime important for a healthy microbiome in captive corals.

[P064] THE INFLUENCE OF MICROBIAL SECONDARY METABOLITES ON MICROBIAL DIVERSITY AND FUNCTIONALITY IN A MARINE MODEL SYSTEM

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Microbial secondary metabolites (SMs) are predominantly regarded as compounds that protect the SM producer from competitors. However, SMs may display a broader spectrum of functionalities and affect gene expression and phenotype in sub-inhibitory concentrations. Through their ability to alter the metabolism of microorganisms, SMs may be strong drivers of microbial community assembly, thus partly responsible for shaping microbial communities in nature. However, the SMs of bacteria and their impact in natural systems have remained unexplored and their true ecological functions in nature have not been elucidated.
Here, we aim to develop a marine microbial model system to study the influence of the SM producer *Phaeobacter* on microbial communities. *Phaeobacter* produces the SM tropodithietic acid (TDA) and is often found associated with microalgae. We will reconstruct a bacterial model community with axenic algae and employ the natural microbiome of the microalgae *Tetraselmis suecica*. To determine the effect of TDA on the model community, the algal-bacterial model will be set-up with TDA-producing *Phaeobacter* and a *Phaeobacter* TDA deletion mutant. Changes in species diversity of the model community will be determined by 16S rRNA gene amplicon sequencing. Modelling of the model community with generalized Lokta-Volterra models will be used to obtain an understanding of the microbial community's structure and response to the SM. The influence of TDA on the communities' genetic potential for SM production will be assessed by targeted amplicon sequencing of biosynthetic gene clusters encoding non-ribosomal peptide- and polyketide synthases. Eventually, this study will aid in our understanding of the natural role of SMs in microbiomes.

[P065] EXPLORING THE POTENTIAL ROLE OF BACILLUS SUBTILIS AS BIOCONTROL AGENT: COLONIZATION OF AGARICUS BISPORUS AND INHIBITION OF TRICHODERMA AGGRESSIVUM

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Trichoderma aggressivum causes green mold disease in the edible mushroom, *Agaricus bisporus.* Its infection can hamper the formation of fruiting bodies and reduce crop yield in mushroom plantation. Recent reports exposed that artificial inoculation of *Bacillus velezensis* prevents *A. bisporus* yield reduction by *T. aggressivum* infection. We hypothesized that biocontrol activity of *B. velezensis* is influenced by the secondary metabolites of *Bacilli*. Since *Bacilli* are known for fungal hyphae attachment capability; it is possible that *Bacilli* can also attach on *A. bisporus* hyphae, and therefore bacterial biofilm on mycelia contributes to the microbial control agent efficacy.
To reveal the biocontrol potential of *Bacillus subtilis* isolates against *T. aggressivum*, the antagonistic activity of *B. subtilis* and their secondary metabolite mutants were investigated with plate assays. Moreover, *B. subtilis* and *A. bisporus* were co-cultivated in liquid medium and visualized under confocal laser scanning microscope (CSLM) to demonstrate *B. subtilis'* hyphal attachment.
Our results revealed that the absence of plipastatin in *B. subtilis* leads to hampered *T. aggressivum* antagonism. In addition, *B. subtilis* readily attaches to *A. bisporus* hyphae, while the absence of biofilm structural genes (Δ*eps* and Δ*tasA*) resulted in lack of hyphal attachment.
The project is connected to the Center for Microbial Secondary Metabolites that is supported by the Danish National Research Foundation (DNRF137).

[P066] ENRICHMENT OF FULL LENGTH RRNA OPERONS FROM ENVIRONEMNTAL SAMPLES

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Fast, accurate and cheap identification of different bacteria in various samples is essential in several research areas, including healthcare and studies of evolution and ecology. The conventional approach is to amplify a region of the 16S ribosomal RNA gene with universal primers and use this region as a fingerprint to reconstruct phylogenies. Inherently this method is biased by primer efficiency and limited in resolution relative to the amplified region. Modern advancements in long-read sequencing enables fast and easy sequencing of the entire rRNA operon at a low-cost, allowing for increased resolution. New gene enrichment strategies utilizing labeled probes or CRISPR/cas9 can potentially overcome the primer bias by designing multiple probes or guide RNAs targeting regions within the entire rRNA operon. This project examines various enrichment strategies for efficient enrichment of full length rRNA operons from environmental samples, with the aim of investigating the microbial diversity in Denmark under the project Microflora Danica. Furthermore, I am experimenting with combining enrichment strategies with unique molecular identifiers (UMIs) to generate long-read low error rate reference sequences on the MinION-platform. This is crucial as low error rate references are essential for the entire rRNA identification system. The database sequences needs to be of the highest possible quality as they represent the true biological sequences. The quality of an identification depends on the quality of the reference database.

[P067] BREAKING THE DEFENSIVE BARRIER: FUNGUS-FARMING TERMITE THREATS FROM FUNGAL DISEASE

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Fungus-growing termites forage in pathogen-rich environments, including grass, decaying wood and animal dung, which suggests that their *Termitomyces* fungal crops should be exposed to antagonists or competitors coming from the environment. However, the fungus gardens appear to remain free from specialized fungal diseases, suggesting that the substrate the termites forage on is either free from antagonists or that defence mechanisms remove them before they enter the nest. To test the first hypothesis, we isolated fungi from 39 forage substrates and isolated more than 400 fungal cultures that were subsequently identified by Sanger sequencing of the ITS region. Fungi are thus abundant within substrates, implying that they could enter termite nests, but their absence in combs suggest that they are dealt with by colony defences. To assess the role of workers in keeping combs clean, we established *Macrotermes bellicosus* sub-colonies from six nests with either 0, 50 and 200 workers. We then sampled a piece of comb from each sub-colony every second day for six days and processed it via ITS amplicon sequencing to characterise the succession of the fungal communities during the eventual collapse of the sub-colonies. In the absence of termite workers, combs were rapidly overgrown by other fungi, primarily from the genus *Pseudoxylaria*, but this was delayed when workers were present. This suggests that termite worker efforts play an important role in the stability of the fungus gardens, and we discuss mechanisms with which they may do so.

[P068] THE UROPYGIAL GLAND BACTERIA AND THEIR POTENTIAL DEFENSIVE ROLES IN GREAT TITS (PARUS MAJOR)

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The Uropygial gland (preen glands) of birds plays an important role in maintaining feather integrity and hygiene. A few studies have demonstrated the potential defensive roles of bacteria residing within the uropygial glands, but our knowledge about bacterial diversity and functions is generally poor. To improve this, we investigated the microbiota of 19 Great tit (*Parus major*) uropygial gland secretions using both direct isolation of bacteria (culture-dependent) and 16S rRNA amplicon sequencing (culture-independent). To evaluate the antimicrobial properties of a subset of these isolates, we performed co-culture experiments of selected isolates identified from both approaches against feather-degrading bacteria (e.g., *Bacillus licheniformis* and *Kocuria rhizophila*) and common fungal pathogens (e.g., *Aspergillus niger* and *Candida catenulata*). Our results show major differences in bacterial communities characterized by culture-dependent vs. -independent approaches, with isolates from 12 bacterial genera (dominated by Firmicutes and Actinobacteria), while amplicon sequencing identified 117 bacterial genera (dominated by Proteobacteria, Firmicutes and Bacteroidetes). Some bacterial isolates (e.g., *Bacillus*, *Dermococcus* and *Staphylococcus*) supressed the growth of multiple antagonists, attesting to a possible defensive role of these bacteria. Our findings suggest that uropygial gland bacteria could play an important role for feather health and that further investigations of these understudied bacterial communities could provide a new avenue to obtain novel antimicrobial compounds.

[P069] PODAXIS' PRESENCE IN HOSTILE TERMITE MOUNDS: ADAPTATIONS TO BIOTIC STRESS

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The basidiomycete fungal genus *Podaxis* consists of a few species; each segregating between two different lifestyles: desert-living and termite associated. They are the only macrofungi observed to grow from grass-harvesting termite mounds (Nasutitermitinae). The genus is a promising model for fungal adaptations to stress, because it either lives in dry desert environments (abiotic stress) or in chemically-defended termite mounds (biotic stress). However, the phylogeny of the genus and *Podaxis'* role and prevalence within termite mounds are not well resolved. Here we used *Podaxis* spores obtained from herbarium/fungarium collections to expand on the current phylogeny of the Internal Transcribed Spacer (ITS) region. The updated phylogeny confirmed earlier work observing several clades segregating between lifestyles, while indicating some geographical structure. We build on this by exploring the prevalence and abundance of *Podaxis* within *Trinervitermes* termite mounds in different locations in South Africa, recording mushroom presence and using metabarcoding of fungal communities within termite colony grass chambers. Finally, in behavioural assays we confronted termites with *Podaxis* to test whether they would be attracted to or avoid the fungus, contributing to providing indications of its role in termite mounds. Our research suggests specialised adaptations to overcoming termite defences and confirms the potential of *Podaxis* as a model to study the interplay between biotic and abiotic stress adaptations in basidiomycetes.

[P070] GASTROINTESTINAL MICROBIAL ECOLOGY OF PIGLETS POST-WEANING

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Weaning of piglets imposes abrupt shifts in diet and environment and often renders the animals susceptible to e.g. *E. coli* mediated diarrhea. To characterize the gut microbial ecology of piglets post-weaning, a total of 110 piglets (13 litters of 8-9 piglet) were weaned at an age of 28 +/- 1 days. The piglets were offered creep feed pre-weaning and were weaned unto the same diet. Piglets were sacrificed at weaning (day 0), day 10 and day 35 post-weaning (1, 4 and 4 per litter, respectively), and digesta samples were retrieved from stomach, distal small intestine, caecum and mid colon. We analyzed digesta organic acids (SCFA and lactate), pH and dry matter, and characterized the digesta microbiota by 16S rRNA gene amplicon sequencing. Stomach SCFA and lactate peaked on day 10, also reflected in low pH. With age, lactate increased in ileum and decreased in caecum. In ileum, caecum and colon, SCFA content increased and pH decreased with age, indicating an overall increase in microbial fermentation. Evaluated by α- and β-diversity indexes, the microbiota was segment-specific. Within segments, there was a clear age-dependent development, with an overall increase in richness and α-diversity (Shannon) per se. In caecum and colon, β-diversity (weighted unifrac) decreased with age, indicating development towards a more similar microbiota among the animals. We observed a segment- and age-dependent pattern in relative abundance of specific taxa. In conclusion, we demonstrated a gender-independent succession of the piglet gut microbiota post-weaning with a, so-to-speak, pre-mature microbiota on day 10 that may be more susceptible to perturbations than the climax microbiota of older animals.

[P071] MULTI-KINGDOM MICROBIAL COMMUNITIES IN EARTHWORMS

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Earthworms are known as 'soil ecosystem engineers' and have a major impact on soil quality as they transport and feed on organic matter. However, little is known about the earthworm gut microbiome. The aim of this study was to assess the gut prokaryotic and eukaryotic microbiome in earthworms, and to explore if the gut microbiome is controlled by the earthworm genera. Using amplicon sequencing of the 16S rRNA gene for bacteria and the 18S rRNA gene for eukaryotes, we profiled gut microbiomes of three earthworm genera sampled from the same grassland soil in Denmark.

The earthworm gut microbiomes consist of a diverse community of bacteria dominated by Proteobacteria, Acidobacteria, Acntinobacteria, Firmicutes, Verrucomicrobia. Within eukaryotic microbial groups the SAR super group (Stramenopiles, Alveolates, Rhizaria) dominated along with fungi and metazoan. Interestingly, the alpha diversity was lower in earthworm genera *Lumbricus* compared to *Aporrectodea* and *Allolobophora.* Correlation based on OTU abundance revealed cross kingdom interactions among eukaryotes and prokaryotes, as several protists OTUs were to be positively correlated to bacterial OTUs in a network analysis.

In conclusion, the earthworm gut microbiome is affected by the earthworm genera. The gut microbiomes of all earthworms have high diversity with potential cross kingdom interactions in gut. Further studies of earthworm feeding habits and secondary metabolite production will increase our understanding of the earthworm gut microbiomes.

[P072] PSEUDOALTEROMONAS REPRESENTS AN UNLOCKED RESERVOIR OF BIOACTIVE POTENTIAL

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The marine environment consists of an underexplored pool of microbial diversity and functionality. The genus *Pseudoalteromonas* is ubiquitous in marine waters and pigmented species of the genus produce a plethora of secondary metabolites. In high concentrations, many of these have antimicrobial activities, however, their natural role is unknown. In marine *Vibrionaceae*, a link between chitin degradation and secondary metabolism has previously been reported. Due to the high chitinolytic capacity of *Pseudoalteromonas* we investigated if this link was present in this genus as well, potentially pointing to ecological functions of the bioactive compounds. Genomic analyses were conducted on 62 pigmented and 95 non-pigmented strains. Glycosyl hydrolase profiles suggested that chitin degradation is a key trait of pigmented species, and that non-pigmented species are rather specialized on algal degradation. We found that pigmented species devote up to 15% of their genome to biosynthetic gene clusters, while for non-pigmented species it was 3% at most. The chemistry of a selected pigmented species was elucidated after growth in chitin or mannose media. Interestingly, we found that the antibacterial compounds tetrabromopyrrole and pseudanes were produced in lesser quantities in chitin compared to mannose. Collectively, this suggests that pigmented *Pseudoalteromonas* play a key role in the degradation or maintenance of chitinous material. However, secondary metabolites may, due to a lower production on chitin, rather act as signals than antimicrobials.

[P073] QUANTIFICATION OF NITROSPIRA CLADE A AND B BY HIGH-RESOLUTION QPCR MELT CURVE ANALYSIS

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Comammox *Nitrospira* are nitrifiers that can completely oxidize ammonia to nitrate, a two-step process not catalysed by other nitrifiers. Comammox *Nitrospira* can be further subdivided into two evolutionarily distinct groups, clade A and B. The *amoA* gene encodes the alpha subunit of ammonia monooxygenase, which catalyses the oxidation of ammonia to nitrite. Recently designed primers targeting the *amoA* gene of both comammox *Nitrospira* spp. clades create distinct peaks in the melt curves corresponding to clade A and B. In this study, we developed and tested a method to use high-resolution melt curve analysis to quantify the relative abundance of clade A versus B comammox *Nitrospira* in environmental DNA samples. The concept was confirmed *in sillico* and was subsequently tested *in situ*. Plasmids containing *amoA* gene fragments of clade A and B were combined in different ratios. The ratio of the melting peak areas showed a clear relationship with the ratio of the respective DNA inputs. A standard curve for each clade was used to calculate the percentages of the two clades in waterworks samples. These results compared favorably to the quantification from *amoA*-based amplicon sequence analysis of the same samples. qPCR amplicon melt curve analysis is a useful tool to distinguish and quantify relative *Nitrospira* clade A and B abundance in environmantal samples. Clade B was consistently more abundant in the investigated waterworks samples.

[P074] MICROFLORA DANICA

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In 1883 Flora Danica, a comprehensive atlas of botany containing all wild plants native to Denmark, was finished. Today, we feel the time is right to add another: the Microflora Danica. By sampling across all natural and cultural habitats across Denmark, including soil, water and sediments, and by applying novel DNA-based sequencing technologies, we wish to generate an almost complete reference database of the microbiome of Denmark. In addition, we hope to contribute with a leap forward regarding illumination of the global microbial dark matter.
The purpose of Flora Danica was to spread the knowledge of botany and thereby to gain a greater knowledge of the useful and harmful properties of the various plants. Not very differently, by establishing the common reference database, Microflora Danica will allow anyone to communicate in the same language about the identification of the microbes and to link to further investigations of their functions and studies of their distribution across habitats. Here, we present an overview of aim, approach, sampling habitats, methods and expected outcomes.

[P075] MIDAS 4: A COMPREHENSIVE REFERENCE DATABASE OF MICROBES IN WASTEWATER TREATMENT SYSTEMS ACROSS THE GLOBE

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We recently developed a method that can produce millions of high-quality, full-length 16S rRNA gene sequences. This method was applied to samples collected at 667 wastewater treatment plants from 33 countries to create the first comprehensive, global 16S rRNA gene reference database for microbes in wastewater treatment systems (MiDAS 4). Sequences in MiDAS 4 were classified based on a new automated taxonomy classification algorithm (AutoTax), which provides a robust classification for all taxonomic ranks from kingdom to species-level for all sequences. AutoTax identified 8075 genera and 27204 species, revealing a much less diverse global community compared to what has previously been predicted. It also revealed some global distribution that partly depended on geography but also type of process design of the treatment plants. MiDAS4 also dramatically improves the specificity and reproducibility of taxonomic classification of amplicons, providing reliable community studies at sub-genus to sub-species-level. It also provides an opportunity to develop highly specific FISH probes that can be used to illuminate the morphological and functional diversity at the sub-genus level *in situ*. Since MiDAS 4 taxonomy provides names or placeholder names for nearly all microbes occurring in wastewater treatment systems across the globe, we anticipate that this will facilitate the sharing of knowledge across the scientific field.

[P076] DOES GUT PASSAGE HELP KEEP FUNGUS-GROWING TERMITE GARDENS DISEASE FREE?

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Fungus-growing termites are rarely infected by antagonistic fungi and their fungus gardens are essentially free of other fungi, with 99% of amplicon reads being of the beneficial fungal symbiont *Termitomyces*. Such an extremely clean farming environment is likely due to a combination of behavioural, physical, and chemical defence mechanisms. Because young fungus-growing termite workers ingest the plant forage material and build fungus garden with faeces, it has been proposed that gut passage could play a key role against infection of the fungus comb, targeting and eliminating mycopathogens present within the plant forage. To test this hypothesis, we collected young minor workers of the fungus-growing termite *Macrotermes bellicosus*, dissected and sectioned their guts into foregut, midgut, and hindgut, and used a culture-based approach to assess viable fungal lineages. We sequenced the ITS region of more than 400 isolates and obtained 52 different fungal genera. The most abundant genera were *Phialemoniopsis, Talaromyces,* and *Penicillium*. We found that neither fungal richness nor fungal community composition significantly differed between gut compartments. This suggests that gut passage alone does not prevent fungi from entering termite nests, implying that the near-axenic conditions observed in fungus gardens must be accomplished by other means.

[P077] CAMPYLOBACTER PHAGES MIMIC THE HOST DEFENSE STRATEGY BY USING PHASE VARIATION TO CREATE NEW PHENOTYPES WITH MODIFIED RECEPTOR BINDING PROTEINS

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Phase variation is a reversible stochastic event that switches the expression of proteins "on or off" in individual cells of a clonal population thereby creating phenotypic heterogeneity. This phenomenon results in a highly dynamic population structure and is an important strategy in bacteria for host and niche adaption including preventing bacteriophage (phage) infection. In *Campylobacter jejuni* phase variation arises from hypermutable homonucleotide G (polyG) tracts located within several genes, often encoding surface structures. We have previously shown that phase variation of such surface structures, including a common phage receptor, is an important phage resistance mechanism in *C. jejuni*. However, it is unclear how the phages adapt to these reversible phase variable population dynamics. Here we show that phages infecting *C. jejuni* mimic the defense mechanism of their host by using phase variation to generate new phage phenotypes that can infect a phage resistant bacterial population. We found that *C. jejuni* phages encode novel accessory receptor binding proteins (RBPs) containing polyG tracts that are phase variable with a mutation frequency equivalent to what has been observed in *C. jejuni.* The expression of the accessory RBPs is associated with a distinct plaque morphology and allow the phages to infect the common phage receptor mutant. Our results demonstrate a novel phage strategy for adaptation to host population dynamics allowing continuous co-existence of both phage and bacteria exceeding the traditional arms race of predator and prey.

[P078] DECHLOROMONAS: TO BE OR NOT TO BE A PAO? THAT IS THE QUESTION!

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Enhanced biological phosphorus removal (EBPR) is a biotechnological process, used in wastewater treatment for phosphorus removal and recovery. It relies on the ability of some organisms, the polyphosphate-accumulating organisms (PAOs), to take up phosphorus and store it intracellularly. *Ca.* Accumulibacter and *Tetrasphaera* are well-known PAOs, globally abundant in EBPR systems. However, several other microorganisms are recognised as putative PAOs. Members of the genus *Dechloromonas* are often abundant in EBPR plants both nationally and worldwide and have long been considered as one of the putative PAOs, since intracellular poly-P has been identified with traditional staining methods. This study applied metagenomics to determine the metabolic potential and Raman microspectroscopy to verify and define the levels and dynamics of important storage polymers in FISH-defined *Dechloromonas* cells. A 99.2% complete metagenome-assembled genome, retrieved from Danish EBPR plants, showed the potential for poly-P, glycogen, and PHA accumulation. *Dechloromonas* cells exhibited *in situ* a *Ca.* Accumulibacter-like phenotype with dynamic levels of poly-P and PHA during feed-famine cycling. In this study, quantitative Raman-FISH analysis was applied to single cells Dechloromonas from full-scale activated sludge plants, which confirmed it to be an important PAO.

Keywords: *Dechloromonas* PAO; Raman-FISH; P mass-balance

[P079] DO BACTERIOPHAGES POSE A PROBLEM FOR MICROBIAL BIOREMEDIATION?

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Many species among the *Spingomonadaceae* have abilities to degrade a variety of xenobiotic compounds and some can even use these as their sole carbon source. This makes them potential candidates for use in bioremediation of polluted groundwater or other contaminated environments. However, it is not unusual, that cultures of sphingomonads used in sandfilters or bioreactors disappear after a short time. A possible explanation could be naturally occurring bacteriophages, which could swiftly eradicate establishing sphingomonads. However, very little is known about bacteriophages targeting sphingomonads with only two genomes found in the NCBI virus database. In this study we present sphingomonads and phages targeting these. The study will proceed with evolutionary experiments on these isolates, to see how phage-host interactions, such as resistance and re-susceptibility evolves, and which mechanisms that contributes to phage resistance. The methods will include cultivation and isolation, sequencing with Illumina and Oxford Nanopore, and various bioinformatic tools will be used in the search for prophages and CRISPR systems in the isolated hosts, and possible spacer rearrangement in CRISPR positive hosts, upon phage encounters.

[P080] PROSPECTIVE EMERGENT PROPERTIES OF MICROBIOME SELECTION IN SOCIALLY COMPLEX HOSTS

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Hosts are strongly selected to wrangle microbial communities into cooperative, stable, and productive partners. Microorganisms may be hosted by groups, such as the cells and tissues of a multicellular organism or distinct individuals of an obligate social group. We contend that complex social insect groups, whom have undergone a major evolutionary transition to higher-level of biological complexity, may manipulate the microbiota as a superorganismal unit, similar to a multicellular organism. Several social insect groups have hosted consistent microbial symbionts over evolutionary time scales during which hosts also exhibited distinct division of labor among group members. The parallel evolution of symbioses and social organization may have selected for an emergent property of the combined effects of division of roles and microbiota, resulting in subgroup-specific microbiota in a colony, similar to body-site specific microbiomes in complex organisms. Additionally, we posit that host mechanisms that foster specific microbial partners, such as regulated immigration, selection through diet and physiology, and compartmentalization to different body regions, may occur at both the level of the individual as well as the superorganism, due to the structured social interactions of colony members. Ultimately, the question remains whether analogies between symbioses in social insect colonies and complex organisms may clarify if similar properties govern microbial associations in higher-level social systems.

Microbial physiology and cultivation

[P081] SYNERGY AT WORK – CO-CULTIVATION OF LACTOBACILLUS BREVIS AND AN ENGINEERED LACTOCOCCUS LACTIS STRAIN FOR SUPERIOR PRODUCTION OF 2-BUTANOL

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Microbial production of 2-butanol has faced limited success due to its toxicity and one key limiting enzymatic activity of butandiol dehydratase. Here, we demonstrate co-cultivation as a promising strategy for generating 2-butanol from microbial fermentation. We found that coupling a meso-2,3-butandiol producing *Lactococcus lactis* with a native *Lactobacillus brevis*, which has coenzyme B12-dependent diol dehydratase activities, could complete the 2-butanol synthesis from glucose, to 2-butanol. We optimized the co-culture fermentation and achieved a production of 5.9 g/L 2-butanol with a yield of 0.61 mol/mol 2-butanol per glucose.
Overall, we have set the stage for promising co-culture production of 2-butanol a much needed biosynthesized alcohol, with high yield and highest titer known so far. Furthermore we explore the potential and application of co-culturing for microbial synthesis of previously unavailable natural metabolic routes.

[P082] RED LIGHT ENHANCES GROWTH OF NEUROSPORA CRASSA

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Fungi as a group share that their cell wall is too rigid to allow the cells to engulf smaller cells and particles for food. This limitation has provided evolutionary pressures to develop effective enzymes to enable uptake of organic food components from the environment. The use of these enzymes have revolutionizing science and industries, for example for breaking up plant polymers into sugars. *Neurospora crassa* is a model fungus. After exposure to blue light or UV radiation, *N. crassa* is known to induce carotenogenesis. Red light is not known to have an effect. In this study, we show that growth of *N. crassa* after three days on solid medium with glucose or carboxymethyl cellulose and lignin is increased 10–30% in red light compared to darkness. Blue light caused accumulation of carotenoids but did not increase growth. Growth was measured by the growth area and by ergosterol extraction. Red-light-induced biomass increase in *N. crassa* was found with both methods. Attempts with more recalcitrant sources of wood in liquid suspensions showed that while the *N. crassa* showed an increase in biomass when degrading beechwood chips in red light, no effect was observed when the plant matter was wheat straw. The observed physiological effects by red light in *N. crassa* is probably mediated by phytochromes. This may have ecophysiological significance in nature and may potentially lead to industrial possibilities using light-induced pathways.

[P083] THE OXYGEN CONSUMPTION RATE OF CABLE BACTERIA

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Cable bacteria are multicellular, filamentous bacteria that naturally occur in marine and freshwater sediment. Oxygen reduction at the surface of the sediment is coupled to sulfide oxidation in deeper, anoxic layers through long-distance electron transport, by means of an as-yet uncharacterized, proteinaceous conductive material. Recently, it has been speculated that oxygen reduction in cable bacteria is performed through a hemoglobin-cytochrome fusion protein able to decouple the oxygen reduction process from energy conservation. Using custom-made glass chambers, microscopy digital video and imaging, and planar optode measurements, the oxygen consumption rate for individual cable bacteria was calculated by observing distortions of the oxygen gradient. The average oxygen consumption rate was 0,86 $pmol_{oxygen}$ $cell^{-1}day^{-1}$, which is ca. 20 folds higher than previous estimates based on several sediment population studies, and possibly higher than any other similar-sized prokaryote. Our results suggest that oxygen reduction in cable bacteria is remotely coupled to energy conservation and is performed by a currently uncharacterized enzymatic machinery.

[P084] DEEP PURPLE: THE BIOLOGICAL DARKENING OF THE GREENLAND ICE SHEET

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Pigmented glacier algae living on the surface of the Greenland Ice Sheet (GrIS) contribute significantly towards the darkening of the ice, thereby increasing its solar absorption and melting rates. The darkening effect of these algae can be mainly attributed to the purple coloured intracellular purpurogallin-like pigment, which absorbs in the visible and UV-light ranges. We still lack a basic understanding on the factors regulating the production of this pigment and promoting the large-scale algae blooms on the GrIS. We performed an incubation experiment on the East coast of Greenland (65°N) with two populations of the abundant glacier algae, *Mesotaenium berggrenii*: one was collected in the field and the other one was previously grown in the lab having lost its original purpurogallin pigment. Using Pulse-Amplitude-Modulated fluorometry (PAM) we revealed an, overall, higher photosynthetic performance (fluorescence yield) at higher irradiances of the population from the field, where purpurogallin is present. The population lacking this pigment showed a significantly lower maximum quantum efficiency (Fv/Fm) in the treatment with VIS+UV light, indicating a photo-damaging effect. Our results highlight the intracellular shading effect of the chloroplast by purpurogallin. Further pigment and metabolomic analysis using Ion Chromatography-Mass spectrometry in addition to transcriptomics will follow, likely yielding additional insights into the processes driving the growth and pigmentation of glacial algae.

[P085] EFFICIENT MEDIA FOR HIGH PRODUCTION OF MICROBIAL LIPASE FROM BACILLUS SUBTILIS (BSK-L) USING RESPONSE SURFACE METHODOLOGY FOR ENANTIOPURE SYNTHESIS OF DRUG MOLECULES

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Background: Microbial lipases are known to depict diverse properties and substrate specificity, making them a potential source for various industrial applications. Several microbial lipases are produced commercially but their high cost and poor stability seem to be a major factor in their successful commercial applications. A crucial factor of industrial prices of these enzymes is the culture media composition that is constantly under review by researchers. In the present study, maximum lipase production by *Bacillus Subtilis* (BSK-L), was achieved using response surface methodology (RSM).

Methods: Culture medium parameters such as low and high cost carbon & nitrogen sources, substrates, temperature, pH, salts, metal ions and incubation time were evaluated. The production of lipase was optimized through RSM based on central composite design. Process optimization involved one variable at time (OVAT), wherein olive oil was used as an inducer and as a carbon source.

Results: Olive oil and peptone were found to be the best carbon and nitrogen sources respectively, whereas, $MnSO_4$ and temperature observed to be most significant factors for production of lipase from BSK-L. Optimized media components obtained from RSM produced satisfactory result of 7500U/g cell biomass and 12-fold increases in lipase production was achieved than that of conventionally optimized conditions (627U/g cell biomass). The lipase produced from indigenous strain (BSK-L) was used for resolution of enantiomers of butyl-ketoprofen via hydrolysis.

Conclusion: In this investigation, significant enhancement in the production of microbial lipases was obtained using statistical tools. The isolated enzyme used for racemic resolution of ketoprofen butyl esters which exhibited better enantiomeric excess of 70% ee compared to commercial available *Candida antarctica* lipase (63% ee). This potential of lipase can be exploited in pharmaceutical industry.

[P086] (P)PPGPP REGULATES A BACTERIAL NUCLEOSIDASE BY AN ALLOSTERIC TWO-DOMAIN SWITCH

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The stringent response alarmones pppGpp and ppGpp are essential for rapid adaption of bacterial physiology to changes in the environment. In *Escherichia coli*, the nucleosidase PpnN (YgdH) regulates purine homeostasis by cleaving nucleoside monophosphates and specifically binds (p)ppGpp. Here, we show that (p)ppGpp stimulates the catalytic activity of PpnN both *in vitro* and *in vivo* causing accumulation of several types of nucleobases during stress. The structure of PpnN reveals a tetramer with allosteric (p)ppGpp binding sites located between subunits. pppGpp binding triggers a large conformational change that shifts the two terminal domains to expose the active site, providing a structural rationale for the stimulatory effect. We find that PpnN increases fitness and adjusts cellular tolerance to antibiotics and propose a model in which nucleotide levels can rapidly be adjusted during stress by simultaneous inhibition of biosynthesis and stimulation of degradation, thus achieving a balanced physiological response to constantly changing environments.

[P087] IDENTIFICATION AND CHARACTERIZATION OF A NOVEL SIGNALING MOLECULE IN SALMONELLA TYPHIMURIUM

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Every year more than 100 million people are infected by *Salmonella* causing 350,000 annual deaths. Multi-drug resistance is an increasing challenge in treating *Salmonella* infections underscoring the need for research into the development of novel antimicrobials to treat *Salmonella* infections. In my group, we have investigated the role of a novel signaling molecule, 3,5-dimethylpyrazin-2-ol (DPO), in *Salmonella* Typhimurium (*S*. Typhimurium). DPO is synthesized as a metabolite from threonine and glycine catabolism. We have constructed a DPO mutant of *S*. Typhimurium that is effectively blocked in DPO biosynthesis and investigated its ability to cause infection of a mouse model of systemic *Salmonella* infections. Our results demonstrate that the presence of DPO, as a consequence of its biosynthesis, is essential for virulence in *S*. Typhimurium. Furthermore, lack of DPO biosynthesis promotes biofilm formation in *S*. Typhimurium, while the addition of synthetic DPO inhibits biofilm formation. Together our results show that DPO functions as a genuine signal in *S*. Typhimurium controlling the switch between a sessile (biofilm) and a virulent lifestyle. Furthermore, we have synthesized a panel of DPO-variants, each characterized by a systematic single-substituent structural modification or addition, relative to the parent compound. These variants are essential for elucidating the molecular mechanism of DPO-signaling by identifying structure activity relationships, and in the discovery of potential lead DPO-antagonistic compounds. The effect of these compounds on biofilm formation will be presented.

[P088] BACILLUS SUBTILIS PERSISTENCE AND SECONDARY METABOLITE PRODUCTION IN ARTIFICIAL SOIL

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Bacillus subtilis is a plant-growth-promoting rhizobacterium proposed as a sustainable alternative to synthetic pesticides in agriculture. It is based, mainly, on its ability to produce a vast array of secondary metabolites (SM) and colonize actively different niche. Those traits have shown to play a pivotal role in plant growth promotion and biocontrol of phythopathogens. However, the fundamental question about the natural role of SMs in the lifestyle of the producing bacteria in soil has been less explored. Therefore, in this work, we explore the population dynamics and secondary metabolites production of *B. subtilis* growing in an artificial soil made of a polymer matrix, either in axenic conditions or under a synthetic microbial community. This approach will allow us to understand, under controlled conditions, the factors that modulate the lifestyles of *B. subtilis* and the SM production in soil.

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[P089] DOMAIN ANALYSIS OF THE CELL WALL PROTEINASE, LACTOCEPIN, OF LACTOCOCCUS LACTIS

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Commercial starter cultures acidify camel milk slowly compared to bovine milk. This is due to reduced digestion rate of casein by the cell wall protease, lactocepin. To find new and better starter cultures to ferment camel milk, we are analyzing the domains of lactocepins and their influence on the digestion of caseins in camel and bovine milk. This study focuses on the cell wall anchor of lactocepins along with the two domains located next to the anchor. To study the characteristics of these three domains we focus on a lactocepin of a *Lactococcus lactis* strain found to acidify camel milk well. Two plasmids have been constructed encoding this lactocepin with and without the three domains. Our research has shown that the acidification rate of bovine milk does not differ between strains carrying this lactocepin with and without the three domains. On the contrary the rate of acidification is reduced in camel milk for the *Lactococcus lactis* strain where the lactocepin lacks the three domains. Further study of the domains includes investigating how the three domains affect specificity, binding of the casein micelle and the uptake of digested peptides. Methods to do so will include further milk acidification experiments and analysis of the peptides digested by the different isogenic lactocepins. Other known lactocepins will also be tested in the same way to see if the same characteristics of the domains are observed.

[P090] FIRST REPORT OF WHITE LEAF DISEASE ON RICE CAUSED BY METHYLOBACTERIUM IN VIETNAM

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[1]*Tokyo University of Agriculture and Technology Fuchu Campus, Biological production of Science, Fuchu, Japan*

First report of white leaf disease on rice caused by *Methylobacterium* in Vietnam

The Vietnam is one of the largest rice-producing areas globally. Mekong Delta of Vietnam provides more than half of rice production in this country (Sebesvari et al. 2012). From December 2014, the new disease rice with white leaves were observed at several provinces in Mekong delta (Supplementary figure 1). The symptom appeared around 15-20 days after seed sowing. The infected plants showed drastically retarded growth and occasionally died.
This study aims to identify the pathogens causing the new disease on rice plants. In December 2017, rice plants exhibiting the symptom from 3 provinces (Vinh Long, Can Tho and Hau Giang) in Mekong Delta, Vietnam were collected and analyzed. Finally, seven pathogens were isolated and demontrated to cause the same original symptom.
All the seven isolates formed pink- or red colonies on MS-methanol media and exhibited characteristics consistent with *Methylobacterium* spp. such as rod shape, motile, aerobic, and Gram-negative. The 16S rRNA and *atpD* gene sequences of these seven isolates were 100% identical to *M. indicum* SE2.11^T in NCBI database. Thus the isolates were identified as pathogenic *M. indicum.*
This is the first report indicate the *Methylobacterium* strains were classified into pathogenic groups and cause white leaf disease on rice plants in Mekong delta of Vietnam.

Figure 1: White leaf disease on rice plants in Mekong delta of Vietnam (Vinh Long province). There were rice plants infected by white leaves with the rate from 10% - 40%. The symptom would be appeared after 15 – 20 days sowing. The infected plants showed drastically retarded growth and occasionally died.

[P091] TEMPERATURE SENSITIVITY OF OSMOADAPTATION SYSTEMS IN RESTING CELLS PREPARED FROM MARINE VIBRIO SP.

Yue Yin[1], Haruo Mimura[1]

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Introduction: Rapid adaptation to salinity changes is crucial for marine bacteria to survive. Accumulation of osmoprotectants, which are biosynthesized in the cytoplasm or transported into the cytoplasm, mitigates osmotic stress. For example, *Vibrio parahaemolyticus* and *Vibrio cholerae* synthesize ectoine in high salinity medium [Ongagna-Yhombi et al., 2013; Pflughoeft et al., 2003]. Transport systems of some osmoprotectants need Na^+ as a coupling ion such as betaine transporter BetP in *Corynebacterium glutamicum* [Ressl et al., 2009], L-proline transporter PutP in *Escherichia coli* and OpuE in *Bacillus subtilis* [Carsten von Blohn, et al., 1997; Pirch, et al., 2002;]. On the other hand, excess accumulation of Na^+ is adverse to viability of bacteria, while abundant existence of K^+ in the cytoplasm maintains the osmotic equilibrium, activities of many enzymes, intracellular pH, and reduces the negative charge of DNA as well. High affinity K^+-transporter of *E. coli* and *Bacillus subtilis* can bind and take K^+ efficiently, even at lower concentration. High concentrations of KCl externally loaded cause the same cationic and osmotic stresses to the bacterial cells as those by NaCl chemically. However, the adaptation mechanisms under hyper KCl stress seem to be different from those under hyper NaCl stress. We studied the survivability under hyper KCl stress by using resting cells.

Experimental methods: *Vibrio* sp. September 1, isolated from a ship's ballast water, was used in this study. Growth at high concentrations of NaCl or 0.8 M KCl and 50 mM NaCl was carried out at 30 and 37°C, and turbidity was measured at given time. Cells grown at early stationary phase were harvested and washed twice with 50 mM HEPES-TMAH buffer, pH 7.5, containing the same concentrations of salts in the growth medium. After washing with the mixture, cells were suspended in the same mixture to prepare resting cells.
The number of surviving cells was counted after exposure to higher concentrations of NaCl alone or KCl and 50 mM NaCl at 30 or 37°C in the absence and presence of an osmoprotectant.

Results and discussion: We examined the survivability of resting cells under hyper salt stress in the presence or absence of betaine at 30 and 37˚C. After 6 h of exposure at 30 or 37˚C in the presence of 1.8 M NaCl, the number of surviving cells increased 1-log cycle by the addition of 50 mM betaine (Fig. 1). The number of surviving cells maintained 1-log cycle by the addition of 50 mM betaine at 37°C when resting cells were exposed to 1.2 M KCl and 50 mM NaCl for 3 h. On the other hand, the value was drastically reduced to less than 1-log order of magnitude when the temperature of exposure was at 37°C. Those results indicated that osmoadaptation system(s) toward hyper KCl stress is temperature sensitive, and 50 mM betaine functions to mitigate hyper KCl stress at 37°C.

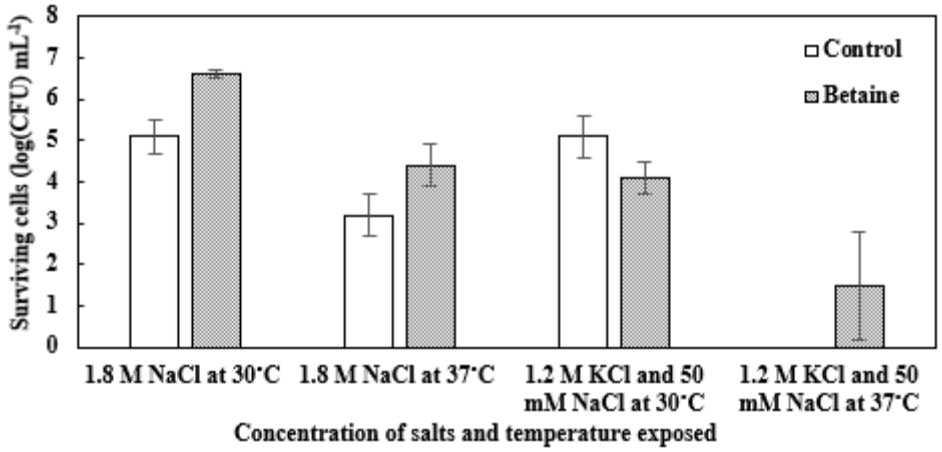

Fig. 1 Effect of betaine externally added on the survivability under hyper salt stress. Each data shows mean value ± SD (n=3). Resting cells were prepared from cells grown in the medium containing 0.5 M NaCl at 30˚C. The number of surviving cells were counted after exposer to 1.8 M NaCl for 6 h at 30 and 37°C or 1.2 M KCl and 50 mM NaCl at 30 and 37°C for 3 h.

Key words: marine *Vibrio* sp., osmoprotectants, hyper KCl stress, osmoadaptation

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[P092] TEMPERATURE SENSITIVITY OF OSMOADAPTATION SYSTEMS IN RESTING CELLS PREPARED FROM MARINE VIBRIO SP.

Yue Yin[1], Haruo Mimura[1]

[1]*Graduate School of Maritime Sciences, Kobe University, Kobe, Japan*

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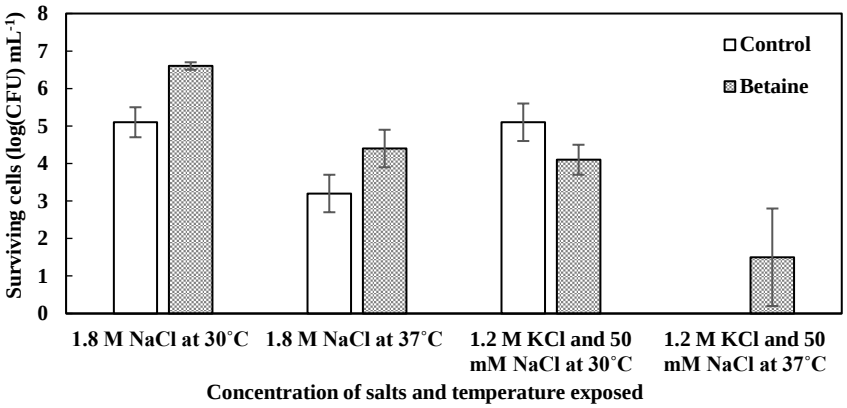

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[P093] DETERMINANTS OF METABOLIC ACTIVITY AND BIOFILM AGGREGATE SIZES AND - DISTRIBUTION IN A NEW IN VIVO-LIKE BIOFILM MODEL

Mads Lichtenberg[1], Sara Louise Worregaard Larsen[1], Kasper Nørskov Kragh[1,2], Dion Terwiel[3], Cristian Picioreanu[3], Peter Østrup Jensen[1,2], Michael Kühl[4], Thomas Bjarnsholt[1,2]

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Bacteria in chronic infections typically consist of relatively small (~20-200 µm wide) cell aggregates embedded in host material and surrounded by polymorphonuclear leukocytes that impose strong O_2 depletion and slow growth of the pathogenic bacteria. However, most *in vitro* studies of biofilms involve use of microtiter plate assays or other surface associated assays, which do not reproduce the mentioned *in vivo* growth patterns. We recently introduced growth of pathogenic bacteria (*Pseudomonas aeruginosa*) in alginate beads as a simple model system for studying embedded biofilm aggregates. The model shows *in vivo*-like growth of pathogenic bacteria, and enables application of a wide range of experimental methods at high reproducibility and replication.
We now have a method for accurately controlling the size distribution of aggregates within the alginate beads by modulating the inoculation density which alters the resource availability during biofilm formation thereby shaping the size and spatial distribution of aggregates.
By implementing these findings in mathematical models, we have developed a framework for addressing questions about the growth patterns observed in chronic infections regarding growth limitation of aggregates, susceptibility toward antibiotics etc.

[P094] PHYSIOLOGICAL RESPONSES TOWARDS PERTURBATION OF INTRACELLULAR ATP AND ENGINEERING OF SACCHAROPOLYSPORA ERYTHRAEA FOR ENHANCED PRODUCTION OF ERYTHROMYCIN AND REDUCTION OF A REDDISH PIGMENT

Xiaobo Li[1], Jun Chen[1], Joakim Andersen[1], Ju Chu[2], Peter Ruhdal Jensen[1]

[1]*Technical University of Denmark, National Food Institute*
[2]*East China University of Science and Technology, State Key Laboratory of Bioreactor Engineering*

Saccharopolyspora erythraea is used for erythromycin production in industry. To explore the physiological role of intracellular energy state in metabolic regulation by *S. erythraea*, we initially overexpressed the endogenous F_1-ATPase in a high erythromycin-producing strain, E3. The F_1-ATPase expression resulted in lower [ATP]/[ADP] ratios, which was accompanied by a drastic increased production of a reddish pigment and a decreased erythromycin production. Subsequent transcriptional analysis revealed that the lower intracellular [ATP]/[ADP] ratios appeared to exert a pleotropic regulation on the metabolism of *S.erythraea*. The lower [ATP]/[ADP] ratios induced physiological changes to restore the energy balance, mainly via pathways that tend to produce ATP or regenerate NADH. The F_1-ATPase overexpression strain exhibited a state of redox stress, which was correlated to the alteration of electron transport at the branch of the terminal oxidases. *S. erythraea* rechanneled the enhanced glycolytic flux towards a reddish pigment in order to reduce NADH formation. The production of erythromycin was decreased which is in accordance with the net ATP requirement and the excess NADH formed through this pathway. Partial growth inhibition by apramycin increased the intracellular [ATP]/[ADP] ratio demonstrated a positive correlation between [ATP]/[ADP] ratios and erythromycin synthesis. Finally, overexpression of the entire F_1F_0-ATPase complex resulted in 30% enhanced erythromycin production and markedly reduced pigment synthesis in E3. The work present a feasible strategy for simultaneous regulation of different secondary metabolism.

Keywords: ATPase; redox regulation; energy metabolism; secondary metabolism; *Saccharopolyspora erythraea*

[P095] ISOLATION OF MICROPOLLUTANT DEGRADING MICROBES FROM PHARMACEUTICAL SPIKED MOVING BED BIOFILM REACTORS

Joseph Donald Martin[1], Selina Tisler[1], Kai Bester[1], Lars H Hansen[2], Carsten Suhr Jacobsen[1], Lea Ellegaard-Jensen[1]

[1]*Aarhus University, Environmental Science, Roskilde, Denmark*
[2]*University of Copenhagen, Frederiksberg Campus, Plant and Environmental Sciences, Frederiksberg, Denmark*

Micropollutants are of increasing concern to both aquatic ecosystems and human health due to their persistent nature and their tendency to bioaccumulate. Micropollutants consist of a broad spectrum of contaminants with concentrations within the ng/L-µg/L range and originate from a multitude of anthropogenic and natural sources. Pharmaceuticals, specifically, are a notable fraction of micropollutants that are most commonly emitted from wastewater treatment plants due to inefficient treatment technologies. Water treatment facilities typically lack the ability to completely remove these pollutants, due to their low concentrations and specific physiochemical properties. Biofilm based treatment methods, however, have shown to influence the removal of micropollutants as a polishing step when applied to standard wastewater treatment plants, methods including moving bed biofilm reactors (MBBR). This study is designed to investigate whether cultivation of a community of microorganisms can be directed towards gaining a greater affinity for degrading pharmaceutical micropollutants. The hypothesis being, organisms that have been in the MBBR system longer will have better adapted to the micropollutants and thus degrade these compounds at a faster rate. In this study, the inflow of a laboratory-scale MBBR is continuously spiked with five specific pharmaceuticals, two antidepressants (Tramadol and Venlafaxine), two x-ray contrast media (Iomeprol and Iohexol), and one anticonvulsant (Carbamazepine). Throughout the spiking process, biofilm covered MBBR carriers, will be sampled and used as inoculum for a series of consecutive enrichment cultures. These enrichment cultures will additionally be used to indicate whether the micropollutant degradation is occurring, based on the rate at which the pharmaceuticals are being degraded. The micropollutant concentrations of these cultures will be routinely monitored through liquid chromatography-mass spectrometry chemical analysis. A full community analysis will be conducted, meaning the role of bacteria, fungi, and protists will all be investigated enabling an in-depth analysis of the complete biofilm ecosystem involved in the micropollutant degradation process.

Others

[P096] BACTERIAL LIFESTYLES IN PREFERENTIAL FLOW PATHS OF A CLAYEY TILL

Frederik Bak[1], Christoph Keuschnig[2], Ole Nybroe[1], Jens Aamand[3], Mette Haubjerg Nicolaisen[1], Timothy Vogel[2], Catherine Larose[2]

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[2]*Ecolé Centrale de Lyon, France*
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Clayey tills contain preferential flow paths such as biopores and fractures that harbour distinct microbial communities compared with the adjacent matrix sediments. Currently, little is known about the life styles of bacterial communities in these flow paths at different depths, and their adaptations to environmental stresses derived from changing exposure to nutrient, oxygen and water. Here, we applied shotgun metagenomics to DNA extracted from biopores and fractures in a clayey till to a depth of 4 meter below ground surface. We found that communities in biopores had higher abundance of genes related to flagellar motility and plant material degradation than communities in the surrounding matrix sediments. Additionally, the shallow biopore microbial communities had more functions related to protection against desiccation and oxygen stress compared to communities in deeper fractures. In the fracture communities, functions enabling microorganisms to resist environmental variation in pH, salinity and low nutrient availability were abundant. Abundant functions in the surrounding matrix sediment communities indicated that microbes in this habitat rely on degradation of amino acids and peptides for carbon and nitrogen sources, and on the synthesis of essential vitamins. Our results indicate that more microorganisms in preferential flow paths are motile and they are better adapted to resist environmental stress than microorganisms in the surrounding matrix sediments.

[P097] EFFECT OF A-HEMOLYSIN PRODUCING E. COLI IN TWO DIFFERENT MICE STRAINS/ BREADS IN A DSS MODEL TO STUDY IBD

Hengameh Chloe Mirsepasi-Lauridsen[1], Hyungjun Yang[2], Carsten Struve[3], Andreas Munk Petersen[4 5], Karen Angeliki Krogfelt[6 7], Xiujuan Xiujuan Wu[2], Caixia Ma[8], Hongbing Yu[2], Kevan Jacobson[2], Bruce Vallance[2]

[1]Statens Serum Institut, Department of Bacteria, Parasites and Fungi, København S, Denmark
[2]BC Children's Hospital, Division of Gastroenterology, Vancouver, Canada
[3]Statens Serum Institut, Department of Bacteria, Parasites and Fungi, København S, Denmark
[4]Hvidovre Hospital, Department of clinical Microbiology, Hvidovre, Denmark
[5]Hvidovre Hospital, Department of Gastroenterology, Hvidovre, Denmark
[6]Roskilde University, Institute of Molecular and Medical Biology, Roskilde, Denmark
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[2]BC Children's Hospital, Division of Gastroenterology, Vancouver, Canada

Background: Phylogroup B2 *Escherichia coli* (*E. coli*) have been associated with Ulcerative Colitis (UC). Studies show, UC associated *E. coli* p19A from B2 phylogroup, harbouring two alpha hemolysin genes, induces cell death and disrupts tight junctions in vitro by dissolving occludin. **In this study the aims are to compare colonization with** p19A in Sigirr−/− and C57BL/6 mouse, to evaluate pathogenic features of *E. coli* p19A and to investigate the role of *E. coli* alpha hemolysin gene in UC mouse model.

Methods: Sigirr$^{-/-}$ and C57BL/6 mice were pre-treated with vancomycin (5 mg per mouse) twice. 3.5% of DSS was added into drinking water for 4 days. UC associated *E. coli* p19A expressing luminescence and GFP, alpha-hemolysin knock out p19A-ΔhlyI, II and non-pathogenic lab *E. coli* DH10B were cultured in LB broth, OD_{600} ≈2.5x 10^8. 100 µl of bacterial culture were orally gavaged into mice. Mice were monitored every day and euthanized on 5th day post-infection. Colonization with p19A WT was visualized using in-vivo imaging system.

Results: Sigirr $^{-}$ / $^{-}$ mouse infected with p19 WT lost ≥15% of bodyweight, while p19A ΔhlyI, II and non-pathogenic *E. coli* infected mice lost ≤5%. Survival analysis of infected p19A WT mice at 5th day post infection shows ≈80 % survival. p19A WT colonized liver and spleen of infected C57BL/6 and Sigirr $^{-}$ / $^{-}$ mouse. p19A WT colonized Peyer's patches and ileum of infected Sigirr $^{-}$ / $^{-}$ mouse.

Conclusion: Sigirr -/- mouse is a better model to study Crohn's disease, where inflammation/infection occurs in ileum and Peyer's patches. UC-associated *E. coli* p19A WT colonizes the intestines of DSS-treated mice and causes extra-intestinal infection. Hemolysin is an important factor in pathogenesis since isogenic hemolysin mutants did not cause the same inflammation.

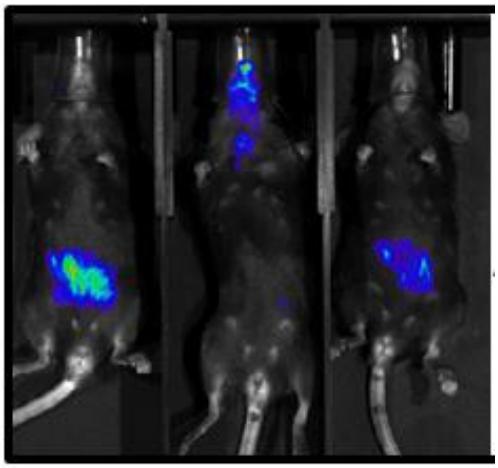

Figure 1 IVIS evaluation of experimental mouse

1) P19A WT infected Sigirr $^{-}$ / $^{-}$ mouse shows luminescent signals from the mouth and intestinal tissues, before and after washing the intestinal contains with PBS.

[P098] EXPLORING ENVIRONMENTAL SOURCES OF ARCHAEAL LIPIDS FOR IMPROVING LIPOSOMES FOR ORAL DRUG DELIVERY

Mette Sloth Bohsen[1], Kenneth Hesseldahl Zipfel[1], Nerea Uri[2], Per Henrik Nielsen[2], Martin Brandl[1], Alexander H Treusch[1]

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While the oral route of drug administration is very cost efficient and has high patient compliance, challenges that especially modern therapeutic protein and peptide drugs face are, among others, premature degradation and poor permeability over the intestinal barrier. The use of nanoparticulate drug carriers like liposomes (spherical vesicles surrounded by a phospholipid bilayer) is a promising way of overcoming these challenges. Advanced liposome formulations often contain enhancers to further stabilize the lipid bilayer, with archaeal tetraether lipids (TELs) being a fairly recently established enhancer. TELs can be found in hyperthermophilic *Crenarchaeota*, however their demanding growth requirements make the biotechnological production of TELs so far uneconomical. Yet unexplored sources of TELs are environmental *Thaumarchaeota*, which can be found in many environments including the ocean, soil, freshwater and also wastewater treatment plants (WWTP). To explore if indeed TELs from *Thaumarchaeota* can be used as enhancers of liposome stability, we collected biomass from different biological treatments at the Ejby Mølle WWTP (Odense) and subsequently optimized protocols for extracting lipids, settling on a modified Blight & Dyer protocol. These efforts were guided by a screening of the biological treatments of the WWTP for *Archaea*, indicating the presence of *Thaumarchaeota*, although often at low abundances. The initial results of these studies will be presented and discussed.

[P099] MICROBIAL CONSORTIUM INVOLVED IN PLANT-MICROBE INTERACTIONS BENEFITS DROUGHT PLANTS AND THEIR EFFECTS ON PLANT GROWTH

Nan Yang[1], Henriette L. Røder[1], Joseph Nesme[1], Zhangli Zuo[2], Morten Petersen[2], Mette Burmølle[1], Søren Sørensen[1]

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[2]Department of Biology, Faculty of Science, University of Copenhagen, Copenhagen, Denmark

Drought is a major restriction on plant production and global warming is causing more drought stress for crops. Hence, it is needed to find sustainable solutions to alleviate such stress. There is increasing information about the ability of special soil microbes to affect drought resistance in plants. Taking advantage of these promising microoganisms to enhance plant's ability to overcome abiotic stresses such as drought is currently considered as the key to development of new strategies for sustainable bio-protection. Here we discovered a microbial consortium consisting of four different bacterial species: *Stenotrophomonas rhizophila*, *Xanthomonas retroflexus*, *Microbacterium oxydan* and *Paenibacillus amylolyticus* from natural soil, is able to synergistically produce more biofilm together than the sum of four single-species biofilm. The objective of this study is to investigate the potential of this four species consortium to help *Arabidopsis taliana* plants cope with drought under *in vivo* conditions based on a hypothesis that such biofilm could protect plants from drought by retaining more water. Preliminary results showed that addition of the four bacterial consortium before drought significantly increased the survival rate and biomass of Arabidopsis under water shortage, which indicated that this consortium could improve plant tolerance against drought.

[P100] IMMOBILIZATION OF MICROBIAL HEPARINASE ENHANCED THE PRODUCTION OF LARGE HEPARIN OLIGOSACCHARIDES

Indu Bhushan Sharma[1]

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Heparin is a naturally occurring anticoagulant and is also used to treat deep vein thrombosis, pulmonary embolism etc. Its major side effects are bleeding and thrombocytopenia. The development of heparin oligosaccharides considered as a better therapeutic agent than unfractionated heparin or disaccharides (dp 2), because of the possibility to reduced non-specific interactions, heterogeneity and have been reported to act as a metastasis in various types of cancers, tumor invasion, inflammation and angiogenesis. Synthesizing longer heparin oligosaccharides takes years of effort and is highly structure-specific. Enzymatic depolymerisation using heparinases is one of the method for production of heparin oligosaccharides. However, uses of heparinases have been hampered due to their undesirable properties such as its efficient catalytic reactions under mild conditions, poor stability and very high cost. Keeping in view the tremendous potential of heparinase for pharmaceutical industries and to make it economical as well as more stable, in this study heparinase was immobilized on CNBr-activated Sepharose, designed immobilized heparinase reactor and and used for production of longer heparin oligosaccharides. Immobilized Heparinase retained high efficiency for depolymerization of heparin at various range of pH (5–8) and temperature (5°–50° C). The optimum parameters i.e., at pH 5.5 and temperature 15°C found better for production of larger proportions of variably sulfated oligosaccharides. Immobilized heparinase had been used 10 cycles for degradation of heparin and observed stability in activity on reuse.

[P101] ONE SIZE DOES NOT FIT ALL; THE GAP BETWEEN STANDARDIZED IN VITRO BIOFILM-INFECTED WOUND MODELS AND IN VIVO CLINICAL SETTINGS

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Introduction: Progressively, biofilms are becoming more recognized as the reason why chronic wounds fail to heal. As a result of this, a whole new market for therapeutic products specifically targeting biofilms in wounds has emerged. In order to develop new antibiofilm-specific wound dressings, a need has arisen for appropriate *in vitro* wound models which simulate the complex environment found in biofilm-infected wounds. Nonetheless, as there is much we still do not know about the variety of factors contributing to the high tolerance found in such biofilms, mimicking these conditions *in vitro* is still a work in progress. In this rapidly evolving field several different models have been, and still are, continuously being created attempting to provide a solid and relevant platform on which new antibiofilm therapeutics can be tested.

Hypothesis and aims: We hypothesize that the current standardized biofilm methods are not correlated with the present knowledge of chronic biofilm infections and our aim was to elucidate the discrepancies which exist between the currently used standardized biofilm models and the translation of these into *in vivo* clinical settings.

Methodology: Clinical observations along with literature research and comparisons to current standardized methods.

Results: There is still no harmonized consensus regarding the use of standard models for testing antibiofilm efficacy of new treatments. To date, only a handful of standardized protocols deals with the elimination of nosocomial relevant biofilms. Yet, the applicability of these models in a clinical situation is often lacking. The gap between the currently recognized *in vitro* standard models and the *in vivo* medical setting is simply too great, and as a result of this, antibiofilm-specific wound dressings often fail to produce satisfying results when applied *in vivo*.

Conclusion: There is a need to recognize that the standardized methods are not suitable for all circumstances; one size does not fit all.

[P102] GROWTH ON CHITIN ALTERS THE METABOLOME OF THE MARINE PSEUDALTEROMONAS. RUBRA S4059

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The marine genus *Pseudoalteromonas* is phenotypically and genetically divided into two groups: pigmented and non-pigmented. Genome mining using antiSMASH has demomonstrated that the pigmented strains harbor 10-25 biosynthetic gene clusters (BGCs) of which the chemistry is only known for a fraction. Pigmented *Pseudoalteromonas* thus represents a rich source of novel bioactive compounds with drug potential. Also, the genus has a highly developed chitinolytic machinery and since we have previously found that growing on chitin may induce biosynthetic gene clusters (BGSs) in secondary metabolite producing Vibrionaceae (Giubergia et al. 2016), we here explore the relationship between the chitinolytic machinery and secondary metabolite production in marine *Pseudoalteromonas*.

We analyzed the genomes of 101 *Pseudoalteromonas* collected on the Galathea3 expedition. All chitinolytic strains contained enzymes of the GH18 family. All the pigmented *Pseudoalteromonas*, which displayed antibacterial activity, harboured GH19 chitinases while these were only found in a few non-pigmented strains. Phylogenetic analyses of the GH19 sequences indicated that the enzymes found in marine Proteobacteria (*Pseudoalteromonas* and *Vibrionaceae*) represent a unique group. We hypothesized that there could be a link between the GH19 chitinases and secondary metabolite potential and therefore constructed a GH19 deletion mutant in a *P. rubra* S4059 strain. Genetic manipulation of *Pseudoalteromonas* is challenging and we here present a conjugation based method allowing manipulation. Surprisingly, the mutant grew as well as the wild-type on both colloidal and crystalline chitin and both strains caused the same size clearing zone on chitin agar. However, LC/MS-analyses of the metabolome found the wildtype produced larger amounts of compounds as compared to mutant when grown on crystalline chitin. Also, several compounds were produced in higher amounts when the wild type was grown on chitin as compared to mannose (an algal monomer). The results suggest that there is a link between production of these compounds and chitin metabolism, especially GH19, in *P.rubra*.

[P103] IDENTIFICATION OF AN INCK PLASMID ENCODING ESC BY BLACMY-2 IN ESCHERICHIA COLI ISOLATED FROM POULTRY IN DENMARK

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A wide range of resistance plasmids carrying AmpC β-lactamase encoding extend-spectrum cephalosporin resistance (ESC) in Gram-negative bacteria plays an important role in transmission antimicrobial resistance causing severe global public problem. The bla_{CMY-2} is the most common plasmid-encoding AmpC β-lactamase gene in *E.coli* worldwide. In this study, twenty-one ESC resistant *E.coli* isolates from Danish poultry production were selected due to identical plasmid replicon type (pMLST) and all encoding bla_{CMY-2}. All these belong to ST-type ST429. Genomic DNA was extracted, fragment libraries were constructed and sequencing by paired-ended MiSeq were constructed (Illumina). All contigs were submitted to the Bacterial Analysis Platform provided by the Center for Genomic Epidemiology, CGE. The sequence analysis of the strains were identified using MLST 2.0. ResFinder v3.1 was used to detect antimicrobial resistance genes. Finally, BLAST analysis of all the draft genomes was performed by using the Gview Server. The results shows that plasmid replicon types IncK was identified for the majority of bla_{CMY-2}-producing *E.coli*. Apart from bla_{CMY-2}, the plasmids also encoded the *aadA1* and *mdfA* antibiotic resistance genes. The twenty-two plasmids had high level of similarity in structure and organization to previously published incK plasmids like pNVI1292 and published plasmids of human and animal origin. All investigated plasmids had conserved backbones with high level of similarity in structure indicating a common origin. Data provided here shows that bla_{CMY-2} producing IncK type plasmids among *E.coli* possibly plays an important role in dissemination of ESC resistance.

[P104] LIFE IN THE DARK: FAR-RED ABSORBING CYANOBACTERIA EXTEND PHOTIC ZONES DEEP INTO TERRESTRIAL CAVES

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Chlorophyll (Chl) *f* and *d* are the most recently discovered chlorophylls, enabling cyanobacteria to harvest near-infrared radiation (NIR) at 700–780 nm for oxygenic photosynthesis. Little is known about the occurrence of these pigments in terrestrial habitats. Here, we provide first details on spectral photon irradiance within the photic zones of four terrestrial cave systems in concert with a detailed investigation of photopigmentation, light reflectance and microbial community composition. We frequently found Chl *f* and *d* along the photic zones of caves characterized by low light enriched in NIR and inhabited by cyanobacteria producing NIR- absorbing pigments. Surprisingly, deeper parts of caves still contained NIR, an effect likely attributable to the reflectance of specific wavelengths by the surface materials of cave walls. We argue that the stratification of microbial communities across the photic zones of cave entrances resembles the light-driven species distributions in forests and aquatic environments.