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Microbiota analysis to reveal temperature abuse of fresh pork

Buschhardt, Tasja; Bahl, Martin Iain; Hansen, Tina Beck; Brejnrod, Asker Daniel; Abu Al-Soud, Waleed; Mortensen, Martin Steen; Sørensen, Søren Johannes; Aabo, Søren

Published in:
The Danish Microbiological Society Annual Congress 2017 - Programme & Abstracts

Publication date:
2017

Document Version
Publisher's PDF, also known as Version of record

Link back to DTU Orbit

Citation (APA):
Buschhardt, T., Bahl, M. I., Hansen, T. B., Brejnrod, A. D., Abu Al-Soud, W., Mortensen, M. S., Sørensen, S. J., & Aabo, S. (2017). Microbiota analysis to reveal temperature abuse of fresh pork. In *The Danish Microbiological Society Annual Congress 2017 - Programme & Abstracts* (pp. 49-49). Article P33 American Society for Microbiology.

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The Danish Microbiological Society Annual Congress 2017

Programme & Abstracts

Monday, 13 November 2017
Eigtveds Pakhus
Copenhagen

DMS Congress 2017 - PROGRAMME

09:00	**Registration, poster mounting and coffee**	
	ROOM II *Second floor*	**ROOM III** *Second floor*
10:00	**Welcome and opening address**	**Welcome and opening address**
10:15	**Understanding the interactive nature of aquatic microbial communities from genomic data** Stefan Bertilsson, *Dept. of Ecology and Genetics, Uppsala University*	**Treating wounds in the face of antibiotic resistance** Rose Cooper, *Cardiff School of Health Sciences, Cardiff Metropolitan University*
10:45	**Coffee and exhibition**	*First & second floor*

	PARALLEL SESSIONS		
	ROOM II *Second floor*	**ROOM III** *Second floor*	**ROOM IV** *Second floor*
	Applied bioinformatics Chair: Mads Albertsen, *Dept. of Chemistry and Bioscience, Aalborg University*	**Clinical microbiology and beyond: recent developments** Chair: Michael Kemp, *Dept. of Clinical Research, University of Southern Denmark*	**Microbiome dynamics in changing ecosystems** Chair: Alexander H. Treusch, *Dept. of Biology, University of Southern Denmark*
11:00	Chair introduction	Chair introduction	Chair introduction
11:05	**Towards a fully populated tree of life** Søren M. Karst, *Center for Microbial Communities, Aalborg University*	**Translational microbiology: from malaria to cancer** Ali Salanti, *Centre for Medical Parasitology, University of Copenhagen*	**Deluged – Soil microbiome responses to the flooding with seawater** Alexander H. Treusch, *Dept. of Biology, University of Southern Denmark*
11:30	**Human gut microbiota – host metabolic interactions in insulin resistance** Helle Krogh Pedersen, *Novo Nordisk Foundation Center for Basic Metabolic Research, Section for Metabolic Genetics, Univ. of Copenhagen Denmark*	**Modelling epithelial infections** Thomas Emil Andersen, *Dept. of Clinical Microbiology, University of Southern Denmark*	**Patterns in microbial community composition and functional capacity in the Bay of Bengal** Beate Kraft, *Nordic Center for Earth Evolution, University of Southern Denmark*
11:45	**Using transcriptomics to elucidate the mode of action of antibiotics** Rikke Heidemann Olsen, *Dept. of Veterinary Disease Biology, University of Copenhagen*	**Large scale introduction of microbiological rapid tests in a clinical setting** Hanne M. Holt, *Dept. of Clinical Microbiology, Odense University Hospital*	**Life in a toxic environment - How do extreme redox conditions impact on N2 fixers in ocean oxygen minimum zones?** Carolin Löscher, *Dept. of Biology, University of Southern Denmark*
12:00	**Flash poster presentations***	**Flash poster presentations***	**Flash poster presentations***

12:15	**LUNCH**	*SALON C, ground floor*
12:15	**POSTERS**	*First floor*
12:15	**EXHIBITION**	*First & second floor*
12:15	GENERAL ASSEMBLY Det Danske Pasteur Selskab **Louis Pasteurs indsats og hans betydning for mikrobiologiens start i Danmark** Niels Høiby *Dept. of Clinical Microbiology, Rigshospitalet*	*ROOM IV*

	PARALLEL SESSIONS		
	ROOM II Second floor	**ROOM III** *Second floor*	**ROOM IV** Second floor
	Second messengers Chair: Tim Tolker Nielsen, *Dept. of Immunology and Microbiology, University of Copenhagen*	**Targeted changes in gut microbiota** Chair: Lars Hestbjerg Hansen, *Dept. of Environmental Science, Aarhus University*	**New perspectives in wastewater treatment by introducing circular economy** Chair: Per Halkjær Nielsen, *Dept. of Chemistry and Bioscience, Aalborg University*
14:15	Chair introduction	Chair introduction	Chair introduction
14:20	**Cyclic di-GMP-mediated multi-tiered regulation of biofilm formation in Burkholderia cenocepacia** Tim Tolker Nielsen, *Dept. of Immunology and Microbiology, University of Copenhagen*	**Metagenomics studies of the human intestinal microbiota: potentials and limitations** Oluf Pedersen, *Faculty of Health & Medical Sciences, Univ. of Copenhagen*	**Microbes are driving the circular economy in wastewater handling** Per Halkjær Nielsen, *Dept. of Chemistry and Bioscience, Aalborg University*
14:45	**Stress induced biofilms of Bacillus subtilis: the role of ppGpp** Ákos T. Kovács, *Dept. of Biotechnology and Biomedicine, Technical University of Denmark*	**Getting from A to B. How to manipulate the gut microbiota of mice and men** Dennis Sandris Nielsen, *Dept. of Food Science, University of Copenhagen*	**Controls of N2O production pathways in nitritation-anammox biomass** Marlene Mark Jensen, *Dept. of Environmental Engineering, Technical University of Denmark*
15:00	**Cyclic di-GMP signaling and proteolytic regulation of biofilm formation in Pseudomonas sp** Morten Rybtke, *Dept. of Immunology and Microbiology, Univ. of Copenhagen*	**Fecal Microbiota Transplantation: past, present and future.** Andreas Munk Petersen, *Gastro Unit, Medical Division, Hvidovre Hospital*	**Production of biochemicals using bacterial cell factories** Alex Toftgaard Nielsen, *Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark*
15:15	**Flash poster presentations***	**Flash poster presentations***	**Flash poster presentations***

15:30	Coffee and exhibition	*First & second floor*
16:00	Travel grant ceremony	*ROOM III, Second floor*
16:15	Probing the soil interactome Jan Roelof van der Meer, *Dept. of Fundamental Microbiology, University of Lausanne*	*ROOM III, Second floor*
17:15	Reception with fermented beverage	*SALON C, ground floor*
18:30	Optional congress dinner	*Spiseloppen, Christiania*

** Please see next page for information about the flash poster presentations*

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Flash poster presentations

	PARALLEL SESSIONS, MORNING		
	ROOM II *Second floor*	**ROOM III** *Second floor*	**ROOM IV** *Second floor*
	Applied bioinformatics	**Clinical microbiology and beyond: recent developments**	**Microbiome dynamics in changing ecosystems**
12:00	**[P2] Novel plasmid metagenome method captures accessory gene carrying plasmids** Tue Jørgensen, *Roskilde University*	**[P14] *Staphylococcus aureus* transcriptional changes during infection development in a guinea pig biofilm infection model** Yijuan Xu, *Danish Technological Institute*	**[P29] Coupling biogeochemical process rates and metagenomic blueprints of Baltic Sea bacterial assemblages in response to environmental changes** Trine Markussen, *University of Copenhagen*
12:05	**[P3] Transcriptomics of the cable bacterium Candidatus Electronema sp. GS** Casper Thorup, *Aarhus University*	**[P12] The molecular mechanism behind Thioridazine Resistance in *Staphylococcus aureus*** Claes Søndergaard Wassmann, *University of Southern Denmark*	**[P30] A novel prosthecate bacterium of the candidate phylum Acetothermia discovered in anaerobic digesters** Liping Hao, *Aalborg University*
12:10	**[P4] DAtest: A tool for comparing differential abundance and expression methods** Jakob Russel, *University of Copenhagen*	**[P84] Retrieval of individual bacterial genomes from complex microbial communities using high-throughput long-read DNA sequencing** Johannes Manner-Jakobsen, *Aalborg University*	**[P72] High Throughput Sequencing of Full-length SSU rRNA Sequences from Complex Microbial Communities without Primer Bias and how it Affects our Ability to Study Microbial Ecology** Jakob Brandt, *Aalborg University*

	PARALLEL SESSIONS, AFTERNOON		
	ROOM II *Second floor*	**ROOM III** *Second floor*	**ROOM IV** *Second floor*
	Second messengers	**Targeted changes in gut microbiota**	**New perspectives in wastewater treatment by introducing circular economy**
15:15	**[P88] Host proteins determine MRSA biofilm structure and integrity** Cindy Dreier, *Interdisciplinary Nanoscience Center (iNANO)*	**[P42] Impact of fungus-farming termite fungal diet on the gut microbiota of omnivorous cockroach Pycnoscelus surinamensis** Kristjan Germer, *University of Copenhagen*	**[P48] Rapid microbial surveillance using Nanopore DNA sequencing** Martin Hjorth Andersen, *Aalborg University*
15:20	**[P60] Evolution of phenotypic heterogeneity in *Bacillus subtilis* biofilms** Marivic Martin, *Technical University of Denmark*	**[P45] Administration of two probiotic strains during early childhood does not affect the endogenous gut microbiota composition despite probiotic proliferation** Martin Laursen, *Technical University of Denmark*	**[P52] Viral indicators for fecal contamination - A one-year viral metagenomic study of treatment efficiency in Danish waste water treatment plants** Maria Hellmér, *Technical University of Denmark*
15:25	**[P38] The role of intergenic mutations in pathoadaptation of *Pseudomonas aeruginosa*** Pavelas Sazinas, *Technical University of Denmark*	**[P46] Effect of fecal microbiota transplantation capsules in clinical studies** Patrick Dennis Browne, *Aarhus University, Roskilde*	**[P47] Some immigrating pathogenic bacteria go straight through full-scale wastewater treatment plants** Jannie Munk Kristensen, *Aalborg University*

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About DMS

The Danish Microbiological Society (DMS) is a professional association in the fields of human and veterinary medical microbiology, general microbiology, food microbiology, environmental microbiology and biotechnology. DMS has existed since 1958, and is dedicated to the advancement of microbiology, both applied and basic, and promotes microbiological information to the public. These aims are achieved by organizing annual congresses, workshops and symposia - and by taking part in the current microbiological debate.
Furthermore, DMS supports students with grants for travelling: applications for the two DKK 5000 grants can be submitted by 1 October each year.

By being a member of DMS, you are part of the advancement of microbiology in Denmark. Additionally, you are entitled to the following benefits from FEMS (Federation of European Microbiological Societies)

- Discounts at FEMS meetings
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- Possibility to apply for FEMS grants (Read more at https://fems-microbiology.org/fems-activities/grants)

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- Lars Bogø Jensen, National Food Institute, DTU
- Kasper Nørskov Kragh, Department of Immunology and Microbiology, University of Copenhagen
- Rikke Louise Meyer, Interdisciplinary Nanoscience Center (iNANO) and Department of Bioscience, Aarhus University
- Michael Thomas-Poulsen, Department of Biology, University of Copenhagen
- Katrine Uhrbrand, National Research Centre for the Working Environment

About the keynote session:
'Probing the soil interactome'
Jan Roelof van der Meer, *Dept. Of Fundamental Microbiology, University of Lausanne, Switzerland.*

Most bacteria in nature live in complex multi species communities (microbiota) rather than in isolation. The rules that control the formation, development, maintenance and evolution of microbiota are still very poorly understood, but are critical for future rational microbiota engineering efforts. Here I will describe key concepts and tools we use in a Swiss multi partner consortium study MicroScapesX to understand microbiota functioning and to redirect microbiota for new functional properties using strain inoculation.

The DMS Congress 2017 is supported by the Federation of European Microbiological Societies (FEMS). Read more about FEMS at www.fems-microbiology.org

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Sample to Insight

Invited talks

[O1] UNDERSTANDING THE INTERACTIVE NATURE OF AQUATIC MICROBIAL COMMUNITIES FROM GENOMIC DATA

Stefan Bertilsson[1]
[1]*Dept. of Ecology and Genetics, Uppsala University*

Metabolic interactions and dependencies can play a central role in structuring natural microbial communities while also defining their function and impact on biogeochemical cycles.

Here I will demonstrate and discuss how we can learn more about such interactions by mapping co-occurences and analysing metabolic features encoded in metagenomes, single cell genomes and metatranscriptomes.

Using examples from ecological observatories in freshwater lakes and aquifers, I will highlight and exemplify the interactive nature of such microbiomes and discuss the role of metabolite exchange, auxotrophies and other interactions in controlling the biogeography and functioning of aquatic microbiomes.

[O2] TREATING WOUND INFECTION IN THE FACE OF ANTIMICROBIAL RESISTANCE

Rose Cooper[1]
[1]*Cardiff School of Health Sciences, Cardiff Metropolitan University*

Today the treatment of overt wound infection relies mainly on antibiotics, but the sustained emergence of antibiotic-resistant microbial strains threatens a return to the pre-antibiotic era where antiseptics were routinely utilised. The range of antiseptics available for topical use on wounds is diverse and includes silver, iodine, chlorhexidine, octenidine and honey. Unlike antibiotics, whose mode of action affects specific cellular target sites in pathogens, antiseptics elicit inhibition in a more generalised manner by acting simultaneously on multiple target sites as oxidising or denaturing agents. This reduces the likelihood of selecting for antiseptic-resistant strains. However, reports of antiseptic-resistance have increased since the 1950s together with a small number of cases of cross-resistance between antibiotics and antiseptics. This indicates that both antibiotics and antiseptics be used judiciously to preserve efficacy. It also demonstrates an urgent need to search for innovative antimicrobial strategies and the need for increased vigilance in preventing wound infection. The role of antimicrobial stewardship in future wound care will be explored in this presentation.

[O3] TOWARDS A FULLY POPULATED TREE OF LIFE

Søren Michael Karst[1]
[1]*Center for Microbial Communities, Department of Chemistry and Bioscience, Aalborg University*

The phylogeny of small subunit (SSU) ribosomal RNA (rRNA) genes have been essential for the improvements in our understanding of microbial evolution and diversity since their discovery by Carl Woese and George Fox more than 40 years ago. Today the SSU gene phylogeny forms the basis for many important techniques used in microbial ecology such as microbial profiling by 16S/18S rRNA amplicon sequencing and single cell techniques using FISH microscopy. However, these techniques are dependent on curated, high quality reference databases of full-length SSU rRNA gene sequences. These databases are underpopulated, ecosystem skewed, and subject to primer bias and chimerism; which gives an incomplete view of the true diversity and undermines the results from methods using the databases. To remedy this we developed a method to generate high quality, full-length SSU rRNA sequences without primers from complex samples using high throughput sequencing. With this method we were able to generate 1.6 million SSU rRNA sequences from diverse range of ecosystems, thereby almost doubling the amount of sequences in the SILVA reference database. We observed a high fraction of novel Bacteria, Eukaryote and Archaea diversity including several deeply branching phylum level lineages related to the Asgard Archaea. This approach will allow expansion of the SSU rRNA reference databases by orders of magnitude and will enable a comprehensive census of the tree of life.

[O4] HUMAN GUT MICROBIOTA – HOST METABOLIC INTERACTIONS IN INSULIN RESISTANCE

Helle Krogh Pedersen[1]
[1]*Novo Nordisk Foundation, Center for Basic Metabolic Research, Section for Metabolic Genetics, University of Copenhagen*

Insulin resistance is a forerunner state of cardiovascular disease and type 2 diabetes. By integrating human gut metagenomics, untargeted serum metabolomics, and diabetes-related host phenotypes in 277 non-diabetic Danish individuals, we show how the gut microbiome impacts the serum metabolome and associates with insulin resistance. The serum metabolome of insulin-resistant individuals is characterized by increased levels of branched-chain amino acids (BCAAs), which correlates with a gut microbiome that has an enriched biosynthetic potential for BCAAs and is deprived of genes encoding bacterial inward transporters for these amino acids. *Prevotella copri* and *Bacteroides vulgatus* are identified as the main species driving the association between biosynthesis of BCAAs and insulin resistance, and in mice, we demonstrate that *P. copri* can induce insulin resistance, aggravate glucose

intolerance and augment circulating levels of BCAAs. Our findings suggest that microbial targets may have the potential to diminish insulin resistance and reduce the incidence of common metabolic and cardiovascular disorders. On the analytical side, the study showcases an approach for extracting biological insight from a multi-dimensional data-cube.

[O5] USING TRANSCRIPTOMICS TO ELUCIDATE THE MODE OF ACTION OF ANTIBIOTICS

Rikke Heidemann Olsen[1]
[1]*Department of Veterinary Disease Biology, University of Copenhagen*

Sertraline is a frequently used human antidepressant. It is, however, also a (weak) antimicrobial. The minimal inhibitory concentration (MIC) for sertraline is 32 mg/Liter for *Escherichia coli*. Furthermore, sertraline may decrease the MIC value of antimicrobial tetracycline in strains of *E. coli* with efflux-pump mediated tetracycline resistance. It has been suggested that sertraline acts synergistically with tetracycline due to efflux-pump inhibition, while the mode of antimicrobial activity for sertraline has not been described at all. In the present study, we used a transcriptome-based analyses to propose a model for the antimicrobial activity as well as the tetracycline-synergistically commotion of sertraline.

[O6] TRANSLATIONAL MICROBIOLOGY: FROM MALARIA TO CANCER

Ali Salanti[1]
[1]*Centre for Medical Parasitology at Rigshospitalet & University of Copenhagen, Institute of Immunology and Microbiology.*

As an immune evasion survival strategy the *P. falciparum* malaria parasite has evolved a protein named VAR2CSA, which mediates parasite binding in the placenta through interaction with uniquely sulfated Chondroitin Sulfate A (CSA) proteoglycans on the trophoblast cells, thereby avoiding circulation in the blood. This unique type of CSA is exclusively found in the placenta and plays a key role in the rapid growth, expansive growth and immune privilege of this organ. These are all features shared with cancer, and our malaria research led us to examine if this same type of CSA could play a role in tumor growth. Using recombinant VAR2CSA (rVAR2) we showed that the vast majority of cancer cell lines (x>200) have this oncofetal CSA (of-CS) and thus binds the malaria protein rVAR2, with no binding to primary non-cancer cells. Pull down experiments and mass spectrophotometry demonstrate that this secondary of-CS modification is present on at least 25 well defined (cancer expressed) proteoglycans. Functional studies demonstrate that VAR2CSA binding to CSA on cancer cells hinders growth, migration and invasion by hindering focal adhesion. VAR2CSA binds specifically to human cancer biopsies with no binding to adjacent normal tissue, and staining

correlates with progression of cancer. In this we have stained more than 3000 biopsies, from very different types of cancer, with more than 95% being positive. These basic findings forms the basis for our work towards a broadly effective cancer treatment, and data from our drug conjugate program, immunotherapy program and diagnostic pipeline will be presented.

[O7] MODELLING EPITHELIAL INFECTIONS

Thomas Emil Andersen[1]
[1]*Dept. of Clinical Microbiology, University of Southern Denmark*

The infectious potential of pathogenic bacteria is closely linked to the ability of the bacterium to effectively colonize and invade the outer barriers of the host. A main site of entrance is the epithelium mucous membranes, where invading bacteria initially must overcome defense mechanisms such as fluid flow and immune effectors. For the past years we have worked on infection models based on host-cell bacteria co-culturing in flow chambers. In the talk, a method will be presented in which epithelial infections by pathogenic bacteria is simulated by seeding of bacteria on flow chamber-grown epithelial cell layers, under conditions that simulate the physiological microenvironment. Quantification of bacterial adhesion and colonization of the cell layers is performed by in *situ time*-lapse fluorescence microscopy and automatic detection of bacterial surface coverage. Three different infection models will be introduced, simulating *S. aureus* endothelial infection and *Escherichia coli* intestinal- and uroepithelial infection. The approach yields information on the fitness of the bacterium to successfully colonize these surfaces under physiological conditions and can be used for evaluating the influence of specific genes, growth conditions and antimicrobial treatment on the adhesive/invasive properties of bacteria.

[O8] LARGE SCALE INTRODUCTION OF MICROBIOLOGICAL RAPID TESTS IN A CLINICAL SETTING

Hanne M. Holt[1]
[1]*Dept.of Clinical Microbiology, Odense University Hospital*

Until recently near-patient testing or point-of-care-testing (POCT) have mostly been used in the areas of clinical chemistry and hematology, for example rapid testing of blood glucose or hemoglobin. POCT for infectious diseases have not been very well established. The most common tests in microbiology have been immune-chromatographic tests, based on the ELISA principle e.g. tests for the detection of beta hemolytic streptococci. Many of these tests have been of inferior quality compared to reference tests. Traditional microbiology analyses are based on culture or advanced molecular methods requiring highly educated laboratory staff.

During the last few years the market for advanced rapid tests has exploded. In general these new tests are built on advanced molecular technology and with a high performance. Hospital departments and general physicians have been flooded with these new analyses. This means that there is a potential for faster diagnoses, more targeted treatment and perhaps faster recovery and discharge. However, there are advantages and disadvantages and pit falls, and it is important to find out in which clinical setting POC-tests are cost-effective and how to organize the education of staff, validation and quality testing, and how to make test results electronically available for the relevant clinicians.

[O9] DELUGED – SOIL MICROBIOME RESPONSES TO THE FLOODING WITH SEAWATER

Alexander H. Treusch[1,2], Kamilla S. Sjøgaard[1,2,] Sebastian S. K. Jensen[1,2], Harald Hasler-Sheetal[1,2], Donald E. Canfield[1,2], Thomas B. Valdemarsen[1], Erik Kristensen[1]
[1]Dept. of Biology, University of Southern Denmark
[2]Nordcee, University of Southern Denmark

Climate change induced rise in sea level will in the future threaten the majority of coastlines in the form of the increased frequency and amplitude of storm surges. As a result, seawater intrusions on low-lying coastal areas are becoming more common, increasing the areas of soils that are short-term or permanently flooded with seawater. Further, 'managed coastal realignment', where coastal areas below sea level are intentional flooded, is an increasingly popular climate change adaption strategy. Consequently, more soils are facing the intrusions of seawater, with especially the microbiome being heavily impacted by the increased water content, salinity and changes in redox conditions. The alteration of the microbiome will also affect the biogeochemical cycling of e.g. carbon, although this is still not well understood.
To explore this, we conducted microcosm experiments where soil cores were artificially flooded with seawater and monitored over longer periods of time. The degradation of labile organic carbon (OC) was followed with biogeochemical methods and environmental metabolomics in parallel to the structure and metabolic potential of the soil microbiome. Rapid OC degradation was observed in the first month and quickly decreased thereafter, rendering the majority of OC refractory towards microbial degradation. A succession in microbial community structure and activity was documented by the changes in abundances of different OC compounds and metabolites over time. Overall our results suggest that limitations in metabolic capabilities of the soil microbiome developing after the flooding with seawater will lead to permanent preservation of the majority of soil OC, potentially creating a negative feedback on the rising sea level by the reduction of greenhouse gas emissions.

[O10] PATTERNS IN MICROBIAL COMMUNITY COMPOSITION AND FUNCTIONAL CAPACITY IN THE BAY OF BENGAL

Beate Kraft[1], Halina E. Tegetmeyer[2,3,4], Michael Forth[1], Alexander H. Treusch[1], Donald E. Canfield[1,5]
[1]*Nordcee, Department of Biology, University of Southern Denmark*
[2]*Max Planck Institute for Marine Microbiology, Bremen, Germany*
[3]*Center for Biotechnology, Bielefeld University, Bielefeld, Germany*
[4]*Alfred Wegener Institute, Helmholtz Center for Polar and Marine Research, Bremerhaven, Germany*
[5]*Villum investigator*

Oxygen levels constitute an important control on the on-going nitrogen cycle dynamics: Low oxygen aquatic environments are major sites of nitrogen loss to the atmosphere. For example, marine oxygen minimum zones account for at least one third of marine nitrogen loss. High-sensitivity oxygen measurements in the Bay of Bengal (BoB), one of the largest low-oxygen environments in the ocean, revealed that the BoB maintains low, but persistent, oxygen levels in the nanomolar range[1]. The BoB is at a tipping point, with global warming and deoxygenation of oceanic water potentially shifting it towards true anoxia and consequentially rendering the BoB a major N_2 sink.
Here, we explore the microbial community structure that allows for the persistence of low-oxygen levels using 16S rRNA gene amplicon sequencing and metagenomic analyses. A focus is set on the functional capacity of the microbial community in the BoB with regard to oxic respiration adapted to low oxygen concentrations and anaerobic respiration. The results presented will provide insights into the community assembly at oceanic oxygen interfaces and will help to generate predictions about the effect of increasing ocean deoxygenation in response to global climate change.

[1]Bristow, LA., et al. (2017), N2 production rates limited by nitrite availability in the Bay of Bengal oxygen minimum zone, Nature Geoscience, vol.10; 24-29.

[O11] LIFE IN A TOXIC ENVIRONMENT- HOW DO EXTREME REDOX CONDITIONS IMPACT ON N_2 FIXERS IN OCEAN OXYGEN MINIMUM ZONES?

Carolin Löscher[1], Donald Canfield[1]
[1]*Nordic Center for Earth Evolution, University of Southern Denmark*

Nitrogen (N) is a key element of life and limits primary production in large parts of the ocean. Still, the factors controlling the oceanic N-budget are largely unclear, particularly in the context of changing climate. Dinitrogen fixation is the biological reduction of dinitrogen gas (N_2) to ammonium. It is quantitatively the most important source of 'new' N to the ocean and

is thought to be limited by the availability of phosphorous and iron (Fe). Global change is predicted to result in the expansion of anoxic waters in the ocean, which in extreme cases can turn sulfidic. While O_2 depletion favors N_2-fixation, the presence of hydrogen sulfide (H_2S) has a direct toxic effect on N_2-fixing organisms and also influences the availability of Fe. To explore the sensitivity of N_2-fixation to changes in O_2 and H_2S in different oceanic regions, an interdisciplinary approach was applied, combining chemical profiling, direct rate measurements and meta-omics including metabolomics. This approach allowed an incomparably detailed monitoring of the response of N_2-fixation to naturally occurring extreme changes in redox conditions. Complementary to the field studies, we explored the potential of N_2-fixing microbes to adapt to manipulated rapidly changing redox conditions in incubation experiments using pure cultures. Our results illuminate the potential of the microbial community involved in N_2- fixation to respond to ocean deoxygenation and sulphidic anoxia, both of which are considered key challenges of the future ocean.

[O12] CYCLIC DI-GMP-MEDIATED MULTI-TIERED REGULATION OF BIOFILM FORMATION IN BURKHOLDERIA CENOCEPACIA

Mustafa Fazli[1], Tim Tolker-Nielsen[1]
[1]*Costerton Biofilm Center. Department of Immunology and Microbiology. Faculty of Health and Medical Sciences, University of Copenhagen*

Recent work indicates that the molecule cyclic diguanosine monophosphate (c-di-GMP) is a second messenger that regulates various cellular processes in bacteria, including biofilm formation, virulence, stress responses and motility. The c-di-GMP content in bacteria is determined by diguanylate cyclases (DGCs) that synthesize c-di-GMP and phosphodiesterases (PDEs) that degrade c-di-GMP. In addition to their catalytic domain the DGCs and PDEs often contain sensory domains that are thought to enable translation of diverse (by and large unknown) environmental cues into c-di-GMP levels. The c-di-GMP binds to effectors resulting in the activation or repression of specific cellular processes. Bacteria can typically synthesize dozens of different DGCs and PDEs, but it is unknown how they can deploy a given subset of them to produce a desired phenotypic outcome without undesired cross talk between c-di-GMP-dependent systems. A detailed understanding of the regulatory mechanisms that are involved in c-di-GMP signaling is essential for the development of measures to control bacteria in diverse settings. Using *Burkholderia cenocepacia* as model system, we have found that c-di-GMP regulates the production of biofilm-stabilizing exopolysaccharide in this bacterium by activating two consecutive transcription events which are induced first by the c-di-GMP effector BerB and then by the c-di-GMP effector BerA. This is an example of multi-tiered regulation of exopolysaccharide synthesis in which two consecutive transcription events are both activated by c-di-GMP.

[O13] STRESS INDUCED BIOFILMS OF *BACILLUS SUBTILIS*: THE ROLE OF PPGPP

Theresa Hölscher[1], Thomas Winkler[2], Veselin Nasufovic[2], Hans-Dieter Arndt[2], Ákos T. Kovács[3]
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Bacteria are known to form complex multicellular communities that are made of specialized cell types and can be formed on biotic or abiotic surfaces. These communities, known as biofilms, confer high resistance to several adverse environmental conditions. The process of biofilm formation in diverse bacteria has been shown to specifically involve the response to self-generated secreted small molecules, i.e. quorum-sensing, but it can also be initiated by diverse signals produced by other organisms living in the vicinity, thus creating an interspecies communication network.
Thiopeptide antibiotics, including the Streptomycetes produced thiostrepton and nosiheptide, have recently been suggested to induce the genes responsible for biofilm formation in the Gram-positive bacterium, *Bacillus subtilis*. Here, we examined how diverse synthetic variants of thiopeptides induce biofilm related gene expression in B*. subtilis*. In addition, we suggest that biofilm induction by thiopeptides is mediated by a stringent response via the alarmone molecule, (p)ppGpp in *B. subtilis*. Indeed, the *relA* mutant strain that is lacking (p)ppGpp hydrolysis, but still having (p)ppGpp synthesis activity by the synthases YjbM and YwaC, shows diminished induction of biofilm genes in the presence of thiopeptides. Our experiments highlight the importance of second messenger signaling in stress induced biofilm development in bacteria.

[O14] CYCLIC DI-GMP SIGNALING AND PROTEOLYTIC REGULATION OF BIOFILM FORMATION IN *PSEUDOMONAS SP.*

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Today, biofilm formation is recognized as the preferred mode of bacterial growth from the environment to chronic infections. A key regulator of this lifestyle is the nucleotide second messenger cyclic di-GMP. In a plethora of species, cyclic di-GMP has been shown to conduct the transformation from motile single-celled growth to multicellular sessile growth within a

biofilm. If environmental conditions become unfavorable the biofilm-embedded bacteria can disperse to repopulate vacant areas more suitable for growth. Dispersal is often preceded by a drop in cyclic di-GMP levels. This talk presents current knowledge on the Large Adhesion Protein (LAP) system present in various *Pseudomonas* species with a focus on previous work done with the environmental soil bacterium *P. putida* and recent work done with the clinically relevant human pathogen *P. aeruginosa*. The LAP system governs the presence of a large adhesin on the cell surface that is involved in biofilm formation. The system functions through proteolytic cleavage of the adhesin membrane anchor by a periplasmic protease that itself is under negative control by a c-di-GMP binding inner membrane protein. The proteases and the c-di-GMP binding regulatory proteins are highly conserved between the two species whereas the surface adhesins share no homology. The LAP system is thus an interesting example on adaptation of a regulatory system between bacteria to comply with the specific need of the individual species.

[O15] METAGENOMICS STUDIES OF THE HUMAN INTESTINAL MICROBIOTA: POTENTIALS AND LIMITATIONS

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About 7 years after the start of the application of next-generation sequencing technology and advanced microbial gene-based bioinformatics, what are the gains for medicine that can be expected? And where are the current major limitations?
In my lecture I will address these general questions from our recent gene-based studies of imbalances of gut microbiota in insulin resistant states, in pre-diabetes and in overt type 2 diabetes.

[O16] GETTING FROM A TO B. HOW TO MANIPULATE THE GUT MICROBIOTA OF MICE AND MEN

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During the last decade it has become evident that the human gut microbiome (GM) plays essential roles in health and disease, with GM dysbiosis being implicated in the development of diseases as diverse as obesity, type 1 diabetes and colon cancer.

Knowing that GM dysbiosis is associated with development of disease, it also becomes of great interest to be able to manipulate the GM in a predictable manner thereby moving the host

away from the disease phenotype. In the present talk examples of feasible ways of manipulating the GM of mice and men (and piglets) will be reviewed with focus on dietary interventions, but also touching upon the use of bacteriophages and fecal transplants.

[O17] FECAL MIRCROBIOTA TRANSPLANTATION: PAST, PRESENT AND FUTURE

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Fecal Microbiota Transplantation (FMT) is a transfer of stool from a healthy donor to a patient with an intestinal dysbiosis. FMT is given in order to restore the patient's microbiota, with the intent to cure or improve the patient's disease.
Already in the 4th century FMT has been described in Chinese Medicine, and in the 17th century in veterinary medicine. In 1958 an American surgeon described how 4 patients with pseudomembranous colitis – today known to be caused by Clostridium difficile infection – were cured with FMT.
In the last 10-20 years a dramatic change has occurred in our knowledge about the intestinal microbiota and its significance for human health. Major projects like the American "Human Microbiome Project" and the European "MetaHIT", have made it possible to map out the human intestinal microbiome and reveal possible associations with many diseases - even non-intestinal diseases such as obesity and diabetes.
This emerging knowledge about the significance of the microbiota in health and disease has made FMT a popular topic even among patients and the media. More and more patients are asking if FMT could be the cure for their diseases. FMT has now been implanted as a possible treatment option for recurrent C. difficile infection in several Danish hospitals.
FMT has already also been evaluated as a possible treatment for other conditions such as obesity, diabetes, hepatic encephalopathy and multiple sclerosis. The major interest for FMT is however still within the gastrointestinal diseases, such as recurrent C. difficile infection, Inflammatory Bowel Disease and Irritable Bowel Syndrome.

[O18] MICROBES ARE DRIVING THE CIRCULAR ECONOMY IN WASTEWATER HANDLING

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The development of circular economy in wastewater treatment includes energy production, resource recovery and production of clean water, all driven my microbial communities. Most of the microbes involved are uncultured and poorly described. Novel understanding about process-critical microorganisms, their identity, physiology, ecology, and population dynamics

improves process optimisation and stability and help finding new approaches and solutions. Combined methodological approaches to study the communities include surveys of community composition by amplicon sequencing, retrieval of genomes by metagenomics and evaluation of metabolic models by Raman microspectroscopy. Focus is on microbes involved in two of the most important processes, recovery of phosphate via the enhanced biological phosphorus removal and improved bioenergy production by anaerobic food webs.

[O19] CONTROLS OF N2O PRODUCTION PATHWAYS IN NITRITATION-ANAMMOX BIOMASS

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Nitrous oxide (N_2O) is an unwanted byproduct during biological nitrogen removal processes in wastewater, as N_2O emissions can contribute substantially to the total CO_2 footprint of a wastewater treatment plant. To establish strategies for N_2O mitigation, a better understanding of production mechanisms and their controls is required. A novel stable isotope labeling approach using ^{15}N and ^{18}O was applied to investigate pathways and controls of N_2O production by biomass taken from a full-scale nitritation-anammox reactor. The experiments showed that heterotrophic denitrification was a negligible source of N_2O under oxic conditions (≥0.2 mg O_2 L^{-1}). Both hydroxylamine oxidation and nitrifier denitrification contributed substantially to N_2O accumulation across a wide range of conditions with varying concentrations of O_2, NH_4^+, and NO_2^-. The O_2 concentration exerted the strongest control on net N_2O production with both production pathways stimulated by low O_2, independent of NO_2^- concentrations. N_2O production by hydroxylamine oxidation was stimulated by NH_4^+, whereas nitrifier denitrification at low O_2 levels was stimulated by NO_2^- at low levels (0.2 mM). In the view of these findings, nitritation-anammox reactors should operated at relatively high O_2 concentrations, which still would allow sufficient anammox activity, and certainly avoid simultaneous low O_2 and high NO_2^-.

[O20] PRODUCTION OF BIOCHEMICALS USING BACTERIAL CELL FACTORIES

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Production of biochemicals from renewable resources is challenging due to a number of factors, including the high cost of fermentable sugars that are the typical substrates for microbial cell factories. Alternative carbon sources are therefore important in order to make

cost competitive biochemicals. Different strategies are currently being implemented in order to increase the number of microorganism with desirable phenotypes that be used as microbial cell factories. Examples will be given on identification of various alternative carbon sources as well as host organisms. This includes for example the use of macro algae as carbon source, fermentation of industrial gasses, as well as consortium based utilization of complex mixtures of carbon sources for production of biochemicals.

[O21] PROBING THE SOIL INTERACTOME

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Bacterial species rarely live in isolation but mostly in more or less complex self-organizing multi-species communities. The self-organization and multi-species nature gives microbial communities a set of inherent and added functionalities, which we can describe but which are neither well-understood nor very well controllable. Here we focus on the simple question of how existing communities can be complemented with a single additional member species carrying a specific functionality thought to be advantageous to the community. This is a useful scenario when, for example, community functions break down as a result of severe contamination. On the example of a contaminated soil and by using transcriptomic tools, we have attempted to better describe and understand the reactions of introduced species to their new home environment, to the resident community and of the resident community to the new incoming member. We have developed further tools to try and measure both global and individual types of growth interactions between introduced members and resident bacteria, in order to predict from this the potential success of the new membership.

Poster abstracts

Applied bioinformatics

[P1] USING NANOPORE SEQUENCING TO GET COMPLETE GENOMES FROM COMPLEX SAMPLES

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The advantages of "next generation sequencing" has come at the cost of genome finishing. The dominant sequencing technology provides short reads of 150-300 bp, which has made genome assembly very difficult as the reads do not span important repeat regions. Genomes have thus been added to the databases as fragmented assemblies and not as finished contigs that resemble the chromosomes in which the DNA is organised within the cells. This is especially troublesome for genomes derived from complex metagenome sequencing. Databases with incomplete genomes can lead to false conclusions about the absence of genes and functional predictions of the organisms. Furthermore, it is common that repetitive elements and marker genes such as the 16S rRNA gene are missing completely from these genome bins. Using nanopore long reads, we demonstrate that it is possible to span these regions and make complete genomes from complex samples with the throughput of a handheld DNA sequencer that plugs into a laptop. Using the kmer sequence composition signatures we demonstrate that machine learning tools can help facilitate pre-assembly sorting of the reads from complex systems. This will ultimately facilitate high throughput generation of high quality reference genomes. This is an essential step for further development of online DNA monitoring and surveillance.

[P2] NOVEL PLASMID METAGENOME METHOD CAPTURES ACCESSORY GENE CARRYING PLASMIDS

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Recent advances in sequencing technology have largely come at the price of end-to-end control of workflow and analysis. In plasmid biology, several studies have been highlighting the laboratory workflow needed to obtain a chromosome-free plasmid metagenome (mobilome) and some has been highlighting the difficulty of assembly of the highly repetitive structures that plasmids are. So far, though, no one has documented and circumvented the bias of laboratory workflows and assembly of plasmids systematically. Here, we present the first such controlled setup of a mock plasmid community mixed with real life complex samples and quantify the biases that each step in conventional mobilome sequencing present. We find that the amplification used in all mobilome studies so far is skewing the proportion of plasmids in favor of small and traitless plasmids to a degree where traces of larger plasmids cannot be found. Further, we show that omission of amplification is possible and that it produces a far more complex and accurate snapshot of the plasmid community than previous methods. From this dataset, we present the largest collection of accessory gene carrying plasmids from a plasmid metagenome to date.

[P3] TRANSCRIPTOMICS OF THE CABLE BACTERIUM CANDIDATUS ELECTRONEMA SP. GS

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Cable bacteria are filamentous members of the *Desulfobulbaceae* family capable of conducting electrons via long distance electron transport. They couple anodic sulfide oxidation and cathodic oxygen or nitrate reduction over centimeter distances in sediments. This process, named electrogenic sulfide oxidation (e-SOx) leads to the development of a suboxic layer, devoid of both sulfide and oxygen. Although supported by biogeochemical analyses, e-SOx as their means of energy metabolism remains paradoxical, as cable bacteria

are phylogenetically affiliated with sulfate reducing bacteria. Accordingly, cable bacteria genomes reconstructed from both single filaments and metagenomes showed the complete sulfate reduction pathway and none of the canonical sulfide oxidation pathways. Reversed steps in the sulfate reduction pathway are therefore proposed to be part of the sulfide oxidation pathway. To test this hypothesis, and to evaluate potential differential gene expressions in the different biogeochemical zones, gene expression of the candidate species Electronema sp. GS via RNA-Seq is compared between sediment zones with either anodic sulfide oxidation, cathodic oxygen and nitrate reduction, or electron transmission only.

[P4] DATEST: A TOOL FOR COMPARING DIFFERENTIAL ABUNDANCE AND EXPRESSION METHODS

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Identifying dataset features that are associated with variables of interest, such as design groups or environmental covariates, is a common procedure in the analysis of microbial marker-gene (e.g. 16S rRNA amplicons), RNA-seq, metabolomics and proteomics data. A myriad of statistical methods exists for conducting these analyses, and they are often applied on data types for which they weren't designed. There are no gold standards and their performances can vary greatly depending on the characteristics of the specific dataset. Here we present an R-package for directly comparing different methods on a dataset of interest, thereby giving the analyst an empirical foundation in choosing a method suitable for that specific dataset. The analysis consists of four steps: First, the variable of interest (say control vs. treatment) is shuffled. Second, randomly selected features are spiked such that they are associated with the shuffled variable. Third, all relevant methods are applied. Finally, false positive rates and AUCs are estimated for each method. The package supports both categorical and quantitative variables, paired/blocked experimental designs and the inclusion of covariates. It contains 25 different methods and is parallelised for fast performance. It is open source and freely available at https://github.com/Russel88/DAtest.

[P5] ANALYZING FUNCTIONAL DYNAMICS IN ENVIRONMENTAL TOTAL RNA WITH NO PCR BIAS

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Aim: To standardize and benchmark a computational approach for studying the functional dynamics in microbial communities in response to stress using Total RNA

Method: We sorted the rRNA and mRNA in silico using state-of-the-art tool SortMeRNA [1] and standardized this pipeline based on robust comparisons using multiple techniques of assembly and data binning tools like Maxbin [2], Trinity [3], Diamond [4] and MetaTrans [5] against various general and environment specific databases such as eggNOG, FOAM, SEED and KEGG to evaluate the sensitivity and specificity of the algorithms. These comparisons help to benchmark tools and pipelines for identification and profiling of complex communities in Total RNA samples. To evaluate the methodology we also simulated synthetic mock communities in silico. Composition included ~5000 genes from 5 species selected to represent distinct yet closely related bacterial families. Simulated with random but supervised manipulation in expression and trained with quality score of HiSeq 2500.
Synthetic Mock Communities were then subjected to similar pipelines to have same biased effects of quality and assembly/binning. We manipulated the expression by dividing the dataset into 2 categories of control/treatment. Treatment samples were then also introduced to gene masking using custom made scripts to introduce low signal data for monitoring the sensitivity of pipeline.

Results/Discussion: We present results from the soil samples with varying concentrations of stress induced. High stress concentration in soil induce pronounced microbial stress responses.

Conclusion: We provide a memory efficient Total RNA pipeline that provides an excellent platform to study the functional and taxonomic dynamics.

[P6] MULTI-OMICS NETWORKS INTEGRATION FOR IDENTIFICATION OF MOLECULAR MEDIATORS OF BACTERIAL INTERACTIONS

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Omics technologies can facilitate observation of bacteria, metabolites and bacterial genetic potential from the same sample. This work integrates 16S amplicon sequence, LC-MS metabolomics and metagenomics to identify molecules with the potential to mediate bacterial interactions. By using highly resolved tandem MS data it is possible to confidently identify an unprecedented amount of metabolites. Augmented by observations of the metagenomic potential of the bacteria to produce them, we attempt to identify molecules actively produced by the bacterial community.
Public data was downloaded from MG-RAST, GNPS and ENA and processed. A weighted correlation network was built from OTU and metabolite abundances. It was hypothesized that metabolites with a high degree of connections to OTUs would be candidates as molecular mediators, and the top 10 were selected. Complementary metagenomes and molecular networking can provide information through pathway analysis of organisms' ability to produce the compounds and will be used to prioritize candidates for in vitro testing.

Clinical microbiology

[P7] EVOLUTION AND DYNAMICS OF ANTIBIOTIC RESISTANCE GENES IN PSEUDOMONAS AERUGINOSA POPULATIONS GROWING IN THE CYSTIC FIBROSIS LUNG

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Understanding bacterial genomic evolution in *Pseudomonas aeruginosa* (*PA*) populations infecting cystic fibrosis (CF) patients may help optimizing antibiotic treatment and prolong life expectancy of the patients. In laboratory experiments, bacterial evolution is often characterized by loss of diversity and random mutation accumulation, however little is known about *in patient* bacterial evolution.
We previously sequenced the genomes of 14 hypermutator isolates of *PA* from a CF patient, spanning 20 years of patient infection history. To investigate antimicrobial resistance genes that are frequently mutated, we analyzed mutation frequencies in 152 genes. Our results revealed high genetic diversity in three genes related to β-lactam resistance (*ampC, ftsI and ampDH3*). To assess population allele dynamics for these well-known antibiotic resistance genes, we performed whole gene sequence analysis directly in sputum sample bacterial populations obtained during five years of chronic infection from two CF patients.
Our results show that evolution is still occurring during antibiotic treatment, and that there is selection and counter-selection of non-synonymous mutations. The full implication of these results is yet to be elucidated. But understanding the driving forces of *in patient* evolution and utilizing antimicrobial treatment strategies that take advantage of the evolutionary paths could help clinicians to optimise treatment strategies and ensure that patients have better clinical outcomes.

[P8] THE CFR AND CFR-LIKE MULTIPLE RESISTANCE GENES - HERE AND THERE AND EVERYWHERE

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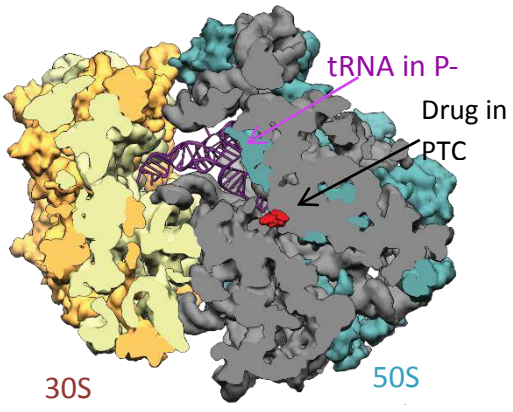
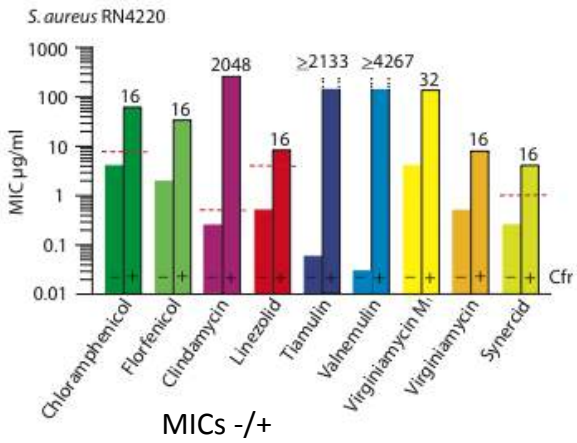
The *cfr* gene encodes an enzyme capable of an RNA methylation - a tiny alteration of the bacterial ribosomes, causing reduced or abolished binding of many antibiotics binding to the peptidyl transferase centre of the bacterial ribosomes It provides multi-resistance to more than six different classes of antibiotics, most of which are in clinical and veterinary use, including oxazolidinones, phenicols, lincosamides, pleuromutilins, streptogramin A, and large macrolides. The *cfr* gene is found in various bacteria and in many geographical locations. It is apparently always placed on plasmids or in transposons.

We have previously shown that there are other similar genes in different bacteria, which provide the same multi-drug resistance. Such genes are now found in well-known pathogens, e.g. *Clostridium difficile* and *Enterococcus faecium*. It is important to clarify how widespread these genes are, what their primary function is, and whether the enzymes are as effective as the original Cfr methyl transferase in providing antibiotic resistance.

Cfr causes methylation at C-8 of A2503 in 23S RNA (*E. coli* numbering). This is sofar the only nucleotide found to be modified with a C-8 adenine methylation. Database searches give some hints to the presence of *cfr*-like genes but do not tell if the coded enzymes have the exact same function as Cfr. More genes thus need to be investigated in details to get an overview of these methyltransferases, their presence, their potential as resistance genes, and also if they modify other nucleotide positions and thus have other functions.





[P9] ISOLATION AND CHARACTERIZATION OF BACTERIOPHAGES ACTIVE AGAINST AVIAN PATHOGENIC E. COLI (APEC)

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Colibacillosis in poultry, which is caused by avian pathogenic *Escherichia coli* (APEC), is an infection responsible for mortality of hens resulting in economic loses worldwide. Furthermore, APEC pose a zoonotic risk and have been associated with urinary tract infections in humans. Antibiotics are used for treatment, however resistance is emerging amongst APEC strains and new treatment options are needed. In this project, we characterized a collection of bacteriophages (n=100) isolated from various environmental sources in three different European countries, and tested their ability to lyse APEC strains representing the most prevalent serotypes causing avian infections in Europe (n=22). Best candidates will later be tested as a phage cocktail to control colibacillosis in different avian infection models. Additionally all phages were whole genome sequenced. Based on the sequences phages belonged to both *Myoviridae* and *Siphoviridae* families and its genome sizes were ranging from 38 Kb to 168 Kb.

[P10] NATURAL TRANFORMATION IN AGGREGATIBACTER

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Natural transformation is a genetically regulated process in which bacteria take up DNA from the environment and incorporate it into the genome. The aim of this study was to investigate the distribution of competence in genus *Agregatibacter*, with particular emphasis on invasive isolates of *Aggregatibacter actinomycetemcomitans*, using a kanamycin cassette as well as DNA from nalidixic-acid resistant strains.
Competence in *A. actinomycetemcomitans* mirrored results previously reported from dental specimens, although two strains of the newly identified clades a' and e' could not be transformed. Competence in the other species of the genus, which have not previously been characterised, is presently investigated.

[P11] THE ROLE OF FIBRIN MEDIATED BIOFILM FORMATION AND DISPERSAL IN S. AUREUS PATHOGENESIS

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The capability of *Staphylococcus aureus* to hide itself in a fibrin biofilm meshwork enables it to form vascular foci that are protected from both the immune system as well as antibiotics during treatment. This poses a huge threat in healthcare settings due to catheter-related bloodstream infections (CRBSI). Dispersing of biofilm fragments together with single cells, can result in new bacteria-induced clots, which can reach more dangerous locations. The exact mechanism linking the *S. aureus* plasma biofilm to dispersal and new foci formation on new vascular sites is not yet fully elucidated.

In this study, the purpose was to elucidate this mechanism. We investigated the role of the genes encoding staphylocoagulase, von Willebrand factor-binding protein, and staphylokinase in bacterial thrombosis during CRBSI. This has been done by constructing single knockout strains of the two coagulation genes, a double mutant, and a single knockout for the *sak* gene. This was followed by phenotypic characterization and quantification of biofilm produced by the bacteria when grown in 10 % human plasma both under static conditions on silicone material and under flow in silicone catheters to mimic the *in vivo* conditions of CRBSI.

Characterization of the mutants revealed a fragile biofilm phenotype for the Δ*coa* mutant compared to the wild type. Furthermore, we saw a significant (95% CF) difference between the amounts of biofilm under static conditions when comparing the wild type with the double mutant. This is, however, not observed under flow, indicating that here additional factors are involved in adherence to the silicone surfaces. Future experiment will further reveal the different contributions of each factor under these conditions

[P12] THE MOLECULAR MECHANISM BEHIND THIORIDAZINE RESISTANCE IN STAPHYLOCOCCUS AUREUS

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Methicillin-resistant *Staphylococcus aureus* (MRSA) have developed resistance to most of the antibiotics used today resulting in an urgent demand for novel approaches such as use of helper compounds. Thioridazine (TDZ) is a potential candidate helper compound as it has been shown to have a synergistic effect in combination with the β-lactam antibiotic dicloxacillin against *S. aureus*. It is postulated that TDZ causes its effect by intercalating in the cytoplasmic membrane, thereby disturbing membrane- and cell wall related processes. However, the precise target of TDZ and mechanism of action is still unknown.

Unpublished work from our research group has shown that by a serial passage experiment *S. aureus* is able to develop resistance towards TDZ. In this study, we investigated the mechanism of TDZ resistance through genotypic and phenotypic changes.

Viability assays and whole-genome sequencing (WGS) were conducted on a set of TDZ-resistant strains of *S. aureus* USA300. Mutations identified through WGS were reproduced by gene knockout in the wildtype strain and further viability assays were conducted with the deletion mutants. TDZ-resistance leads to a loss of synergy between TDZ and the β-lactam antibiotic dicloxacillin. Through WGS, 11 mutations in nine different genes were identified where of most were either cell-wall or cytoplasmic membrane related. A knockout mutant of the cardiolipin synthase gene (*cls*) exhibited reduced susceptibility towards TDZ, indicating that cardiolipin may play a major role in the bactericidal effect of TDZ. Furthermore, the TDZ resistant strain did also show at least two-fold increased MIC value for daptomycin compared to the wildtype indicating mechanism of action in the cytoplasmic membrane.

[P13] DO GENOME SEQUENCES OF BACTERIAL PATHOGENS PREDICT THE OUTCOME AND SEVERITY OF INFECTIONS?

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Employing a collection of about 500 *Pseudomonas aeruginosa* isolates, covering 1-10 years of persistent colonization of more than 30 cystic fibrosis patients, we have a unique and high-resolution window into pathogen adaptation.

Using measures of 16 different phenotypes including whole genome sequences of all isolates, we searched for general trends of phenotype evolution and phenotype-genotype links. To get insight into the adaptation to the CF airways we used Generalised Additive Mixed Models to evaluate the change in phenotypes over time of infection, and sought to link phenotypes with specific genetic mutations.

We observed the following trends: 1) An overall decrease in antibiotic sensitivity over time, but generally a low degree of resistance development; 2) no immediate correlation between non-synonymous mutations in genes, previously associated with specific phenotypic effects from laboratory experiments, and the resulting phenotype of the clinical isolate; 3) evolution towards a decreased growth rate in both Luria Broth and Artificial Sputum Medium.

Using this collection of isolates and the linked patient information, the correlation of phenotype and genotype appears to be much more complex than expected from a one-gene-one-function assumption. It supports the theory that the ability of a pathogen to establish a persistent bacterial infection is far more complex than solely its acquisition of antibiotic resistance.

[P14] STAPHYLOCOCCUS AUREUS TRANSCRIPTIONAL CHANGES DURING INFECTION DEVELOPMENT IN A GUINEA PIG BIOFILM INFECTION MODEL

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Introduction:
Staphylococcus aureus is a major human pathogen. The aim of this study was to identify the *in vivo* transcriptional changes of a clinical *S. aureus* strain during infection development in a guinea pig biofilm model. Additionally, we studied the correlation between the obtained data with the published *in vitro* and human prosthetic joint infection transcriptome data of the same strain.

Method:
Four implants (Teflon cages) were placed subcutaneously in each of six animals 14 days prior to inoculation with *S. aureus*. Cage fluid and cages were collected for RNA-sequencing analysis at day 3, 7 and 9 after infection with *S. aureus*.

Results:
Principal component analysis showed that the *in vivo* gene expression profiles were distinctly different from *in vitro* cultures of *S. aureus*, whereas the data from the guinea pig experiments grouped together with the human infection.
Differential gene expression showed that 16 virulence genes were upregulated at infection day 3 (INF3) compared to infection day 9 (INF9), while all genes involved in arginine deiminase pathway and urea degradation pathway were upregulated at INF9. This indicated decreased virulence expression and response to the increasing acidic environment during infection development.
Furthermore, there was no difference between groups INF7 and INF9 indicating no further development of the infection from day 7 to day 9.

Conclusion: *S. aureus* changes its gene expression to adapt to altered host environment during infection.

[P15] THE ELUSIVE PERSISTER PHENOTYPE: PSEUDOMONAS AERUGINOSA IN CYSTIC FIBROSIS PATIENTS

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Characterization of phenotypic variants of bacteria which are tolerant to antibiotics (persisters) is critical for the understanding of treatment failure, disease latency and re-emerging infections. We have investigated a collection of 583 *Pseudomonas aeruginosa* (*P.a*) isolates collected over a period of more than 10 years from the cystic fibrosis (CF) Clinic in Copenhagen using high-throughput screening methods. We have focused on the early stages of infection, and based on MIC determinations for all isolates we observe only little antibiotic resistance development. Nevertheless, antibiotics fail to fully eradicate the bacteria. Our objective is to understand why antibiotic treatment fails in CF patients infected with *P.a*. by investigating isolates with high, intermediate and low persister phenotypes and the corresponding data from whole genome sequencing (WGS).

Our results show that approximately 26 % (Fig. 1A) of the bacteria display a high-persister phenotype with respect to ciprofloxacin treatment *in vitro*. We have used data from different phenotypic observations with respect to the growth rate, lag time, biofilm-forming capacity and length of infection, to correlate the persistence phenomenon in these isolates and have found significant differences in the growth between the high and low persisters (Fig.1B). The WGS data revealed 27 distinct genes which are mutated with higher frequency in the high persister population. In addition, several pairs of clonal isolates from individual patients are investigated for fitness changes associated with variant persister phenotypes, using competition experiments.

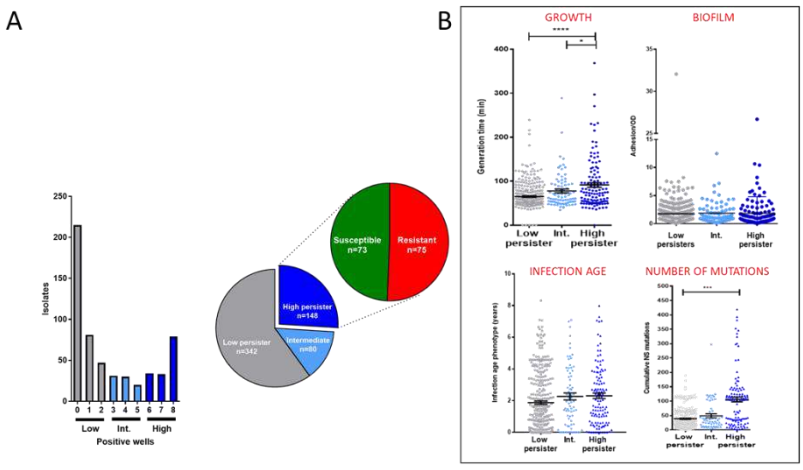

Fig. 1 High-throughput screening for clinical isolates with ciprofloxacin tolerant phenotype

[P16] ANTIBODY TESTING FOR MYCOBACTERIUM AVIUM COMPLEX INFECTION IN CYSTIC FIBROSIS

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Mycobacterium avium complex (MAC) is recognized as an important and severe pathogen among patients with cystic fibrosis (CF). Early signs of pulmonary disease with MAC can be missed in patients with CF and a serological method could help stratify patients according to risk. The objective of this study was to test the diagnostic accuracy of a method for investigating IgG activity against MAC. Combining antibody measurements with culture results can yield simultaneously good diagnostic sensitivity and specificity. One approach is to comprehensively screen CF populations for antibodies and then reserving frequent sputum culturing and additional diagnostic modalities, for those who are antibody positive. In CF research, serodiagnosis for nonturberculous mycobacteria (NTM) has been examined using a BCG based antigen A60 and recently by our group in patients with *Mycobacterium abscessus* complex (MABSC), which showed to accurately identify patients with pulmonary disease caused by MABSC. The aim of this study was to investigate antibody activity against MAC determined by ELISA. This was performed based on a clinical data from CF patients from Copenhagen CF Center and serum samples for antibody determination which were collected at least once a year. Patient cases were retrospectively identified and assigned one of four defined groups depending on a review of their NTM culture results and patient files. Results showed that anti-MAC antibody levels were correlated to whether patient fulfil established criteria for MAC pulmonary disease. Our study shows the potential for a clinically useful anti-MAC antibody test to be used in combination with mycobacterial culture for accurately identifying patients with pulmonary disease caused by MAC.

[P17] OXYGEN AFFECTS THE ACTIVITY OF AMIKACIN ON MYCOBACTERIUM ABSCESSES BIOFILM

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The multi-resistant *Mycobacterium abscessus* (MABSC), frequently cause difficult-to-treat chronic lung infections in cystic fibrosis patients (CF). MABSC infections in CF lungs are difficult to successfully treat, due to their natural resistance towards most clinically available antibiotics. MABSC biofilms have been observed in CF lung sputum where oxygen (O_2) consumption caused by polymorphonuclear leukocyte activity creates anaerobic conditions. Accumulating evidence suggests that the efficacy of several bactericidal antibiotics, such as aminoglycosides, is enhanced by stimulation of pathogens' aerobic respiration and decreased by lack of O_2. Current experiments aim to elucidate the role of cell aggregation (biofilms) and study the bactericidal killing of MABSC by amikacin during oxic and anoxic conditions.

MABSC isolates grown in Mueller Hinton broth with 5 % Tween 80 (MH) were added to oxic and anoxic MH media in 20 ml glass vials to achieve 10^5 cells/ml. Samples were incubated at 37^{o}C, 150 rpm. Samples with various concentrations (%) of Tween 80 were evaluated by CLSM on a Zeiss LSM 880 confocal microscope running Zen 2.1 together as well as by micro-respirometry of O_2.
The number of bacterial colony forming units (CFU) was determined after 1, 3 and 5 days of incubation. Time-kill curves were generated for amikacin treatment in four-fold dilutions from 2 to 512 mg L^{-1}.

Bacterial disaggregation increased amikacin efficacy suggesting that aggregation contributes to antibiotic tolerance in a fashion similar to other biofilm infections. Low O_2 consumption in MABSC aggregates create slow-growing or dormant subpopulations protected against amikacin activity.

[P18] INFLUENCE OF BIOFILM AGE, MEDIA AND EXPOSURE TIME ON BIOFILM REMOVAL BY VANCOMYCIN AND TOBRAMYCIN

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Osteomyelitis is an inflammatory process with bone destruction caused by infecting microorganisms. Chronic osteomyelitis is especially difficult to treat due to biofilm formation. In this study we tested influence of biofilm age, growth media, and antibiotics exposure time on biofilm removal.
Staphylococcus aureus and *Pseudomonas aeruginosa,* the common pathogens in osteomyelitis, were tested with vancomycin and tobramycin, respectively. We used MBEC™ assay for growing both 1 and 3 days old biofilms. The biofilms were exposed to antibiotics for 1, 2, 4 and 7 days in Tryptic Soy Broth (TSB) or Cation-adjusted Mueller Hinton Broth (CAMHB). Minimum inhibitory concentration (MIC), minimum biocidal concentration (MBC) and minimum biofilm eradication concentration (MBEC) were determined in each test.
We found that choice of media influences MIC, MBC and MBEC values, which were lower in CAMHB than TSB for both bacteria. Furthermore, old biofilms were more difficult to remove than young biofilms. Prolonged exposure of *S. aureus* biofilm to vancomycin significantly reduced MBEC value (>100 fold reduction when exposure extended from 1 day to 7 days), while, MBEC of *P. aeruginosa* biofilm remained constant at 5 µg/ml in TSB and 1.25 µg/ml in CAMHB with different exposure time. Our data suggested that it was possible to eradicate local biofilm infection with high local dose of antibiotics.

[P19] PLASMID CARRIAGE IN MYCOBACTERIUM CHIMAERA

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Several recent studies have identified water tanks of Heater–cooler units (HCUs) as potential sources of *Mycobacterium chimaera*, causing a number of surgical site infections. Recently, we identified *M. chimaera* in HCUs from all five thoracic surgery departments in Denmark and subjected a total of seven *M. chimaera* isolates to whole-genome sequencing. From each sequenced isolate, we were able to recover circular genomes of 4-6 extrachromosomal elements (plasmids) ranging between 13-157 kbp in size. By analyzing the plasmid profiles of our isolates and other *M. chimaera* isolates retrieved from the European Nucleotide Archive (ENA) we were able to establish that 97% of sequenced isolates contained one or more plasmids identical to plasmids present in Danish isolates. Remarkably, all *M. chimaera* isolates associated with the Sorin Stöckert 3T HCUs carried a

plasmid not seen in any other isolates. Thus, we have established that *M. chimaera* can carry distinct plasmid profiles that can be tied to specific isolation sources.

[P20] THE EFFECT OF AZITHROMYCIN ON P. AERUGINOSA BIOFILMS

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Azithromycin (AZM) is extensively used in the treatment of patients with *Pseudomonas aeruginosa* biofilm infections in the lung in cystic fibrosis patients, due to its anti-virulence and anti-inflammatory properties. The macrolide drug was thought to be inactive against *P. aeruginosa*, because the highest achievable concentration in the lungs is far below the minimum inhibitory concentration (MIC). However, the bactericidal effect of AZM on stationary phase *P. aeruginosa* has been described.

Here we investigated in flow-cell biofilms the emergence of MexCD-OprJ efflux-mediated resistance (AZM is a substrate of this pump) in wild-type and Δ*mutS* hypermutable strains using PAO1-*mCherry*-P_{CD}-*gfp*+ and Δ*mutS*-*mCherry*-P_{CD}-*gfp*+ monitors, respectively. The viability of wild-type PAO1 and PAOΔ*mutS* biofilm populations exposed to sub-MIC concentrations AZM was also studied, by live dead staining.

The results show no detectable expression of the MexCD-OprJ efflux pump in flow-cell biofilms exposed to AZM, which is in contrast to results reported in static biofilm systems. The effect of AZM on biofilm viability showed that there is no significant reduction of the PAO1 and PAOΔ*mutS* biomass. However, a superficial subpopulation of dead cells was observed only in PAO1, but not in PAOΔ*mutS* biofilms. Our study shows that AZM has no effect on the biomass of 3 days old flow-cell *P. aeruginosa* biofilms and that the beneficial effect of AZM in *P. aeruginosa* biofilm-infections is probably mainly due to its anti-virulence and anti-inflammatory effect.

[P21] HYPERBARIC OXYGEN BOOSTS THE EFFECT OF TOBRAMYCIN ON PSEUDOMONAS AERUGINOSA BIOFILM

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In cystic fibrosis (CF) patients, *P. aeruginosa* biofilms cause a state of chronic infection in the lungs, where zones of O_2 depletion have been measured. The tolerance of biofilms to antibiotics is enhanced under hypoxic conditions. Accumulating evidence suggests that oxidative stress contributes to the bactericidal effect of antibiotics. The antibiotics perturb metabolism and respiration in bacteria which will lead to increased formation of lethal hydroxyl radicals.
We investigated the combined effect of hyperbaric oxygen treatment (HBOT) and tobramycin on agarose-embedded *P. aeruginosa* PAO1 biofilms. The MIC of tobramycin on PAO1 cells was measured to 1 mg/L. In mature anaerobic biofilms the $MBIC_{90}$ was measured to 45 ± 31 mg/L and the MBEC was determined to be 683 ± 296 mg/L. By combining tobramycin treatment with HBOT (100 % O_2, 2.8 bar for 1.5 hours) in mature anaerobic biofilms, $MBIC_{90}$ was reduced to 12 ± 10 mg/L and the MBEC was reduced to 256 ± 0 mg/L.
Using cyanide (KCN), aerobic respiration was decoupled and O_2 consumption of exponentially growing PAO1 cells was measured. An inverse correlation between increasing concentrations of KCN and decreased levels of bacterial O_2 consumption was observed. A checkerboard assay with increasing concentrations of KCN and tobramycin was performed, which revealed that the O_2-dependent killing by tobramycin is reduced when KCN is added to aerobically growing PAO1 cells.
In conclusion, re-oxygenation by HBOT sensitizes *P. aeruginosa* biofilms to tobramycin. Tobramycin has O_2-dependent as well as O_2-independent killing. The exact mechanisms improving the bactericidal activity of tobramycin when combined with HBOT have yet to be established.

[P22] OXYGEN ACCUMULATION BY NITRIC OXIDE REDUCTASE MUTANT PSEUDOMONAS AERUGINOSA

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Pseudomonas aeruginosa expresses a flexible metabolism including utilisation of nitrogen oxides (NOx) as electron acceptors for anaerobic respiration by denitrification contributing to chronic infection in cystic fibrosis lungs. The toxic denitrification intermediate nitric oxide (NO) is removed

in the presence of O_2 by flavohaemoglobin activity and by nitric oxide reductase of the denitrification pathway in the absence of O_2. In a nitric oxide reductase mutant however, O_2 depletion initiates accumulation of NO, inhibiting aerobic terminal oxidases and allowing O_2 accumulation from the atmosphere. Here, nitric oxide reductase mutant *P. aeruginosa* grown in airtight vials displayed rapid depletion of O_2 from normoxic media (~200 µM O_2) with subsequent accumulation of O_2 peaks of up to 13.3 µM despite the lack of atmospheric O_2 access. Augmentation of O_2 peaks was achieved with physiological level NO_3^- or NO_2^- supplementation and 1 mM NO_2^- stimulated peaks of up to 54.7 µM O_2. NO_3^- stimulated O_2 accumulation could be potentiated 2 fold by inhibiting aerobic respiration with 2 mM potassium cyanide. These results are suggestive of an oxygenic denitrification pathway existing simultaneously with aerobic respiration which could afford *P. aeruginosa* further flexibility in adapting to growth in conditions of dynamic, low O_2 availability.

Microbiomes in ecosystems and ecology

[P23] FLUORESCENCE REPORTER GENE PLATFORM APPLICABLE FOR THE DETECTION AND QUANTIFICATION OF HORIZONTAL GENE TRANSFER IN ANOXIC ENVIRONMENTS

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Background: The study of horizontal gene transfer (HGT) in microbial communities has been revolutionized by advances in cultivation-independent methods based on fluorescence reporter-gene technologies. However, the use of fluorescent markers like green fluorescent protein (GFP) and mCherry is limited by environmental constraints that affect the correct maturation of their fluorophores, such as oxygen availability and pH levels. Few studies have focused on elucidating their impact, and the sheer amount of distinct protein variants requires each system to be examined in an individual fashion. The wealth of ecologically and clinically relevant oxygen-deprived micro-habitats in which bacteria thrive, calls for the urgent development of suitable tools that permit their study.

Objectives: Development of an aerobic fluorescence recovery method for mCherry and GFPmut3, as well as characterisation of the impact the pH has on their fluorescence intensities. Validation of the dual-labelling system for the study of HGT in anoxic milieus.

Methods: The time-course fluorescent recovery of mCherry and GFPmut3 *in vivo*, as well as the effect of the extracellular pH on fluorescence was monitored through flow cytometry. The applicability of the findings was validated in anaerobic filter mating experiments.

Conclusions: The findings present a solution to an intrinsic problem that has long hampered the utilization of this system to study HGT in environments devoid of oxygen, highlight its pH limitations, and provide the experimental tools that will help broaden its horizon of application to other fields.

[P24] THE EFFECT OF INCREASED LOADS OF DISSOLVED ORGANIC MATTER ON ESTUARINE MICROBIAL COMMUNITY COMPOSITION AND FUNCTION

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Increased river loads are projected as one of the major consequences of climate change in the northern hemisphere, leading to elevated inputs of riverine dissolved organic matter (DOM) and inorganic nutrients to coastal ecosystems. The objective of this study was to investigate the effects of elevated DOM on a coastal pelagic food web from the northern Baltic Sea, in a 32-day mesocosm experiment. In particular, the study addresses the response of bacterioplankton to characteristically different types of DOM. The supplied DOM differed in stoichiometry and quality and had pronounced effects on the recipient bacterioplankton, driving compositional changes in the bacterial communities. DOM additions stimulated protease activity and caused a release of inorganic nutrients, suggesting that DOM was actively processed. The release of re-mineralized carbon, nitrogen and phosphorus was associated with bacterial processing and corresponded to 25-85 % of the supplied DOM. The DOM additions had a negative effect on phytoplankton with decreased Chl *a* and biomass, particularly during the first half of the experiment. However, the accumulating nutrients likely stimulated phytoplankton biomass which was observed to increase towards the end of the experiment. This suggests that the nutrient access partially outweighed the negative effect of increased light attenuation by accumulating DOM. Our experimental data suggest that parts of future elevated riverine DOM supply to the Baltic Sea will be efficiently mineralized by microbes. This will have consequences for bacterioplankton and phytoplankton community composition and function, and significantly affect nutrient biogeochemistry.

[P25] THE CHRISTMAS TREE RHIZOSPHERE BACTERIAL COMMUNITY

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Abies nordmanniana is one of the most important Christmas tree species in Europe, but production is hampered by prolonged and unevenly growth. The rhizosphere represents a diverse source of plant growth promoting bacteria; hence growth of *A. nordmanniana* may be influenced by the rhizosphere bacterial communities. The *A. nordmanniana* rhizosphere community has so far not been characterized and the ability of this rhizosphere to select a distinct community from different soils remains unknown. The aims of this study were to characterize the bacterial communities associated with roots of *A. nordmanniana* at the nursery stage, and determine if comparable rhizosphere bacterial communities develop in different soils. The composition of the bacterial communities from bulk soil and rhizosphere from tall and small plants at different sampling sites was compared by 16S rRNA gene sequencing. We found clear differences in community composition between rhizosphere and bulk soil. In addition, we found a significant effect of the sampling site on both the rhizosphere and the bulk soil communities. *Proteobacteria* dominated in the rhizosphere independently of the site, while even *Actinobacteria*, *Acidobacteria* and *Bacteriodetes* were abundant in rhizosphere. The same phyla dominated in bulk soil, but the relative abundance of *Acidobacteria* was higher while that of *Proteobacteria* was lower than in rhizosphere. We did not find a core microbiome specifically associated with roots of tall plants.

[P26] THE ELECTROMICROBIOME OF OXYGEN MINIMUM ZONES AND ITS BIOGEOCHEMICAL SIGNIFICANCE.

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The electromicrobiome may play a crucial role in the production of CH_4 via Direct Interspecies Electron Transfer (DIET) and in the biogeochemical cycling of iron in marine Oxygen Minimum Zones (OMZs). Recent studies show that electroactive *Geobacter* species and *Methanosarcinales* methanogens can form syntrophic associations where direct exchange of electrons via electrically conductive E-pili, rather than H_2 transfer promotes CH_4 generation. *Geobacter* are arguably the most important dissimilatory Fe (III) reducers in anoxic terrestrial and freshwater sediments but have been regarded as unimportant or absent in marine environs. New -omics data from the Peruvian OMZ show that *Geobacter* are in fact abundant in fully marine anoxic sediments and in the water column, along with methanogens that are known to take part in DIET. Molecular signatures of DIET including the Pil A pilin monomer of e-pili and C type cytochrome oxidase genes from *Geobacter* are

also present. This is a significant discovery that suggests electron flow from oxidation of organic carbon may be directed
towards methanogenesis via DIET as an electron sink, or alternatively to extracellular iron oxides with the concomitant release of Fe(II) and trace elements. Release of Fe(II) is crucial to N-cycling and primary production in surface waters but if enough electron flow is directed into methanogenesis by DIET, it could have profound effects on C-cycling and biogeochemical cycling. Discerning the role of DIET in OMZ´s is key to understanding their expansion as the world's oceans become deoxygenated due to changes in oceanic temperature, chemistry and circulation and will facilitate our understanding of how anthropogenic climate change will respond.

[P27] NOVEL METHOD FOR ASSESSING DISPERSAL IN ENVIRONMENTAL COMMUNITIES REVEALS A NARROW PHYLOGENETIC DISTRIBUTION OF THE ABILITY TO EFFICIENTLY DISPERSE UNDER LOW HYDRATION CONDITIONS

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Dispersal is a key process of bacterial community assembly. Yet, very few attempts have been made at assessing bacterial dispersal at the community level as focus has previously been on pure culture studies.
In this study, we developed a method that provides community-level surface motility profiles under controlled hydration conditions from environmental samples and enables us to isolate and uncover the diversity of the fastest bacterial dispersers. The method expands on the Porous Surface Model (PSM), previously used to monitor dispersal of individual bacterial strains in liquid films at the surface of a porous ceramic disc. The current procedure targets complex communities and captures the dispersed bacteria on a solid medium for growth and detection.
The method was first validated by distinguishing dispersal patterns of mixtures of fluorescently-tagged motile and non-motile strains of *Pseudomonas putida* KT2440. Applying the method to a soil bacterial community showed that community-scale dispersal rate declined as conditions became drier. However, dispersal was detected even under low hydration conditions (-3.1 kPa), previously proven too dry for *P. putida* KT2440 motility.
We were then able to specifically recover and characterize the fastest dispersers from the inoculated communities. 16S rRNA gene amplicon sequencing revealed that the fastest dispersers were substantially less diverse than the total communities. The dispersing fraction of the soil microbial community was dominated by *Pseudomonas* which increased in abundance at low

hydration conditions. The results gained in this study provide a valuable contribution to our knowledge of the dispersal ability within complex communities.

[P28] LEGACY EFFECTS OF CHROMATED COPPER ARSENATE CONTAMINATION ON BACTERIAL COMMMUNITIES

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Multi-element contaminated soils are complex habitats confronting microbial life with a range of stresses, but it remains a challenge to elucidate the main factors selecting microbial communities in such environments. Total metal contamination is a poor predictor of effects on soil microbiota as metal bioavailability depends on chemical metal speciation. Hence, it is crucial to use a multi-disciplinary approach to elucidate the major determinants of microbial community structure and functioning in contaminated soils. This study investigates the long-term effects of multi-metal contamination on soil bacterial community diversity, function and metal tolerance. Top soil contaminated with varying concentrations of chromium, copper and arsenic due to the use of chromated copper arsenate (CCA) more than 40 years earlier were sampled at a former wood impregnation site in Denmark and characterized by soil physical and chemical tools as complemented by the use of recombinant, whole-cell bacterial bioreporters. Bacterial community composition, function and metal tolerance were characterized with molecular and microbiological tools such as next-generation Illumina sequencing of the 16S rRNA gene, high throughput qPCR array targeting microbial biogeochemical cycling genes (nitrogen, phosphorus, carbon and sulfur genes) and pollution-induced community tolerance (PICT) assays. Overall, our results indicate that of the three metal contaminants, copper was the main driver of bacterial community composition, function and metal tolerance. Insights from this study will provide valuable knowledge on the intricate interactions between soil physicochemical properties, contamination and microbial communities.

[P29] COUPLING BIOGEOCHEMICAL PROCESS RATES AND METAGENOMIC BLUEPRINTS OF BALTIC SEA BACTERIAL ASSEMBLAGES IN RESPONSE TO ENVIRONMENTAL CHANGES

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Bacteria are major drivers of biogeochemical nutrient cycles and energy fluxes in marine environments, yet how bacterial communities respond to environmental change is not well known. Metagenomic approaches allow examination of genetic responses of the entire microbial community to environmental change. However, it is challenging to link molecular information directly to microbial biogeochemical process rates. Future changes in climate conditions are expected to increase the input of dissolved organic matter (DOM) and inorganic nutrients to the Baltic Sea with potential implications for carbon turnover.

Here, we investigate metagenomic responses in natural bacterioplankton communities to well-defined environmental stressors in the Baltic Sea, including increased river input, increased nutrient concentrations, and reduced oxygen level. This allowed us to identify informative prokaryotic microbial gene markers responding to environmental perturbation. Our results demonstrate that metagenomic and metabolic changes in bacterial communities in response to various environmental stressors is influenced by initial community composition and biogeochemical factors shaping the functional response. Furthermore, the different types of DOM originate the bacterial functional response and had the largest impact on metagenomic blueprint. Most prominently, changes in DOM loads influenced specific transporter types reflecting the substrate availability. The results provide new knowledge for developing models of ecosystem structure and biogeochemical cycling in future climate change scenarios and advance our exploration of the potential use of marine microorganisms as markers of monitoring environmental conditions.

[P30] A NOVEL PROSTHECATE BACTERIUM OF THE CANDIDATE PHYLUM ACETOTHERMIA DISCOVERED IN ANAEROBIC DIGESTERS

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Members of the candidate phylum Acetothermia are globally distributed and have been detected in various habitats. However, little is known about their physiology and ecological importance. In this study, an OTU belonging to Acetothermia was detected at high abundance in two full-scale anaerobic digesters at wastewater treatment plants. Specific probes were designed to target it by fluorescence *in situ* hybridization (FISH). FISH combined with confocal laser scanning microscopy, raman microspectroscopy and atomic force microscopy revealed an unusual morphology composed of a central rod body with long bipolar prosthecae. The first closed genome from this phylum was obtained by differential coverage binning of metagenomes and scaffolding with nanopore sequences. Genome annotation and metabolic reconstruction suggested an anaerobic chemoheterotrophic lifestyle in which the bacterium are predicted to obtain energy and carbon via fermentation of peptides, amino acids, and simple sugars to acetate, formate and hydrogen. A survey into Danish digesters showed that predominance of this prosthecate bacterium might be related with low phosphate concentration. We hypothesize that the prosthecae allow for increased nutrient uptake by greatly expanding the cell surface to volume ratio, providing a competitive advantage to the cell under some nutrient-limited conditions.

[P31] THE HYPHAE-MICROBIOME OF THE PHOSPHATE SOLUBILIZER PENICILLIUM BILAIAE UNDER REAL SOIL CONDITIONS, IDENTIFIED USING A NEWLY DEVELOPED MICROCOSM

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Hyphae-associated bacteria have great potential as members of bacterial-fungal consortia in environmental biotechnology. Fungi have been found to have a general effect on the size and composition of bacterial communities in mycosphere soil; however, no information is available on microbiomes firmly attaching to fungi in soil systems. This is mainly due to experimental challenges. In the present study we developed a novel type of baiting microcosm, where fungal hyphae interact with bacteria under close to natural soil conditions. We used this baiting microcosm approach to determine, for the first time, the composition of the bacterial community associating in the soil with hyphae of the phosphate-solubilizer, *Penicillium bilaii.* The hypha-associated bacterial community

was dominated by *Proteobacteria*, accounting for 93% of the sequences. In contrast, *Proteobacteria* accounted for 10 % of the soil community. Hence, *Proteobacteria* had a significantly higher relative abundance in the hypha-associated community than in the soil community (P=0.0023, Tukey test). Other phyla identified were *Bacteroidetes*, *Actinobacteria* and *Firmicutes* accounting for only 0.5 – 3% of the sequences. Within the hyphae associated *Proteobacteria*, *Burkholderia* accounted for 89% of the sequences. The strong selection for a distinct bacterial community by the fungal hyphae was further supported by a significantly lower diversity, estimated by the Shannon H index (P=0.00078, Tukey test) and a significantly lower richness as estimated by the Chao1 index (P=0.043, Tukey test). In conclusion, the study provided valuable information that can be used for a targeted approach towards identifying *in situ* compatible fungal-bacterial consortia.

[P32] MICROBIOME DYNAMICS IN HYDROCARBON CONTAMINATED SOILS AS A RESPONSE TO NITROGEN AMENDMENT - A STRATEGY FOR ENGINEERING THE MICROBIOME FOR ENHANCED BIODEGRADATION?

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Nitrogen is an important factor shaping bacterial communities often increasing the relative abundance of *Actinobacteria* and β-*Proteobacteria*. These genera harbors several groups known to degrade alkanes, and it is hypothesized that N can be used in a targeted approach to enhance alkane degradation.
Soil microcosms were established containing an aged diesel-contaminated soil amended with NH_4NO_3 and spiked with either dodecane or octadecane, representing alkanes with different bio-accessibility. Microcosms without nitrogen amendment served as controls.
Microbial community structure was evaluated by 16S rRNA gene amplicon sequencing after 0, 26, 69, 102 and 154 days. Nitrogen amendment significantly altered the community structure resulting in less diverse microbial communities dominated by *Achromobacter*, *Rhodococcus* and *Arthrobacter* after 69 days. In contrast, the chain-length of the alkanes had no impact on the microbial community structure. At the functional level, nitrogen amendment also changed the initial kinetics of alkane mineralization; however, whether the amendment resulted in a shorter or longer lag phase before initiation of alkane mineralization, was alkane-dependent.
Abundance and expression of *alkB* genes were quantified by qPCR. Nitrogen amendment stimulated proliferation and expression of *alkB* genes within the first 70 days in both dodecane- and octadecane-amended soil, but expression of *alkB* was not detectable in any of the microcosms not amended with nitrogen. In conclusion, nitrogen rather than the alkanes is driving the changes in abundance and activity of the *alkB* harbouring bacteria. We furthermore show that *alkB* abundance and expression are not reliable biomarkers for alkane biodegradation.

[P33] MICROBIOTA ANALYSIS TO REVEAL TEMPERATURE ABUSE OF FRESH PORK

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Violations of temperature regulations in the meat chain may affect meat safety. Methods are lacking to estimate whether meat has been subjected to temperature abuse. Exposure to too high temperatures may lead to systematic changes in the diverse bacterial communities of fresh meat. We investigated whether temperature induced changes in the community composition on fresh meat surfaces can reflect the temperature-history (combination of time and temperature). Sterile pieces of pork were inoculated with a carcass swab homogenate, to which *Salmonella* was added. Changes in the meat microbiota were monitored during aerobic chill-storage (4 °C and 7 °C) and temperature abuse (12 °C and 16 °C) for 96 hours, by culture-based methods and 16S rRNA gene sequencing. Bacterial genera that dominated during prolonged temperature abuse were *Acinetobacter*, *Serratia* and *Pseudomonas,* whereas chill-stored meat was dominated by *Pseudomonas* only. We also showed that the initial community affects subsequent changes during storage. The results suggest that principal coordinate analysis of beta diversity could be a useful tool to reveal temperature abused meat. Sequence data and culturing data revealed a strong positive association between growth of *Escherichia coli* and growth of *Salmonella*, which suggests that *Escherichia coli* can be used as indicator of temperature-history supporting growth of *Salmonella* on fresh pork surfaces.

[P34] THE VINEYARD'S MICROBIAL MAP

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In the wine milieu, the word "*terroir*" means the set of all the human and environmental factors, which affect the quality and characteristics of the wine produced in a specific region.
The soil type and other edaphic factors, such as pH, mineral composition and climate, can affect the microbiota harbored on the soil surface and in deeper soil layers. This microbiota may play an important role on plant health and further in the wine production. Based on this and on the relations

the microbes can have with a specific wine region, the aim of this study is to enlighten the microbial fingerprint that characterizes different vineyards around the world.
A further aim is to design a *"map"* of the different vineyards based on their soil microbial composition. This may facilitate, in the future, the possibility of follow the evolution along the years of these microbial communities accordingly with season, agricultural practices and weather conditions in the different wine regions with specific focus on pathogens and microbes of oenological interests.
We applied both a 16s library amplicon based Illumina sequencing approach and qPCR followed by High Resolution Dissociation curve on soil coming from different vineyards; This data will be analyzed in order to build this map and assess the existence of a *unique* microbial *terroir*, for each field and/or region. Until now, different "*cores*" of microbes that contributes to the community's fingerprint attributable to a specific place have been revealed.

[P35]ISOLATION OF ANTIMICROBIAL DRUG-PRODUCING BACTERIA FROM SOCIAL SPIDERS

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Antimicrobial resistance in pathogens is an ever-increasing problem for human health, and calls for an extraordinary effort to meet the needs for antimicrobial substances.
In this study, we aim to discover novel antimicrobial compounds from host-associated bacteria found in highly inbreed social spiders (*Stegodyphus dumicola*), which are abundant and widespread in Southern Africa. Despite their low genetic diversity, these spiders seem not to be affected by pathogens, indicating that a highly efficient microbial defence system might be in play. Using aerobic incubation on complex media, we isolated 49 bacterial strains from the nesting material of ten spider colonies; additional isolates from spider body surface and haemolymph are currently under progress. The isolates were characterized morphologically, classified by 16S rRNA gene sequencing, and screened for antimicrobial activity.
Two novel genera were identified, and three isolates (two that shared 99% identity with *Bacillus subtilis*, and one with 98% identity to *Chryseobacterium* sp.) were recorded to be antibacterial against *Staphylococcus epidermidis*. One isolate was also antibacterial against *Escherichia coli*, but none were active against *Pseudomonas putida*. Our preliminary results indicate that the microbial inhabitants of social spider nests might have a protective role against external pathogens.

[P36] SOIL CONDITIONS AND *PENICILLIUM* SPECIES INFLUENCE HYPHOSPHERE MICROBIOME ASSEMBLAGE IN SOILS

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Fungal hyphae are unique selection niche for particular microbial communities during fungi-bacteria interaction. However, the extent to which microbial communities recruited by hyphae is influenced by fungal species or soil conditions, as well as their functional differences are unclear. Here, we studied the impact of fungal species and soil conditions on selection of hyphae-associated bacterial communities and their functional traits through 10 independent soil microcosms consisting of two *Penicillium* strains with different phosphate solubilizing capacities and five types of soil with organic and inorganic phosphates amendments. The microbiomes from two fungal hyphosphere as well as soil background were analyzed using high-throughput sequencing of 16S rRNA gene amplicons. The composition of hyphal attached bacterial communities was significantly distant from soil communities ($p<0.001$). Compared with soil communities, hyphosphere communities showed decreased diversity, abundance, and less variation for taxonomic structure, but increased abundance of specific bacterial phyla irrespective of fungal species and soil types, such as Proteobacteria, Bacteroidetes and Firmicutes which are well known phosphate solubilizing phyla. Soil types exhibited strong impacts on hyphosphere bacterial communities ($p<0.001$), whereas the effect of fungal species was not significant ($p=0.4869$). Despite the fact that no differences were observed at community level, fungal hyphae from two *Penicillium* species were enriched for specific OTUs and discriminative taxa. Furthermore, functional prediction by PICRUSt revealed that hyphae-selected microbiomes with particular functional profiles are involved in phosphonate and phosphinate metabolism, signaling and transcription, suggesting active fungi-bacteria interaction activities in hyphosphere. To validate if hyphosphere bacterial communities exhibited specific functional profiles on P metabolism, the abundance of functional genes related to phosphorus cycling was examined via quantitative PCR (qPCR) for soil and hyphosphere communities. In general, most phosphorus cycling-related genes were more abundant in hyphosphere communities than in soil communities, and the distribution and abundance of genes in hyphosphere communities varied among soil microcosms and fungal species. Accompanied by an increased proportion of potential iPSB strains identified in hyphosphere and an enriched pathway involved in phosphonate and phosphinate metabolism, these findings indicated higher and more varied microbial phosphorus metabolic processes in hyphosphere. Taken together, our results provide strong evidence that hyphosphere represented a unique niche for selection of microbiome with concentrated metabolic functional traits. In this niche, soil types and fungal species together contribute to orchestrate hyphosphere microbiome assemblage.

Gene regulation, communication and second messengers

[P37] A FAMILY OF SRNAS PLAYS A ROLE IN THE RESPONSE OF LISTERIA MONOCYTOGENES TO HEME TOXICITY

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Backgrounds

At present, over 200 putative small non-coding regulatory RNAs (sRNAs) have been identified in *Listeria monocytogenes*. Interesting, several sRNAs have been identified as being induced in human blood. *L. monocytogene*s has the ability to lyse erythrocytes, remove heme from hemoglobin and liberate Fe^{2+} from heme. Even though iron is essential for life, it is highly toxic under aerobic conditions, as it reacts with oxygen species forming free radicals. Thus, *L. monocytogenes* needs to find a way to overpass the unfavorable conditions it encounters in the presence of excess heme.

Objectives

To understand why some sRNAs are highly induced in human blood, we hypothesized that this induction could be caused by high levels of heme in this environment. Therefore, the aim was to investigate the role of heme-induced sRNAs in response to excess heme and look for their putative targets in *L. monocytogenes.*

Methods

Wild-type cells were subjected to increasing concentrations of hemin and sRNAs levels were determined via Northen Blot analysis. To verify their role in the prevention of heme toxicity, growth of a strain lacking sRNAs was compared to the wild-type strain. Finally, a search was performed to identify possible targets of the sRNAs under hemin stress.

Conclusions

A family of sRNAs was greatly induced by hemin, and a strain lacking the sRNAs showed impaired growth in the presence of hemin, suggesting a fine-tuning role for these sRNAs in the prevention of heme toxicity. Putative target genes were identified and the mechanisms underlying the regulation by the sRNAs are under investigation.

[P38] THE ROLE OF INTERGENIC MUTATIONS IN PATHOADAPTATION OF PSEUDOMONAS AERUGINOSA

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Bacterial pathogens evolve during the course of infection, driven by different selective pressures inside the host. Studies of pathogen adaptation have focused predominantly on molecular evolution within coding regions, whereas the role of adaptive mutations in intergenic regions has been investigated to a considerably lesser extent. As a consequence, the level to which intergenic mutations contribute to pathogen adaptation inside the host remains unclear.
We had previously analysed the presence of nucleotide-level variants in intergenic regions in 44 clonal lineages of the opportunistic pathogen *Pseudomonas aeruginosa*, isolated from cystic fibrosis patients. We identified 88 intergenic regions in which parallel molecular evolution occurs in multiple lineages or isolates. The functional impact of a subset of mutations in specific intergenic regions was subsequently investigated. By generating reporter fusion constructs and allelic replacement strains, we can show that these mutations affect transcriptional regulatory interactions and that they facilitate the evolution of important pathogenic features such as iron acquisition and antibiotic resistance.
Our results highlight the strength of using a combined bioinformatics and functional genomics approach to improve our understanding of the role that intergenic mutations play in bacterial adaptation to the host.

[P39] DEVELOPMENT OF AN AGAR-BASED RNA-SEQ ASSAY FOR STUDYING THE INTERACTOME OF NASAL STAPHYLOCOCCI STRAINS.

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Background *Staphylococcus aureus* is well-known as a human pathogen but also lives as a commensal in the human nose in approximately one fifth of the population. Carriage is usually asymptomatic but the nasal cavities can function as reservoirs for infection e.g. during surgical procedures. The human nose hosts a multi-species community and it has previously been reported that *Staphylococcus epidermidis* strains can modulate *S. aureus* phenotypes. Intriguingly the cohabitation has been shown to result in inhibition of nasal colonization and biofilm formation of *S. aureus*. Even though some *S. epidermidis* products have been implicated in this inhibitory effect, a comprehensive understanding of the interactions between nasal Staphylococci isolates is needed.

Objectives The aim of this study was to establish a robust method to map the transcriptome profiles of nasal *S. aureus* and *S. epidermidis* focusing on the differences between mono- and co-culturing.

Methods Staphylococci strains were isolated from nasal cavities of healthy Danish individuals. A pair of *S. aureus* and *S. epidermidis* strains isolated from the same nose were used for a novel agar-based RNA-seq method enabling investigation of their interactome. The strains were grown on agar surfaces as mono- and co-cultures, respectively, and RNA was harvested after 24 hours of incubation. RNA-seq was performed and enables mapping of the expression profiles of the two nasal Staphylococci isolates.

Conclusion Our preliminary results indicate this novel RNA-seq method can be applied to study interactions between *S. aureus* and *S. epidermidis* isolated from nasal swaps of a healthy Danish adult.

[P40] EXPRESSING FLUORESCENT SURFACE PROTEINS IN STAPHYLOCOCCUS EPIDERMIDIS.

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Staphylococcus epidermidis is considered one of the most frequent leading causes of nosocomial infections competing in rank with *Staphylococcus aureus*. These bacteria, commonly found in normal human flora, although, *S. epidermidis* is not usually pathogenic but patients with compromised immune systems are at risk of developing infection and form pathogenic communities resistant to normal antibiotics therapy. Their ability to form a protective matrix, called a **biofilm**, allows the community to grow on surfaces and eventually disperse, with supervised interference from the outside. The biofilm formation is believed to be mediated by the cell surface proteins which are responsible for cell adhesion, with focus on the Extracellular matrix binding protein (Embp) that has been directly recognized as a virulence switch. Embp is a large surface protein produced in many *S. epidermidis* strains. Little is known about the control of Embp expression, if Embp strictly locates to the cell surface or whether it is detached from the surface (proteolytic cleavage). The GFP sequence was inserted after the C terminal and before the N terminal on the Embp sequence in parallel, by first building an insert DNA sequence through a series of PCR, followed by integration into the pIMAY shuttle plasmid, an intermediary step of *E. coli* transformation and *S. epidermidis* transformation. This study aims to tag Embp with GFP which will establish a track for the investigation of this protein during biofilm formation.

Composition and manipulation of gut microbiomes

[P41] COMPARATIVE ANALYSES OF THE DIGESTIVE TRACT MICROBIOTA OF TROPICAL PASSERINE BIRDS

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The digestive tract microbiota plays important roles for their hosts, including nutrient absorbtion, vitamin synthesis, immune response to pathogens and detoxification. To fully understand the roles of the microbiota in individual digestive tract compartments, we explore the composition of microbiotas, and possible changes in its composition from the crop to the cloaca in passerine birds (Aves: Passeriformes). We focus on seven species of tropical passerine birds representing clades that span the entire passerine radiation, multiple dietary guilds and adaptation to different elevations. First, we obtained regurgitated samples collected along an elevational gradient in New Guinea. Secondly, we dissected alcohol specimens from the same elevational gradient and isolated six compartments along the digestive tract. Thirdly, during fieldwork in 2017, I collected cloacal swabs to determine the microbiota in the terminal digestive tract compartment. DNA will be extracted and MiSeq amplicon sequencing of the 16S rRNA gene will be used to identify and compare microbial communities. We anticipate that this study will improve our understanding of the microbiota of passerine birds by identifying compositional differences across digestive tract compartments. Furthermore, this research project will shed light on microbial roles and their importance for adaptation of their hosts to particular environments, and ultimately the distribution of species in space and time.

[P42] IMPACT OF FUNGUS-FARMING TERMITE FUNGAL DIET ON THE GUT MICROBIOTA OF OMNIVOROUS COCKROACH PYCNOSCELUS SURINAMENSIS

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Fungus-farming termites (sub-family Macrotermitinae) engage in an obligate mutualism with fungi in the genus *Termitomyces*, which degrade plant material and are the main food source for the termite host. These termites host a diverse gut microbiota that is more similar in composition to those of the ancestral cockroaches than to other termites. This convergence in gut community structure has been suggested to be due to the fungal diet of the farming termites, and recent findings show that feeding the omnivorous cockroach *Pycnoscelus surinamensis* with a strain of

Termitomyces for four weeks systematically shifted gut community compositions. To build on this, and to examine if the *Termitomyces* species provided as a fungal diet is important for community structure in the gut, we compare gut compositions of cockroaches feeding on eight different *Termitomyces* fungal strains (three species) from three major termite genera. After four weeks of *Termitomyces* feeding, we dissected guts, extracted DNA and used MiSeq amplicon sequencing of the 16S rRNA gene to examine gut microbial composition. Furthermore, we evaluated whether the gut microbiota would remain stable, or shift further, if the cockroaches were kept on a *Termitomyces* diet for an additional four weeks and whether the gut microbiota composition would reverse to the original composition if the cockroaches returned to their natural leaf-litter diet.

[P43] MAPPING THE MICROBIOME OF SOCIAL SPIDERS (STEGODYPHUS)

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Owing to an obligate inbreeding mating system, social spiders of the genus *Stegodyphus* have one of the lowest species-wide genetic diversities ever found in an animal species. In spite of this, each species has a wide geographical distribution range characterized by steep climate gradients. We propose that symbiotic bacteria contribute to the phenotypic variation of their spider hosts, acting as a 'non-genetic' mechanism to facilitate host adaptation. Furthermore, the spiders live in large nests in close contact with almost genetically identical nest-mates, making them vulnerable to diseases. We propose that pathogen defense could be facilitated by symbionts. The first step in finding out whether microbial symbionts contribute to the spiders' ability to adapt to the environment and to resist pathogens, is to map the microbiome of social *Stegodyphus*. We collected >200 spiders from 85 nests from multiple populations of each of three social *Stegodyphus* species in India and South Africa/Madagascar. Using bacterial 16S rRNA gene amplicon sequencing, we identified the core microbiota as well as symbionts which varied in presence and abundance between spider species. The microbiomes of all 3 spider species were dominated by the same 10-15 symbionts. The symbiont composition of individual spiders within a nest was almost identical, but the variation within populations was large, and there was no correlation between beta-diversity and geographical distance. Analysis of spiders sampled along a measured temperature gradient is currently in progress, as is identification and localization of the core symbionts by fluorescence in situ hybridization.

[P44] COLITIS AND MICROBIOTA - A STUDY ON WEANED PIGLETS WITH DEXTRAN SODIUM SULFATE - INDUCED COLITIS

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IBD's are complex and multifactorial diseases with unknown etiology but believed to result from both environmental and genetic factors. Among these factors, the GI-tract microbiota is thought to play an important role in the pathogenesis and etiology. It is well known that nutritional factors play a central role in the composition and function of the GI microbiota. However, there is a lack of knowledge regarding which microorganisms are involved in the development of these diseases.
This master aims to investigate the changes occurring in weaned piglets when developing colitis induced by administering dextran sodium sulfate (DSS) as compared to control piglets with regard to composition and activity of the intestinal microbiota. To the knowledge of the author, this is the first time a DSS –induced colitis model has been used on weaned piglets. In addition, the impact of diet, by adding beef meat to the feed will be studied. Registration of macroscopic lesions of the colon showed a higher incidence of colitis in piglets fed with added beef meat compared to those fed a control diet. How high levels of dietary meat affects the gut microbiota compared the control diet will be investigated in the current master project.
Microbiota composition will be analyzed by 16S rRNA gene amplicon sequencing and the number of sulphate-reducing bacteria by qPCR. Metabolites reflecting both carbohydrate and protein microbial fermentation will be measured including short fatty acids, biogenic amines, indoles para-cresol, etc; and sulphide concentration will be determined. The results are expected to contribute with knowledge on the involvement of microbiota in the development of IBD.

[P45] ADMINISTRATION OF TWO PROBIOTIC STRAINS DURING EARLY CHILDHOOD DOES NOT AFFECT THE ENDOGENOUS GUT MICROBIOTA COMPOSITION DESPITE PROBIOTIC PROLIFERATION

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Probiotics are increasingly applied to prevent and treat a range of infectious, immune related and gastrointestinal diseases. Despite this, the mechanisms behind the putative effects of probiotics are poorly understood. One of the suggested modes of probiotic action is modulation of the endogenous gut microbiota, however probiotic intervention studies in adults have failed to show significant effects on gut microbiota composition. The gut microbiota of young children is known to be unstable and more responsive to external factors than that of adults. Therefore, potential effects of probiotic intervention on gut microbiota may be easier detectable in early life. We thus investigated the effects of a six month placebo-controlled probiotic intervention with Bifidobacterium lactis (BB-12®) and Lactobacillus rhamnosus (LGG®) on gut microbiota composition and diversity in Danish infants, as assessed by 16S rRNA amplicon sequencing and we assessed probiotic proliferation by qPCR. Probiotic administration did not significantly alter gut microbiota community structure or diversity as compared to placebo. The probiotic strains were detected in 91.3% of the fecal samples from children receiving probiotics and in 1% of the placebo treated children. Baseline gut microbiota was not found to predict the ability of probiotics to establish in the gut after the six month intervention. Within the probiotics group, proliferation of the strains LGG® and BB-12® in the gut was detected in 44.7% and 83.5% of the participants, respectively. A sub-analysis of the gut microbiota including only individuals with detected growth of the probiotics LGG® or BB-12® and comparing these to placebo revealed no differences in community structure or diversity.

[P46] EFFECT OF FECAL MICROBIOTA TRANSPLANTATION CAPSULES IN CLINICAL STUDIES

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Many human disorders, including irritable bowel syndrome (IBS) and ulcerative colitis (UC) are linked to dysbiosis of the intestinal microbiota. Fecal microbiota transplantation (FMT) is a

promising treatment for many such disorders and can be even more effective than traditional medical therapies. Our consortium aims to develop the use of encapsulated fecal microbiota (FMT capsules) to simplify FMT treatment and obviate the need for invasive medical procedures such as nasoduodenal tube insertion, enema or colonoscopy.

Here we used microbiome analysis to assess the effects of FMT capsules on the microbiotas of patients. In the case of IBS patients, we showed that patients had lower fecal microbial biodiversities than healthy donors, despite the non-correlation between IBS severity and alpha-diversity. We showed that FMT capsules increased the fecal biodiversity of patients in a placebo-controlled experiment. Furthermore, there was strong evidence for certain operational taxonomic units from fecal donors becoming established in patients. Future work will focus on optimizing FMT capsules against UC and IBS.

Waste water treatment

[P47] SOME IMMIGRATING PATHOGENIC BACTERIA GO STRAIGHT THROUGH FULL-SCALE WASTEWATER TREATMENT PLANTS

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Background:
Bacteria immigrating to wastewater treatment plants (WWTPs) are usually considered to be absorbed to the activated sludge flocs or die off. Therefore, it is assumed that highly abundant bacteria in the effluent comprise primarily of those that grow in the plant. However, standard methods for detecting bacteria in the effluent are based on culture dependent methods, which may underestimate the bacteria that are not removed. The aim of this study was to determine the fate of immigrating bacteria by culture independent methods.

Methods:
Net growth rates and cell numbers were calculated for specific OTU's using bacterial mass balances based on 16S rRNA gene amplicon sequencing of samples from influent, process tanks and effluent at 14 Danish full–scale WWTPs.

Results:
The microbial community composition in influent wastewater was very similar across the 14 WWTPs investigated. Some genera were found in high relative abundance in both the influent and effluent but not in process tanks, this indicates that specific genera stay in the water phase and are discharged to the environment. One of these is the genus *Arcobacter*, of which some are known pathogens. This poses a potential health risk and may indicate a need for implementing methods to remove bacteria such as *Arcobacter* before they are discharged with the effluent.

[P48] RAPID MICROBIAL SURVEILLANCE USING NANOPORE DNA SEQUENCING

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Wastewater treatment plants depend heavily on microbial communities to clean sewage water, which has to pass strict nutrient requirements before the effluent goes into waterways. The

biological processes are generally stable. However, problems do occur occasionally, can arise quickly and lead to process breakdown. To mitigate this, operators have to act fast to control problematic microbes. With current methods, it is often impossible to predict a system crash before it is too late. Monitoring the microbial community for critical changes is tedious, as the process from sample to results take several days and requires expert knowledge as well as expensive lab facilities.
In this project, we developed rapid protocols to make a profile of all bacteria and detect problematic microorganisms, such as pathogens or process critical bacteria from wastewater treatment plants, onsite in a matter of hours. This will provide actionable information to plant operators in time to mitigate a process breakdown. The key to this is the development of simple, cheap and easy to use protocols that will ultimately allow plant operators to monitor and report the microbial status as a routine measurement alongside simple process characteristics such as pH and temperature.

[P49] RAMAN SPECTROSCOPY BASED ABSOLUTE QUANTIFICATION OF POLYPHOSPHATE, GLYCOGEN AND PHA IN POLYPHOSPHATE ACCUMULATING BACTERIA IN FULL-SCALE WASTEWATER TREATMENT PLANTS

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Enhanced biological phosphorus removal (EBPR) process is an integral part of wastewater treatment, in terms of removal and recovery of phosphorus (P). The typical polyphosphate accumulating organism (PAO) phenotypes such as the genera Candidatus *Accumulibacter* and *Tetrasphaera* are well-characterised in terms of their P – metabolism taking place under anaerobic/aerobic “feed”/”famine” cycles induced in the EBPR process. Under the aerobic “famine” conditions of the EBPR process, typical PAO phenotypes accumulate large amounts of P in the form of intracellular granules of polyphosphate. The quantitative aspects of P accumulation in PAOs are poorly understood. This is partly due to shortfalls in currently available quantitative chemical analyses methods.

This study utilised a non-invasive and non-destructive Raman spectroscopy based method to quantitatively evaluate intracellular polyphosphate, glycogen and PHA contents of single PAO cells, defined by fluorescent *in-situ* hybridisation from full-scale Danish EBPR plants.

Ca. Accumulibacter cells were able to hold relatively larger quantities of polyphosphate per cell, compared to *Tetrasphaera* cells. The dynamics of glycogen and PHA followed existing metabolic

models for *Ca*. Accumulibacter but not for *Tetrasphaera*. Raman micro-spectroscopy was successfully used to quantitatively characterise single PAO cells from full-scale plants and is suitable for further studies on the ecology of PAOs.

[P50] IDENTIFICATION OF NOVEL FOAMING MICROBES IN ANAEROBIC DIGESTERS

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Foaming is a major operational problem in anaerobic digesters (AD) at wastewater treatment plants (WWTP). Specific filamentous bacteria are often described to be responsible for the foaming. It may be bacteria coming with the surplus activated sludge, or it may be some that grow in the digesters. In one type of digesters, no living microbes are coming with the feed and that is when the thermal hydrolysis process (THP) is used as a pre-treatment step. High temperature and pressure break down the structure of extracellular polymeric substances, destroy cell walls and kill all of the microorganisms. The objective of this study was to identify putative foaming-forming microorganisms growing in ADs with THP.
The top foaming layer and digester sludge sample were taken from a foaming event at Fredericia WWTP with THP in Denmark. 16S rRNA gene amplicon sequence and fluorescence in situ hybridization where applied to identify organisms potentially responsible for foaming.
Foaming in Fredericia WWTP digesters was caused by hitherto unknown filamentous organisms which accumulated in the top foaming layer. Members of the A6 phylotype (phylum Chloroflexi) and a novel phylotype classified to the family Ruminococcaceae (Firmicutes) were identified as potential candidates. This study is the first to identify putative foam forming bacteria actually growing in anaerobic digesters and further studies on their physiology and ecology should provide control measures.

[P51] THE IN SITU CHARACTERIZATION OF THE CHLOROFLEXI COMMUNITY OF FULL-SCALE ANAEROBIC DIGESTERS AND THE INFLUENCE OF IMMIGRATION.

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Filamentous Chloroflexi are often abundant in activated sludge and anaerobic digesters, where they have an important role in floc formation and degradation of complex matter. They are also well-known to cause bulking and foaming problems. Some members of the phylum have been isolated and characterized *in situ*, however, more insight is needed to understand their function. The aim of

this study was to characterize the Chloroflexi community in anaerobic digesters, identify novel taxa and design FISH probes for their *in situ* detection. Furthermore, as it was concluded that the microbial community in anaerobic digesters was strongly influenced by migration, the goal was to investigate the survival of Chloroflexi abundant in the activated sludge and potentially able to survive in anaerobic conditions, when fed into the digesters. 16S rRNA amplicon sequencing was used to identify the most abundant phylotypes in 32 Danish anaerobic digesters. T78 and *Leptolinea* were the dominant Chloroflexi in mesophilic digesters, and RB349 and SJA-170 were the most abundant in thermophilic digesters. Phylogenetic analysis showed their affiliation with the Anaerolineae class, which contains several cultured filamentous bacteria with anaerobic metabolism. All the novel phylotypes were short and thin filaments, often found on the surface of sludge flocs. This specific spatial arrangement may be linked to their functional role, as they likely contribute to the hydrolysis of complex matter. Filamentous Chloroflexi fed into the digesters with activated sludge were quantified with q-FISH and were shown to die off once they reach the new environment, suggesting detrimental effect of high temperature and/or salt concentration.

[P52] VIRAL INDICATORS FOR FECAL CONTAMINATION - A ONE-YEAR VIRAL METAGENOMIC STUDY OF TREATMENT EFFICIENCY IN DANISH WASTE WATER TREATMENT PLANTS

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Viral pathogens in irrigation water are a major threat to public health due to their possibility to cause disease in humans. When using reclaimed water for irrigation it is therefore important to make sure that the water is free from pathogens which can contaminate the crops. In this study we are therefore using metagenomics sequencing with the aim to map the viriome in different water sources. In addition we investigate the possibility to use Human Adenovirus (HAdV) or JC Polyomavirus (JCPyV) as indicator for human fecal contamination.
Water has been sampled monthly throughout the treatment process from two urban waste water treatment plants in Copenhagen. All samples are investigated for their viral content and the presence of pathogens by metagenomic sequencing and analyzed specifically for HAdV, JCPyV, norovirus GI and GII (NoV GI and GII) using quantitative (q)PCR.
Preliminary qPCR results showed that the average concentration for HAdV within a sample is higher than the average concentration of NoV GI and GII. HAdV could therefore be a good indicator for human fecal contamination in water. The initial analysis of the metagenomic data identifies viruses in all water sources. However, the number of identified pathogenic viral species decreases with treatment of the waste water. Further bioinformatic analyses will investigate the seasonal variations of viral composition within a sample as well as the effect of the treatment system. Updated qPCR and metagenomics data will be presented.

[P53] DEVELOPEMENT OF ONLINE DNA SEQUENCING METHODS TO MONITOR BACTERIAL COMMUNITIES

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Wastewater treatment plants rely on complex microbial communities to clean wastewater. However, despite their central role, the microbial communities are not routinely monitored at wastewater treatment plants.
In this presentation, we will demonstrate our progress at implement on-site library preparation and real-time DNA sequencing using the Oxford Nanopore MinION sequencing platform. The DNA sequencing can be used to identify the bacterial species, either through whole genome or 16S rRNA amplicon sequencing.
The identified species are coupled to functional information from the MiDAS Field Guide, which is a database of microorganisms found in Danish wastewater treatment plants and their main functional role. The development of an on-site, real-time, fast, simple and robust procedure from which bacterial species can be detected at the wastewater treatment plant, will enable knowledge driven decisions in the daily operation. Currently, a "suit-case" solution have been developed, and further focus is on simplifying procedures that needs to be conducted at the wastewater treatment plants and the bioinformatic interpretation of the data.

[P54] ACTIVITY OF PAO BACTERIA IN ANAEROBIC DIGESTERS AND THEIR SIGNIFICANCE FOR P RECOVERY

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Optimization of phosphorus (P) recovery methods has recently attracted considerable attention due to quickly depleting P rock reserves. Wastewater is a potential source of P, from which it can be efficiently recovered by the use of the Enhanced Biological Phosphorus Removal process, where polyphosphate accumulating organisms (PAO) bacteria take up excess amounts of P from the wastewater and subsequently release it to the bulk liquid in anaerobic digester, from which it can be precipitated and used as a fertilizer. The process requires further optimization since our knowledge of biological P release potential and the type of microorganisms involved is limited. In this study we investigated the factors influencing the metabolism and anaerobic P-release in model PAO bacteria *Ca.* Accumulibacter after it enters anaerobic digesters, with the focus on the effect of

temperature as well as high salt and ammonia concentrations. The dynamics of P release was monitored during short (4 h) and long-term (21 d) incubations where ortho-P was measured in the bulk liquid and the changes in *Ca*. Accumulibacter abundance were analysed using 16S rRNA amplicon sequencing. We observed that the conditions encountered in anaerobic digesters had a profound effect on biological P release activity in *Ca*. Accumulibacter, with the observed P release patterns being strongly dependent on the incubation temperature. Other factors like high salt and ammonia concentration also had a significant effect on the metabolic response of the investigated PAO. More studies are needed to confirm the results for other types of PAO present in full-scale WWTPs, however, these observations can have important implications for the future design of P recovery process from the digesters.

Other

[P55] GROWTH PARAMETER ESTIMATES OF LISTERIA MONOCYTOGENES IN COOKED CHICKEN: EFFECT OF PREPARATION OF INOCULUM

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In this study, it was demonstrated that even with very low inoculation volumes (0.44 ± 0.11%), estimates of lag times (λ) and growth rates (μ_{max}) can be significantly affected by the procedure used for preparation of the inoculum. Estimates of λ and μ_{max} from growth curves for *Listeria monocytogenes* on slices of sous-vide cooked chicken breast were compared for four inoculum preparation procedures. Overnight cultures were grown in BHI and sub-cultured in four different ways; i) dilution in fresh BHI (direct30), ii) chilled incubation for 3 d before dilution in fresh BHI (coldBHI), iii) chilled incubation for 3 d before dilution in Maximum Recovery Diluent (MRD) (coldMRD) and iv) dilution in fresh BHI followed by chilled incubation for 3 d (direct8). Direct30 and direct8 were tested against coldBHI at 8 and 19 °C, whereas the coldMRD procedure was compared to coldBHI in the temperature range from 6 to 24 °C. Lag times (λ) and max specific growth rates (μ_{max}) were fitted and used for statistical analyses. At 19 °C, signs of injured cells repairing during the first 3 h were found for the direct30 procedure. However, no clear statistical differences were found for λ ($P = 0.07$) or μ_{max}-values ($P = 0.50$). At 8 °C, results obtained for direct30 and coldBHI were identical. The direct8 procedure resulted in longer λ at 19 °C ($P = 0.04$) but similar μ_{max} ($P = 0.12$). At 8 °C, growth curves obtained using the direct8 procedure appeared to have no lag time when fitted. However, the curves showed signs of injured cells repairing during the first 5 – 7 h suggesting that the obtained λ-values of 0 could be an artefact resulting from the curve-fitting procedure. The coldBHI procedure resulted in 12% higher μ_{max}-values than obtained for coldMRD and below 10 °C, shorter λ-values were found.

[P56] ENRICHMENT, CONSTRUCTION AND CHARACTERIZATION OF AN ENVIRONMENTAL MICROBIAL CONSORTIUM DISPLAYING EFFICIENT KERATINOLYTIC ACTIVITY

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Keratin refers to a group of insoluble and tough protein materials. Currently, keratin byproducts are either buried, burned or treated with thermochemical processes prior to incorporation in animal feed formulations. Learning from nature, utilization of keratinolytic microbial consortia stand as a cost efficient and environmental friendly way to valorize this recalcitrant biomass resource. In this study, we applied directed experimental evolution to enrich for soil-born microbial consortia growing on α-keratin medium in sequential batch cultivation (n = 7). Along with keratinolytic activity

measurement, consortia evolution was monitored via 16S rRNA gene amplicon sequencing. A promising keratin degrading microbial consortium, “KMCG6”, displaying efficient keratinolytic trait was obtained, yielding ~80% degradation of raw material, and featuring mainly members of Bacteroidetes and Proteobacteria phyla. Comparative composition analysis reveals that low sulphur-containing protein turned into soluble substance preferentially. This work represent a significant advance in the field of α-keratin degradation, with potential applications in animal feed and agriculture.

Keywords: keratin degradation, enrichment cultivation, microbial consortia, biotechnology

[P57] EVALUATION OF STRAIN SPECIFIC QUANTITATIVE PCR FOR DETECTING BACILLUS STRAINS IN SWINE FAECES

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Bacillus sp. spores are being used in probiotic products and as direct feed additives for pigs and poultry. However, the general knowledge regarding the intestinal fate of *Bacillus sp.* spores after ingestion is still limited, particularly due to difficulties in detecting and differentiating strain-specific spores and vegetative cells in the complex microbiota of the gastro-intestinal tract (GIT). Questions we want to answer are: do the spores germinate and resporulate after ingestion and to what extent? How long does it take for spores to germinate and resporulate? How many spores and vegetative/ active bacterial cells are at different time points and at different segments along the GIT? Where in the intestinal segments of the pig GIT do the relevant germination and resporulation take place?
To address these questions a two-step approach has been initiated: i) strain-specific quantitative PCR (qPCR) assays were developed for several *Bacillus* sp. strains using their genome sequences. The assays were experimentally validated with respect to efficiency and specificity. The assay efficiencies are close to 100%. The primer specificities were tested on 260 *Bacillus* sp. strains available in Chr. Hansen culture collection. The data showed that it is possible to perform strain-specific qPCR. ii) pig faecal samples were spiked with increasing amounts of specific *B. subtilis* strains as either vegetative cells or spores. Three different DNA extraction kits were tested, and an evaluation of the qPCR method for detection in faeces was performed. The qPCR results were compared with traditionally plating techniques.

[P58] A GLASS OF WINE WITH ICE AND PHAGES PLEASE" -REASSESSING THE ROLE OF PHAGES FOR THE WINE INDUSTRY

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Consumers' demand for wine of enhanced organoleptic attractiveness that should result from sustainable practices during all stages of wine making is increasing the challenges for the wine industry. Thus, the development of efficient biological treatments is more than urgent and relevant, so as to control the bacteria that are affecting the final quality of wine.

In light of these, the aim of this ongoing study is the isolation and characterization of lytic and lysogenic bacteriophages against the "new generation" starter of wine fermentation, *Lactobacillus plantarum*.

Screening tests with a wide range of samples that included wine samples from the beginning, mid- and end of malolactic fermentation have been conducted using the two -so far commercially available- wine starters of the species. More than 20 phages, the first against *L. plantarum* wine strains, have been isolated and are currently being sequenced and characterized. Those bacteriophages found to infect *L. plantarum* can be used for the development of phage cocktails to control the result of malolactic fermentation and can be studied to prevent future stuck fermentations in view of the rising popularity of wine starters.

[P59] BACILLUS SUBTILIS SURFACTIN INFLUENCES ASPERGILLUS NIGER MORPHOLOGY AND ACTIVATES THE CELL WALL INTEGRITY PATHWAY

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Bacteria and fungi can coexist and interact in nature in various forms spanning from symbiosis to producing antimicrobial compounds that impair or kill the other organism. Previous study on *B. subtilis* and *A. niger* interaction revealed that genes involved in metabolism and putative antimicrobial production are differentially expressed upon close contact of the two organisms.
Here, we examined the morphological changes of fungal hyphae that were observed during co-cultivation of *B. subtilis* with *A. niger*, when growing hyphae form a blown up, rounded structure, which we termed 'bulbous cells'. We were interested which bacterially produced compound is responsible for this cell shape alteration and how the cell biology of *A. niger* is altered. For this,

various *A. niger* reporter strains were co-cultivated with supernatant of *B. subtilis* or its mutants lacking production of certain secondary metabolites.
We identified surfactin, a powerful surfactant and antibiotic of *B. subtilis* as the predominant agent causing the morphological changes in fungal hyphae. *C*onfocal microscopy using *A. niger* cell organelle reporters showed that secretory vesicles are miss-localized in bulbous cells. Furthermore, bulbous cells are more abundant in *A. niger* Δ*rlmA* strain. RlmA is a part of the cell wall integrity pathway, which induces cell wall biosynthesis and remodeling. Activation of the cell wall integrity pathway of *A. niger* was measured using a luminescence reporter gene construct, which showed increased cell wall stress, when *A. niger* is exposed to supernatant of *B. subtilis* wild type, but not when surfactin production by the bacterium was impeded.

[P60] EVOLUTION OF PHENOTYPIC HETEROGENEITY IN BACILLUS SUBTILIS BIOFILMS

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The pellicle biofilm matrix in *Bacillus subtilis* is predominantly composed of exopolysaccharide (EPS) and amyloid fiber (TasA). These components are synthesized by enzymes or composed of proteins coded in the *epsA-O* or *tapA-sipW-tasA* operons, respectively. In response to various signals, these operons are heterogeneously expressed, yielding only a subpopulation of cells expressing the *eps* and *tasA* genes. Our recent study shows that cells lacking both *eps* and *tasA* are not able to stick to the pellicle formed by the wild type cells and are outnumbered by the producer population. Contrary, single mutant strains (*eps* and *tasA*) are able to mix with wild type *B. subtilis,* increase their relative frequency in the pellicles and act as cheaters. However, it is uncertain how the presence of cheaters alters the bi-stability of matrix gene expression in producer strains and how such mixed population behaves in an evolutionary time scale. In this study, pellicles formed by the co-culture of producer (wild type) and cheaters (*eps* mutant) were repetitively re-inoculated every two days to follow alterations in biofilm gene expression heterogeneity and population ratio dynamics. Interestingly, certain populations of these co-cultures showed increased number of cells expressing the matrix gene while control populations in which wild-type strain evolved alone demonstrated high number of cells with matrix expression switched OFF. Further, co-cultures with producers in the overproducing state allowed cheaters to increase in number eventually resulting in population collapse. This study points towards the alteration of phenotypic heterogeneity when cheaters invade the biofilms of *B. subtilis*

[P61] INFLUENCE OF CHLORIDE AND PHOSPHATE IONS ON THE ANTIMICROBIAL EFFICACY OF A NOVEL ELECTROPLATED COATING AGAINST STAPHYLOCOCCUS AUREUS

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Self-disinfecting surfaces in health-care settings are receiving increasing attention as additional strategies to the normal disinfection practices, which often lack effectiveness due to personnel issues, failure to follow manufacturer's recommendations and inadequate antimicrobial activity. We have developed a novel copper-silver electroplated coating for frequently touch items in hospital rooms and have demonstrated its antimicrobial effectiveness. We attribute part of the antimicrobial effect to a slow silver-driven release of copper, however, further studies are required in order to understand the precise mechanism of its antimicrobial action. In particular, in presence of phosphate buffered saline, a common biological buffer solution, copper can complex with chlorine and phosphorus and this can influence its antimicrobial activity. These compounds are common in sweat and in standard detergents and we therefore decided to investigate the antimicrobial effect of the novel copper-silver electroplated coating in presence and absence of chlorides and phosphates containing buffer solutions. We used *S. aureus* 8325 as test organism and determined the antimicrobial effect of the surfaces on the bacterium when suspended in different buffers and dilutes. We also included electrochemical measurements and live/dead staining in order to directly quantify and visualize the killed bacteria.

[P62] IMPACT OF RECEPTORS ON HOST RANGE OF SALMONELLA-INFECTING PHAGES

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Salmonella enterica consist of more than 2400 serotypes and highly diverse. Except certain model phages, the extent of the host range of phages that can infect wild type *Salmonella* has been poorly described. In this work, we have isolated, sequenced and determined the receptor and the host range of a large number of phages for associating phage families and the receptors with the host range potential of *Salmonella*-infecting phages isolated from the environment.
In total, 50 phages were isolated on 10 different isolation hosts (7 serotypes) and subjected to large host range analyses using 71 Danish *Salmonella* isolates. Using *S.* Typhimurium LT2c mutants, we

found the receptors of 43 phages. Subsequently, 40 of these phages were sequenced using MiSeq platform, allowing us to group 31 distinct phages into phage families and associate these with host ranges. Members of *Jerseyvirus* infect only up to 12 strains, whereas among other O-polysaccharide (O-PS)-dependent phages, three phages of *Vi1virus* infected the most strains (up to 20). Interestingly, none of the O-PS-dependent phages could infect all strains of a certain serotype, indicating the absence of serotype specificity. The B_{12}-uptake channel (BtuB)-dependent phages identified as *T5virus* had the broadest host range, infecting up to 37 different strains.
Statistical analyses of the host range showed that the receptor is the foremost influencer of the host range compared to the isolation host or sample or assigned family. BtuB-dependent members of *T5virus* have higher potential to cover the diversity of food-associated *Salmonella* strains, making them better candidates for biocontrol purposes.

[P63] CORRELATION BETWEEN PIGMENT PRODUCTION AND CHITIN DEGRADATION IN MARINE PSEUDOALTEROMONAS SPECIES

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Chitin is the most abundant polymer in the ocean and an important source of carbon and nitrogen for marine bacteria. The genus *Pseudoalteromonas* is divided into a non-pigmented and a pigmented clade and many species are highly chitinolytic. In addition, the pigmented strains are commonly known for production of antimicrobial compounds. Here, we investigated and whole-genome sequenced a collection of 98 *Pseudoalteromonas* strains consisting of 52 pigmented and 46 non-pigmented strains. We found that all pigmented strains degraded chitin, whereas only half of the non-pigmented strains degraded chitin. Furthermore, pigmented strains displayed an almost immediate onset of chitin degradation, whereas non-pigmented strains showed a delayed onset of chitin degradation. On a genetic level, we saw clear differences between the clades. Both clades harbored a chitin degradation cluster consisting of two chitinases from glycosyl hydrolase (GH) family 18 and a lytic polysaccharide monooxygenase. Some pigmented strains harbored two of these clusters. Apart from that, all pigmented strains encoded one or more chitinases belonging to the GH family 19, which are less common in bacteria. Here, we identified several genes that might explain the faster chitin degradation in pigmented species. These results suggest that pigmented *Pseudoalteromonas* play a key role in degradation of chitin-containing material in the marine environment.

[P64] COEVOLUTION BETWEEN SALMONELLA TYPHIMURIUM AND A OPS-DEPENDENT PHAGE

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The use of phages as alternatives to antibiotics is becoming increasingly attractive, due to the rise and spread of resistant bacteria. Nevertheless, bacteria can develop resistance to phages too. These adaptation strategies are less known and need to be investigated to be able to foresee how phages will impact the diversity and the balance of microbial communities.
In this project, we studied the evolution of an environmental phage, S118, dependent on the O-polysaccharide as bacterial receptor, in presence of his host, the Gram-negative foodborne pathogenic bacterium, *Salmonella* Typhimurium. *Salmonella* resistance mechanisms were investigated by transferring coevolved phages and bacteria every 24h, for 9 days. The experiment was performed as 3 biological replicates by inoculating 3 microcosms with 10^6 cfu/ml *Salmonella* cells and 10^8 pfu/ml phages. For each transfer, 5 different colonies were isolated from the coevolved *Salmonella* populations and their resistance were tested against phages from each transfer.
Our results showed so far: I) the emergence of resistance mechanisms to S118 after 24h, mainly due to OPS change; II) the increasing number of *Salmonella* colonies completely resistant to all phages from past, present and future transfers; III) the change of plaque morphology after 24 h in the evolution experiments and after 48 h in the coevolution; IV) the reduced infectivity (but not the extinction!) of evolved and coevolved S118 against coevolved *Salmonella*.
The identification of the mechanisms responsible for *Salmonella* resistance will be identified by adsorption assays and whole genome sequencing. The fitness cost of these resistance mechanisms will be important to evaluate the impact of phages in microbial communities.

[P65] INFLUENCE OF THE CELLULAR METABOLIC STATE ON REPLICATION INITIATION FREQUENCY

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In *Escherichia coli* and most other bacteria, chromosome duplication is regulated by ATP activation of the initiator protein DnaA. This fact suggests a coupling between the cell cycle and energy metabolism in such a way that under growth conditions where the cell has ample energy supply, chromosome duplication is promoted. Our objective is to establish and characterize the connection between the energy charge of the cell and chromosome replication in bacteria.

To test the role of the ratio ADP to ATP in the cell in controlling DnaA activity, we have induced the expression of an ATP synthetase that is decoupled and whose activity is to deplete ATP (Koebmann *et al*., J Bacteriol, 2002). We have studied the effect of this low energy charge situation on mutants affecting initiation of replication (*DARS1* and *DARS2* sequences, involved in DnaAATP rejuvenation). The effect on cell cycle properties and initiation of replication has been analyzed by flow cytometry. We can conclude that lowered cellular ATP/ADP ratio has no influence on initiation frequency in the presence of any of the mechanisms reactivating DnaA. Nevertheless, when the cellular energy is reduced at least one of the DARS regions is required to ensure the accumulation of active DnaAATP. We suggest accumulation of DnaAATP as the absolute limiting factor for initiation of replication.
Fellowship support for B. M-C from Fundación Alfonso Martín Escudero of Spain is gratefully acknowledged.

[P66] ADAPTIVE LABORATORY EVOLUTION OF ANTIBIOTIC RESISTANCE USING DIFFERENT SELECTION REGIMES LEAD TO SIMILAR PHENOTYPES AND GENOTYPES

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Antibiotic resistance is a global threat to human health, wherefore it is crucial to study the mechanisms of antibiotic resistance as well as its emergence and dissemination. One way to analyze the acquisition of *de novo* mutations conferring antibiotic resistance is adaptive laboratory evolution. However, various evolution methods exist that utilize different population sizes, selection strengths and bottlenecks. While evolution in increasing drug gradients guarantees high-level antibiotic resistance promising to identify the most potent resistance conferring mutations, other selection regimes are simpler to implement and therefore allow higher throughput. The specific regimen of adaptive evolution may have a profound impact on the adapted cell state. Indeed, substantial effects of the selection regime on the resulting geno- and phenotypes have been reported in the literature. In this study we compare the geno- and phenotypes of *Escherichia coli* after evolution to Amikacin, Piperacillin and Tetracycline under four different selection regimes. Interestingly, key mutations that confer antibiotic resistance as well as phenotypic changes like collateral sensitivity and cross-resistance emerge independently of the selection regime. Yet, lineages that underwent evolution under mild selection displayed a growth advantage independently of the acquired level of antibiotic resistance compared to lineages adapted under maximal selection in a drug gradient. Our data suggests that even though different selection regimens result in subtle genotypic and phenotypic differences key adaptations appear independently of the selection regime.

[P67] DECREASING ACIDIFICATION TIME IN FERMENTED MILK BY ADDING BACILLUS SP.

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Bacillus species are widespread bacteria in nature, and are used as both enzyme producers, probiotics for animals and humans and as an important part of indigenous fermented food. However, within milk-based fermented foods, bacilli are often seen as contaminants, either being pathogenic, like *B. cereus*, or spoiling the product, e.g. *B. licheniformis*. The typical starter culture in fermented milk products consists of mainly three species of lactic acid bacteria (LAB): *Lactococcus* sp., *Streptococcus thermophilus* and/or *Lactobacillus* sp.

We wanted to investigate whether a beneficial effect on e.g. acidification and texture could be obtained by co-culturing LAB and bacilli.

Results obtained showed that milk co-inoculated with *Bacillus* sp. reached pH 4.5 faster compared to controls with only LAB added. The effect seemed to be more dependent on the LAB used and to a smaller extent the species of *Bacillus* added. Milk with only *Bacillus* added did not acidify. General aspects of *Bacillus* in milk with different LAB strains were investigated; off flavor, sugar consumption, production of volatiles and small acids, syneresis, and effect of storage. The results showed that *Bacillus* seem to boost growth of LAB, decrease acidification time and add to texture. No adverse effects could be found: there was no off flavor, severe syneresis or strange compounds formed when adding *Bacillus* to milk together with LAB strains.

The concept of *Bacillus* in milk thus seems promising: faster acidification, increased texture, and no adverse effects found so far.

Reference:
WO2017/005601 A1

[P68] ISOLATION AND CHARACTERIZATION OF BACILLUS SUBTILIS STRAINS FROM DIFFERENT ECOSYSTEMS

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Bacillus subtilis is well-known for its biofilm formation and during the last years, it become also interesting for plant root colonization, as biofilm formation is linked to attachment in the rhizosphere. The extracellular matrix of *B. subtilis* is mainly composed of exopolysaccharides (EPS) and the protein TasA. To understand the abundance, the biofilm formation ability and biocontrol properties of *B. subtilis* isolates, diverse soil ecosystems were targeted. In this study, eight out of 95 sporulating soil isolates from different ecosystems were phenotypically characterized with

biochemical tests and genetically confirmed as *B. subtilis* strains. Importantly, the abundance of *B. subtilis* depended on the soil ecosystem used for sampling. The soil isolates behaved in biofilm formation and colonization of *Arabidopsis thaliana* roots similar to the undomesticated *B. subtilis* type strain NCIB 3610. The root colonization assays were performed under low rotation to encourage root colonization and to prevent pellicle formation. Consequently, this study confirmed the ability of *B. subtilis* to colonize plant roots after 18 h of incubation. The screening of antimicrobial compounds demonstrated an inhibition of the phytopathogenic fungus *Alternaria alternata* due to the majority of the soil isolates. We propose that *B. subtilis* isolates from soil ecosystems are promising targets for biocontrol against plant pathogens.

[P69] ANTIMICROBIAL PROPERTIES AND COMPOSITIONAL ANALYSES OF TERMITE SOLDIER DEFENSIVE SECRETIONS

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Termites are eusocial insects with division of labor between worker and soldier individuals (castes), where the soldier caste is responsible for colony protection. This can take the form of mechanical defense, with soldiers of many termite genera having enlarged mandibles, or chemical defense, in which defensive oral secretions deter predators. While there has been only limited work exploring the exact compositions and diverse functions of termite defensive secretions, several studies suggest that they contain antimicrobial compounds. The defensive secretions of termite soldiers may thus also play a role in defense against microbial pathogens of the termites. We explored the antimicrobial potential of oral secretions from soldiers of two termite taxa, *Coptotermes formosanus* and *Mastotermes darwiniensis*, by performing inhibition assays against putative fungal and bacterial antagonists. Preliminary results indicate that the oral secretion of *C. formosanus* soldiers inhibits the growth of the entomopathogenic fungus *Beauveria bassiana*. Antimicrobial activity assays will be complemented by compositional analysis of the secretions using proteomics and liquid chromatography-mass spectrometry (LC-MS) based metabolomics.

[P70] AUTONOMOUS MONITORING OF MICROBIAL ABUNDANCE IN THE DESERT OF SAUDI ARABIA

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In the oil industry, undesired growth of microorganisms can result in reservoir souring and plugging, and microbial influenced corrosion (MIC) of carbon steel. Monitoring the microbial status provides information on the needs for action. However, manual monitoring campaigns are costly, and there is a constant demand for new methods that reduce the effort, while increasing sampling frequency and data quality.
Saudi Aramco is the world's largest oil company, producing approx. 12.5 million barrels per day, generating more than $1 billion a day in revenues. To sustain oil production, seawater, for injection into the oil reservoirs, is transported through a network of pipelines through the desert to the production sites. Maintaining pipeline integrity and avoid reservoir plugging is of utmost importance to sustain production and avoid costly breakdowns.
To monitor the degree of microbial fouling in the injection water system Saudi Aramco and Danish Technological Institute have collaborated on developing an autonomous microbial sensor. The sensor is designed to endure the harsh Saudi Arabian desert environment with occasional sandstorms, temperature fluctuations between 5 and 65°C, and highly saline liquid of 55‰, and further, to run for a month without need for maintenance and replacement of reagents.
With this sensor technology, Saudi Aramco can today monitor their injection seawater for microbial abundance, without visiting the remote sites more than once per month.

[P71] GREENLANDIC ENZYMES FOR BIOCATALYSIS OF PREBIOTIC OLIGOSACCHARIDES

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Prebiotics are oligosaccharides that stimulate growth of beneficial gut bacteria like *Bifidobacteria* and *Lactobacilli*, while reducing growth of pathogenic bacteria such as *E. coli* and *Salmonella enterica.*
The aim of this study was to discover and apply bacterially produced enzymes from hot and cold environments in Greenland for production of prebiotic oligosaccharides.
Bacteria were isolated from ikaite columns in the Ikka Fjord (<5°C, pH 10.4), and from hot springs in East Greenland (49-60°C, pH ~7). Strains were isolated and screened for enzymatic activities. Selected enzymes were expressed in *E. coli* and assayed using TLC and HPLC.

We have characterized a cold-active β-galactosidase from the bacterium *Alkalilactibacillus ikkensis* isolated from an ikaite column. This enzyme shows high hydrolytic as well as transglycosylation activity at 0-20 °C, making it an attractive enzyme for the dairy industry in production of fresh milk products.
Heat-stable enzymes for production of prebiotics in preserved foods are desirable due to increased solubility of lactose at high temperatures. Therefore, we screened thermo-tolerant bacteria for enzymes able to catalyse the formation of human milk oligosaccharides (HMOs) for use in milk formula. This resulted in the isolation of a thermostable α-L-fucosidase from *Paenibacillus dendritiformis*, which is able to hydrolyse α-L-fucoside bonds and catalyse the formation of fucosylated oligosaccharides at 50 °C.
In conclusion, we have shown that bacteria from Greenland have scientific as well as industrial potential due to the broad variety in physical and chemical conditions in the environment, affecting the properties of the bacteria and the bioactive compounds that they produce.

[P72] HIGH THROUGHPUT SEQUENCING OF FULL-LENGTH SSU RRNA SEQUENCES FROM COMPLEX MICROBIAL COMMUNITIES WITHOUT PRIMER BIAS AND HOW IT AFFECTS OUR ABILITY TO STUDY MICROBIAL ECOLOGY

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The small subunit (SSU) ribosomal RNA (rRNA) genes have been used to study microbial diversity and evolution for the last 30 years. Today, databases containing full-length SSU rRNA reference sequences remain fundamental for many core analyses in microbial ecology, such as community profiling using 16S/18S rRNA amplicon sequencing and in-situ studies based on fluorescence in situ hybridization (FISH) microscopy. The quality of the data produced relies heavily on the reference databases used, and it is widely recognized that the current databases are underpopulated, ecosystem skewed, and subject to primer bias. Here we present a method that combines reverse transcription of polyadenylated full-length SSU rRNA molecules with Illumina based synthetic long-read sequencing to obtain high quality, full-length SSU rRNA sequences in a high throughput manner. We applied the approach to complex samples from seven different ecosystems and obtained more than 1,000,000 SSU rRNA gene sequences from all domains of life with an estimated raw error rate of 0.17%. We observed a high fraction of novel diversity including several deeply branching phylum level lineages. Here we describe how the method works and demonstrate how the access to comprehensive ecosystem specific SSU databases affect our ability to study microbial ecology.

[P73] ELUCIDATING PRIORITY EFFECTS IN MULTISPECIES BIOFILM ASSEMBLY

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The arrival order of different species to a specific niche may strongly impact community assembly and functionality. Timing of colonizing species influence how the different species interact with each other and the environment. In this study, we used a model consortium composed of four soil isolates (*Stenotrophomonas rhizophila, Xanthomonas retroflexus, Microbacterium oxydans* and *Paenibacillus amylolyticus*) to determine if arrival order matters in the development of a multispecies biofilm grown under different *in vitro* conditions. We screened our four species individually for exerting priority effects on later arriving species by evaluating physical differences in biofilm formation. The general procedure for all experiments was to pre-inoculate one species in the system, incubate it for 6, then add all four species and incubate for either 18h Calgary Devices (CD) and Drip-Flow Reactors (DFR) or 14h-34h (IBIDI). We quantified overall biofilm production in CD by crystal violet staining. Species ratios in the different biofilms grown in DFR were determined by plate count. Further, the spatial localization of fluorescently-tagged *S. rhizophila* and *X. retroflexus* in biofilms grown in IBIDI slides were assessed by confocal microscopy. Our preliminary data indicate that arrival order play a role in early multispecies biofilm assembly. Prior arrival by specific species resulted in distinct spatial structure of biofilms. The quantity and quality of the biofilms formed in the different systems also differed depending on which species had been pre-inoculated.

[P74] ASCHIP: A COMPREHENSIVE HIGH-THROUGHPUT QPCR CHIP FOR INVESTIGATING MICROBIAL ARSENIC RESISTANCE AND BIOTRANSFORMATION GENES IN ENVRIONMENTS

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Arsenic (As) is a ubiquitous toxic element adversely affecting human health worldwide. Quantitative PCR (qPCR) is currently considered the gold standard for precise monitoring of genes in environmental samples and has been applied to quantify genes involved in As biogeochemical cycling. We here report the development of a novel high-throughput qPCR (HT-qPCR) based AsChip for comprehensive monitoring of As related genes. This novel AsChip contained 81 taxa-specific primer sets targeting 19 As genes with hundreds species covered at nanoliter reaction scale. High specificity, sensitivity and efficiency of AsChip were revealed by computational and experimental validations. AsChip was successfully applied to track As genes on soil samples from a chromated copper arsenate contaminated site located north of Copenhagen. A totally of 58 As genes were

detected and its total abundance was up to 0.76 copies per cell with *arsC* gene involving As (V) resistance process contributing most. Our novel AsChip will allow for comprehensive, cost-effective molecular analysis of genes involved in microbial As cycling.

[P75] IMAGING TRANSCRIPTION DURING MICROBE-MICROBE INTERACTIONS

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Microbial interactions underpin many important biotechnological applications spanning medicine, food development and processing, bioremediation and biocontrol. In order to study microbial interactions a broad range of technologies exist including genomics facilitating the identification of pathways and genes coupled with transcriptomics and mass spectrometry imaging to study gene expression and metabolites exchanged during interactions. While these methods advance our understanding of microbial interactions, they also have limitations namely the requirement for destructive sampling. Here, we report the development of a microplate reader-based system for visualizing gene expression dynamics in living bacterial cells in response to a fungus in space and real-time. A bacterium expressing the red fluorescent protein mCherry fused to the promoter region of a regulator gene *nunF* indicating activation of an antifungal secondary metabolite gene cluster was used as a reporter system. Time-lapse image recordings of the reporter red signal and a green signal from fluorescent metabolites naturally produced by the bacterium combined with microbial growth measurements showed that *nunF*-regulated gene transcription is switched on when the bacterium enters the deceleration growth phase and upon physical encounter with fungal hyphae. The established non-destructive method has many advantages notably the ability to provide detailed space and time information on the transcription of target genes in living organisms. Importantly, the technique is an alternative but complementary tool to the many technologies already used for studying microbial interactions.

[P76] THREE EARTHWORM SYMBIONTS WITH THREE EVOLUTIONARY PATHS

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The excretory organs (nephridia) of earthworms harbor specific, vertically transmitted, extracellular symbiotic bacteria. *Verminephrobacter* benefits host reproduction and has co-evolved with lumbricid earthworms during the past 100 million years. Genome comparison with closely related free-living organisms showed that *Verminephrobacter* is impacted by bottleneck-induced drift although there are no signs of genome reduction or AT-bias.

The common compost worm, *Eisenia andrei*, is colonized by *Verminephrobacter* and two additional uncultivated symbionts; *Ca.* Lumbricidophila and *Ca.* Nephrothrix. We obtained genomes of the latter two symbionts by sequencing a cocoon metagenome. Compared to closely related organisms *Ca.* Lumbricidophila has a reduced genome (2.85 Mb compared to 4-5 Mb) and its GC content is reduced by 14%. Furthermore, analysis of dN/dS ratio and codon usage bias in >1000 orthologous genes showed a much stronger impact of bottleneck-induced drift than in *Verminephrobacter*.

The genome of *Ca*. Nephrothrix could only be assembled in 1220 scaffolds due to a high microdiversity within this symbiont group. This microdiversity, and thereby greater genetic potential within this group, might enable *Ca.* Nephrothrix to evade the impact of bottleneck-induced drift altogether.

Although the three symbionts co-inhabit the same host and transmitted together from one host generation to the next, the symbiotic lifestyle has impacted their evolution in markedly different ways.

[P77] BIOLOGICAL CONTROL OF SOFT ROT CAUSING BACTERIA USING PHAGE THERAPY

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The increased demand for food, following the global growth of the human population, requires that overall crop yields increase. However, crop diseases critically impact the possibility of increasing crop yields, thus calling for new, and preferably environmentally sustainable, approaches to tackling these diseases. One such approach is bacteriophage (phage) therapy of crop plants.
Pectobacterium and *Dickeya* spp. are known to cause the soft rot disease of potatoes (*Solanum tuberosum*). Phages infecting these soft-rot causing phytopathogens were isolated and a subgroup was subsequently characterized using next-generation sequencing. The phages were then applied in bioassays with potatoes to assess the viability of the phages for biological control. Furthermore, a novel approach to phage isolation, where living potatoes were used as substrate for the phytopathogens during isolation, was investigated for its potential in specifically isolating phages virulent under *in planta* conditions and thus potentially more pertinent to an authentic crop setting. Understanding the interactions between phages and their bacterial hosts in the infected plants can assist in evaluating the capability of alternative approaches to the control of pathogens, while also providing valuable insights into the evolutionary mechanisms, which are in play during these interactions.

[P78] DISCOVERY OF NOVEL ALGAE-DEGRADING ENZYMES FROM MARINE BACTERIA

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Algal cell wall polysaccharides, and their derived oligosaccharides, display a range of health beneficial bioactive properties. Enzymes capable of degrading algal polysaccharides into oligosaccharides may be used to produce biomolecules with new functionalities for the food and pharma industry. Some marine bacteria are specialized in degrading algal biomass and secrete enzymes that can decompose the complex algal cell wall polysaccharides. In order to identify such bacteria and enzymatic activities, we have used a combination of traditional cultivation and isolation methods, bioinformatics and functional screening. This resulted in the discovery of a novel marine bacterium which displays a large enzymatic potential for degradation of red algal polysaccharides e.g. agar and carrageenan. In addition, we searched metagenome sequence data and identified new enzyme candidates for degradation of fucoidan – a fucose-containing cell wall polysaccharide of brown algae with wide biomedical potentials. Putative enzymes were produced recombinantly in *E. coli* and biochemical analyses documented their ability to degrade algal polysaccharides.

[P79] CHANGES IN CYTOCHROME C REDOX STATE SHOW ELECTRON TRANSPORT IN INDIVIDUAL CABLE BACTERIA

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Various lines of indirect evidence indicate that multicellular cable bacteria can conduct electrical currents along the longitudinal axis of their centimeter-long filamentous bodies. This observation extends the known distance for biological electron conduction by orders of magnitude but, at present, direct experimental confirmation of long-distance electron transport is lacking. Here, we used resonance Raman microscopy to determine the redox states of c-type cytochromes along individual filaments of living cable bacteria. Cytochromes are known to function as electron shuttles, and Raman spectra showed that the dominant cytochrome type in cable bacteria resembles that of bacteria capable of extracellular electron transport. Scanning individual cable bacteria over a length of 4 mm, stretching from a sulfidic zone towards an oxygen front, we observed a gradual change from reduced to more oxidized cytochromes. Disrupting the electron transport by cutting cable bacteria filaments with a laser caused a rapid shift to more reduced cytochrome states in the cable end now disconnected from oxygen, while a cyclic exposure to oxic-anoxic conditions at the cathodic end induced concomitant cycles in the cytochrome redox state towards the anodic end. These findings are consistent with a model where cytochromes are indicators of a redox potential gradient along a conducting structure inside cable bacteria, thus providing the first direct evidence for microbial long-distance electron transport.

[P80] COMPARISON OF DIFFERENT COMBINATIONS OF FILTERS AND DNA EXTRACTION METHOD FOR QUANTITATIVE AIRBORNE BACTERIA ANALYSIS

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Molecular biological methods such as quantitative PCR that are based on nucleic acids extracted from environmental samples can be used to study population dynamics through the quantification of specific genes with a high sensitivity and resolution. These methods require an efficient and reproducible extraction method, especially in designing DNA detection of low density air samples. In this study, different types of filters and DNA extraction kits were examined to find the method that gives the best extraction and purification for quantitative analysis. Air samples were collected from a roof station located at KU and a municipal WWTP (Roskilde) by commercial vacuum cleaners (Karcher) to capture airborne bacteria into a collection buffer. Filtration of the buffer using either a polyethersulfon membrane filter (Millipore) or a nuclepore track etched filter (Whatman) followed by the extraction with the Powerwater DNA isolation kit (Mobio) outperformed than other combination. The method was found to be reproducible with average DNA concentration of 0.7-1.6 ng/µl for WWTP samples and the extracted DNA was reliable for qPCR analysis. The information can

be useful when low density samples are treated and easy and fast extraction without requiring specialized equipment or complex processes is preferred.

[P81] TRANSMISSION OF EXTENDED-SPECTRUM CEPHALOSPORIN (ESC) RESISTANCE THROUGH THE BROILER PRODUCTION SYSTEM IN DENMARK

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The prevalence of extended-spectrum cephalosporin (ESC) producing *Escherichia coli* in the two Danish broiler production systems was investigated. Samples were collected at central breeding facilities, at hatcheries and at broiler production using different sampling techniques. From 2015 to 2016 285 samples were collected. Twenty two isolates of the twenty four ESC positive *E. coli* were sequenced. The data revealed two distinct dominant clones; one in each of the tested production systems. One clone carried the $bla_{TEM\text{-}52B}$ gene on related plasmids while the other clone carried $bla_{CMY\text{-}2}$ on identical plasmids. Data presented here suggest that the ESC resistant *E. coli* enters the Danish broiler production at the top of the breeding system and is transferred vertical downwards. No data obtained indicates that house specific clones exist and persist in the production system or that horizontal transmissions occur inside the production system. Both bla_{CMY2} and bla_{TEM52B} has in previously studies been detected in parent and broiler flock and even in broiler meat.

[P82] FORMATION AND DYNAMICS OF CIRCULAR DNA ELEMENTS DERIVED FROM SINGLE-STRAIN MOBILOMIC SEQUENCING

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While the bacterial genomics have reached a level of maturity where complete genomes of single strains are achieved with relative ease, these assemblies only provide a snapshot of a bacterium's genetic material. Although this can provide important information on the organization of an organism's genome and genes encoding interesting traits, it also gives researchers an impression of a static genome in a fixed configuration. However, this does not represent the natural state of genomes that should be considered more dynamic. A novel approach, single-strain mobilomic sequencing, was applied in this study, in order to investigate the on-going molecular evolution of the pesticide-degrading model organism *Sphingobium herbicidovorans* MH. This method is based

on exonuclease digestion of linear DNA fragments and subsequent sequencing of remaining circular DNA. Most DNA molecules are naturally circular in bacterial genomes but larger molecules such as chromosomes and megaplasmids will break during handling of DNA and will therefore be digested during exonuclease treatment. Single-strain mobilomic sequencing revealed the presence of many small circular DNA molecules originating from various IS elements, including the excised and circular state of a catabolic gene cluster with high importance to pesticide degradation. These circular DNA elements have the potential to be inserted into new genomic locations and aid in propagation of important genes on other replicons. The formation rate of the circular DNA molecules could furthermore be estimated using this approach, highlighting that this method holds great potential for studying the dynamics of genomes and providing snapshots of gene movement.

[P83] BIOFOULING OF MEMBRANES: FILAMENTOUS YEASTS - AN OVERLOOKED FACTOR?

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Background: Reverse Osmosis (RO) membranes are increasingly being used in the food industry and biofilms may present a challenge in terms of clogging or up-concentration of microorganisms. The biofilm may form in spite of regular cleaning using a Cleaning-In-Place system

Aim: To investigate the microbiota on fouled RO membranes and its response to cleaning and disinfection with special emphasis on newly isolated yeast species.

Methods: Biofilm was visualized using light microscopy. 16S and 26S rRNA sequencing, physiological, biochemical, macro-and microscopic observation were used for identification. Broth cultures of filamentous species were exposed to time-temperature tolerance assays. Tolerance to CIP solutions was tested on biofilms created under static conditions on RO coupons.

Results: A dense network of clotted, filamentous yeast covering a great area as well as budding yeast cells and bacteria was observed on several occasions. Filamentous yeast species were identified as the closely related genera *Saprochaete clavata* and *Magnusiomyces spicifer* of similar physiology and cell morphology. Isolates from both genera exhibited heat tolerance to ≤ 75◦C/15 min and survival after CIP treatment.

Conclusion: Highly stress tolerant filamentous yeast species have been shown to constitute part of RO membrane biofilm, covering large areas even in low numbers. Hyphae structure may facilitate their attachment and survival. Clotting ability, slow growth compared with the bacteria present and the lack of a quantification method may have led previous studies to overlook them. More research on their source and role is needed and a strategy for their removal should be developed.

[P84] RETRIEVAL OF INDIVIDUAL BACTERIAL GENOMES FROM COMPLEX MICROBIAL COMMUNITIES USING HIGH-THROUGHPUT LONG-READ DNA SEQUENCING

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Genomes are the blueprint of a microbe's physiological properties, and knowledge hereof makes it possible to reconstruct potential metabolisms, and establish hypotheses for evolution, function and ecology. However, it is extremely difficult to cultivate the majority of microbes in the laboratory – also known as the "microbial dark matter (MDM)".
Currently, to obtain genomes from environmental samples, the standard approach is to use short-read metagenomic sequencing. Due to the short reads, assembly is computationally heavy and mapping is not always possible. However, the rapid development in long-read DNA sequencing has made it possible to close more genomes.

In this presentation, we will show our progress to optimize DNA extraction methods for ultra-long-read DNA sequencing, and apply these methods to obtain genomes from anaerobic digesters using the Oxford Nanopore sequencing technology. The retrieved genomes will be used to describe the potential functions of the microbes in anaerobic digesters. Furthermore, phages and plasmids will be tied to the microbe they reside in through application of the newly developed Hi-C method.

[P85] POLYSACCHARIDE HYDROLYZING ENZYMES FROM MARINE COLWELLIA SP.

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Several bacteria isolated from the marine environment are known to encode enzymes with the ability to degrade algal sulfated polysaccharides. The catalytic mechanisms and structures of these specific enzymes is important, since these enzymes can modify the biochemical properties of sulfated poly- and oligosaccharides, which are reported to have health promoting effects.
The aim of this study was to identify and characterize novel enzymes with the ability to hydrolyze the sulfated polysaccharides from red algae. A marine bacterium was isolated from sea urchins and selected based on its ability to dig into solid agar. Genome mining identified several candidate enzymes, which were then selected for cloning and expression in *E. coli*. Enzyme activity was characterized using a reducing sugars assay (MBTH assay), Thin Layer Chromatography (TLC) and Fluorophore Assisted Carbohydrate Electrophoresis (FACE).
This led to the discovery of several enzymes, for example: (i) a β-agarase showing hydrolytic activity on agar, agarose and neoagarooligosaccharides (NAOS), (ii) an α-neoagarobiase with activity against the NAOS releasing 3,6-anhydro-L-galactose (AHG), and (iii) a κ-carrageenase, which hydrolyzed both κ- carrageenan and furcelleran and two novel furcelleranases which were capable of hydrolyzing furcelleran.

In conclusion, we isolated a novel bacterium affiliated to the genus *Colwellia,* and characterized several enzymes with industrial application potentials.

[P86] AMINOBACTER SP. MSH1BIODEGRADATION OF 2,6-DICHLOROBENZAMIDE (BAM) IS STIMULATED IN MEMBRANE TREATED RESIDUAL WATER

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Groundwater is a precious resource in Danish drinking water production. Yet, abstraction sites are threatened by accumulation of recalcitrant pesticide residues, resulting in well closure. Thus, development of strategies for pollutant removal are encouraged. To this end, bioaugmentation applied to rapid sand filters (RSF) in drinking water treatment plants (DWTP) has been suggested as a promising method. However, introduced degraders are usually alien to the RSF environment, where nutrients are scarce, and are thus subject to competition and predation by the native RSF community, leading to decline in population size and hence degradation capacity.
We propose a novel approach where water is treated in two steps. First, membrane filtration separates the water into an ultra-pure fraction and a residual fraction, where pollutants, carbon, minerals etc. are concentrated. Second, the residual fraction then act as feed for a RSF with degrader bacteria, or consortia, for degradation of unwanted pollutants. Here we show results of batch experiments, with untreated and residual membrane water from three DWTP's, using the BAM degrading bacteria *Aminobacter* sp. MSH1. Results show an increased BAM degradation potential of MSH1 in residual vs untreated water, along with increased numbers of MSH1 in the early stages, which together holds promise for future flow-system experiments.

[P87] EVALUATION OF A DIRECT LYSIS METHOD FOR THE EXTRACTION OF NOROVIRUS RNA FROM VARIOUS SOFT FRUIT AND VEGETABLES

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Consumption of soft fruits contaminated with noroviruses (NoV) frequently causes disease outbreaks. To ease surveillance and control viruses in fruit and vegetable supply chains, current detection methods need increased simplicity and sensitivity.
For the extraction of NoV RNA on soft fruit and vegetables, we evaluated a reference method A, ISO/TS 15216, comprising viral elusion-concentration-extraction, and a method B, encompassing only direct lysis of viruses. The aim of this study was to assess the efficiency of these 2 methods to recover spiked modelvirus, mengovirus (MC_0), from frozen samples of raspberries, strawberries, blueberries, blackberries, mango, spinach, and 4 different types of fruit mixes, while eliminating inhibitors for the subsequent RT-qPCR detection of NoV.
The extraction efficiency of MC_0 from raspberries, strawberries, blueberries, blackberries, mango, spinach, and fruit mix a, b, c and d was 0-18% using method A; while method B resulted in 11-91%. The PCR inhibition during NoV detection for the same matrices using method A was 0-96%; while a decreased inhibition of 0-22% was obtained by using method B.
Method B showed better recovery rate in all sample types and less co-concentration of RT-qPCR-inhibitors in all matrices but fruit mix a and c, which exhibits better sensitivity of the method over method A. Thus, we propose study and implementation of method B in detection and surveillance of NoV in soft fruits and vegetables.

[P88] HOST PROTEINS DETERMINE MRSA BIOFILM STRUCTURE AND INTEGRITY

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Human extracellular matrix (hECM) proteins aids attachment and initiation of an infection, by specific binding to bacterial cell surface proteins. However, the importance of hECM proteins in structure, integrity and antibiotic resilience of a biofilm is unknown. This study aims to determine how specific hECM proteins affect *S. aureus* USA300 JE2 biofilms.
Biofilms were grown in the presence of synovial fluid from rheumatoid arteritis patients to mimic *in vivo* conditions, incorporating hECM proteins into the biofilm matrix. Difference in biofilm structure, with and without addition of hECM to growth media, was visualized by confocal laser scanning microscopy. Two enzymatic degradation experiments were used to study biofilm matrix

composition and importance of hECM proteins: removal of specific hECM proteins from growth media, before biofilm formation, and treatment of 24-hour-old biofilms.
hECM addition changed the overall biofilm structure, with larger dispersion of cells within the biofilm matrix. Fibrin, elastin, and collagen were important in forming and maintaining the biofilm structure. Their absence, from growth media, reduced biofilm formation 5-fold, indicating that they are important for biofilm initiation. Their enzymatic degradation, in an established biofilm, caused dispersal, showing that these proteins are critical for structural integrity.
We conclude that while hECM proteins are an integral part of the biofilm matrix, we find no evidence that these matrix components are directly responsible for the biofilm's unique antibiotic resilience. When utilizing *in vitro* biofilm models, we recommend addition of hECM proteins to standard growth media, in order to mimic biofilm properties and structure seen *in vivo*.

[P89] BACTERIOPHAGES USE HYPERMODIFIED NUCLEOSIDES TO EVADE HOST'S DEFENCE SYSTEMS.

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Since the very beginning of life, primitive cells were forced to face selfish genetic elements like viruses or plasmids. Bacteria, continually exposed to infections, developed several phage resistance mechanisms e.g. restriction-modification and CRISPR-Cas systems. On the other hand, bacteriophages developed several strategies to evade these defence mechanisms. Ultimately, this led to the oldest and still running arms race - microorganisms vs. their molecular parasites.
We here describe a remarkable new strategy used by the recently isolated *Escherichia coli* phage CAjan belonging to *Seuratvirus* genus. CAjan contains a set of genes with a high degree of similarity to 7-deazapurine biosynthesis genes. 7-deazapurines are hypermodified nucleosides, which are present in some classes of RNAs in bacteria and eukaryotes. CAjan show a remarkable resistance to restriction endonucleases. In order to investigate this mechanism in detail we have used several methods including transcriptomics, direct plaque sequencing, restriction endonuclease analysis and CRISPR-Cas genome editing. Through generation of specific mutants, we were able to introduce a restriction sensitive phenotype in the CAjan bacteriophage providing new insight on use of alternative bases by bacteriophages.

[P90] SINGLE-MOLECULE OPTICAL MICROSCOPY FOR THE INVESTIGATION OF STAPHYLOCOCCUS AUREUS BIOFILM FORMATION

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Staphylococcus aureus biofilms are a leading cause of implant associated infections, which are notoriously difficult to treat using antibiotics. Understanding the role various molecules play in matrix development could lead to new insights in treating biofilm infections. A bespoke fluorescence microscope is under development which is capable of imaging single molecules inside *S. aureus* biofilms on a millisecond time scale, and will be used to shed light on how various molecules contribute to matrix formation. Biofilms are challenging to image using fluorescence microscopy because they strongly scatter light; the microscope overcomes this using transverse illumination with a single Bessel beam to improve the signal to noise ratio. A Bessel beam is used because it is non-diffractive and self-reconstructing, giving an improved depth of imaging and maintaining a high enough intensity to allow for millisecond imaging of single molecules. Host derived fibrin is a key component of the matrix in coagulase positive *S. aureus* biofilms: the microscope will be applied to investigate how two secreted coagulases, von Willebrand factor binding protein and coagulase, contribute to fibrin formation. Fluorescent fusion proteins are currently being developed for the two coagulases, and will be used for microscopy under *in vivo* like conditions in knock out mutants of *S. aureus* that lack the gene for one of the two coagulases.

[P91] USING VOLATILE ORGANIC COMPOUNDS (VOCS) AS INDICATORS OF FUNGAL GROWTH AND FOOD SPOILAGE

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Microbial spoilage of vegetables is a global problem, estimated to cause loss of 15-20% of produce before reaching the consumer. Hence, there is focus on developing tools to monitor and manage spoilage microorganism at long-term storage facilities, in order to diminish food loss. One option is employ the volatile secondary metabolites that microorganisms produce. Often, a high number of VOCs is produced, constituting a VOC profile specific for a given microorganism. In the present project we studied three *Fusarium oxysporum* and three *F. proliferatum* strains isolated from diseased onions, and known to cause heavy economical losses in the postharvest chain. The fungi were screened for production of VOCs, while grown at similar conditions in onion medium, to study the similarity between their VOC profiles and to identify potential *Fusarium* VOC biomarkers. Likewise, *Fusarium* isolates were grown at different densities to establish an eventual relationship between VOCs and fungal biomass measured by total DNA content or by RT-PCR. The results showed

that the different isolates produced the same VOCs, in general. However, PCA analysis also revealed that the two species had distinct VOC profiles that were discernible even at the isolate level. The amount of VOC produced correlated positively with fungal biomass, indicating that the VOCs have potential to serve as a non-destructive indicator of *Fusarium* sp.

[P93] COMPARISON OF DNA EXTRACTION METHODS TO DETECT FUNGAL DIVERSITY FROM ALCOHOLIC FERMENTATION WINES

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In light of both on-going advances in next-generation sequencing (NGS) technologies and dropping sequencing prices, there is a growing interest in genetically profiling the microbial communities involved in winemaking. Although a number of studies have characterised the microbes of grapes, must, leaves and soils, few have studied those active within the actual fermentation process. This is partly due to the presence of large amounts of polyphenol and polysaccharides within the fermenting must, thus rendering it difficulty to extract DNA. Hence, the development of efficient and reliable DNA extraction methods from this substrate is of considerable interest. Here we present a comparison of the efficacy of 3 DNA extraction protocols for application to the alcoholic fermentation stage of winemaking: a commercial protocol (FastDNA Spin Kit for Soil), a CTAB-chloroform based protocol, and a depurinated DNA-phenol-chloroform based protocol. The test was performed in duplicate on Riesling ferments derived from 4 German vineyards, across 4 fermentation time points. Fermentation was carried out in microvinification under laminar workflow. PCR amplification of the fungal ITS2 region was initially performed to characterise the extraction efficiency of the yeasts within the must. Subsequently shotgun sequencing of the extracted DNA was also performed on selected samples to give a deeper overview of the results. Overall we anticipate that our results will be useful for those that wish to better understand the alcoholic fermentation process in wine.

[P94] INFLUENCE OF NUTRIENT COMPOSITION ON ASSEMBLY AND SUCCESSION IN MULTISPECIES BIOFILMS

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It is widely recognised that bacterial cells live in biofilms, consisting of an array of different species, surrounded by a self-produced matrix. The assembly and functionality of biofilms in a specific niche are widely changeable depending on the resources present. This project will investigate succession in a multispecies biofilm using a model consortium composed of four soil isolates (Stenotrophomonas rhizophila, Xanthomonas retroflexus, Microbacterium oxydans and Paenibacillus amylolyticus).
We screened biofilm formation in minimal media supplemented with 10 different simple carbon sources using the Calgary biofilm device. The biofilms were grown for 24h followed by crystal violet staining to quantify the biomass produced. Minimal media supplemented with sucrose was chosen as growth medium for further experiments as the species displayed similar signs of synergy in biofilm formation as had previously been observed when grown in Tryptic Soy Broth.
Growing a single strain alone for 6h and adding all four strains for 18h lead to a decreased formation of biomass, indicating that all four species has an important role in the biofilm assembly. Interestingly, when X. retroflexus was grown alone in TSB for 6h, after which all four strains were added for 18h, the amount of biomass formed came close to that of all four species grown for 24h. This was also observed in minimal media supplemented with sucrose, when P. amylolyticus was the primary coloniser of the biofilm.
Future experiments include growing the biofilms in a continuous flow system coupled with confocal laser scanning microscopy, allowing for real-time investigation of the succession and spatial structure of the biofilms.

[P95] DNA AND RNA SIP REVEAL NITRIFIERS IN GROUNDWATER FED BIOFILTERS

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Nitrification, the enzymatic process converting ammonia to nitrite and nitrate, plays a central role in the global nitrogen cycle. A few clades within the *Proteobacteria, Thaumarchaeota*, *Nitrospira* and *Chloroflexi* are known to carry out nitrification. Based on our earlier findings, we hypothesized that non-classical nitrifiers drive ammonium oxidation in groundwater-fed biofilters. Hence, we applied DNA and RNA stable isotope probing (SIP) coupled to next-generation sequencing to lab-scale biofilter columns fed with NH_4^+ or NO_2^- and ^{13}C labelled or unlabelled HCO_3-. Allylthiourea (ATU) and sodium chlorate were added for specific inhibition of autotrophic bacterial ammonia- and nitrite-oxidizing bacteria respectively. Our results reveal that comammox *Nitrospira* contributed to

ammonium oxidation in the biofilter. Higher $^{13}CO_2$ uptake by *ABS-19, Pedomicrobium, Rhizobacter,* and *Acidovorax* with higher DNA and RNA sequence abundance shifts than *Nitrosomonas* and comammox *Nitrospira* suggest that these taxa may be related to nitrification, providing a plausible explanation for their high abundance in the investigated RGSF. Metagenomic evidence revealed the presence of *amoA* genes in the identified taxa. Canonical *Nitrospira* drives nitrite oxidation in the filter. Archaeal ammonium oxidation was not detected.

[P96] ACTIVATION OF THE STRINGENT RESPONSE BY LOADING OF RELA-TRNA COMPLEXES AT THE RIBOSOMAL A-SITE

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RelA/SpoT Homologs (RSHs) are ubiquitous bacterial enzymes that synthesize and hydrolyze (p)ppGpp in response to environmental challenges. Bacteria cannot survive in hosts and produce infection without activating the (p)ppGpp-mediated stringent response, but it is not yet understood how the enzymatic activities of RSHs are controlled. Using UV crosslinking and deep sequencing, we show that Escherichia coli RelA [(p)ppGpp synthetase I] interacts with uncharged tRNA during steady-state cell growth without being activated. Amino acid starvation leads to loading of cognate tRNA•RelA complexes at vacant ribosomal A-sites. In turn, RelA is activated and synthesizes (p)ppGpp. Mutation of a single, conserved residue in RelA simultaneously prevents tRNA binding, ribosome binding, and activation of RelA, showing that all three processes are interdependent. Our results support a model in which (p)ppGpp synthesis occurs by ribosome-bound RelA interacting with the Sarcin-Ricin Loop of 23S rRNA.

[P97] REGULATION OF PHAGE RECEPTOR OMPK BY QUORUM SENSING SIGNAL AT THE SINGLE-CELL LEVEL AND POTENTIAL ANTI-PHAGE DEFENSE STRATEGIES IN *VIBRIO ANGUILLARUM*

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Quorum sensing (QS) is a process of cell-cell communication by which bacteria can use signaling molecules to coordinate gene expression at the population level. Our previous study showed that the environmental *Vibrio anguillarum* isolate PF430-3 employs QS to regulate phage-host interactions. Specifically, *V. anguillarum* PF430-3 mutant cells that are locked in the low-cell-density state (Δ*vanT* mutant) activated the phage receptor OmpK expression, but meanwhile they showed an aggregation phenotype that protected them from phage infection by creating physical barriers. By contrast, cells locked in the high-cell-density state (Δ*vanO* mutant) did not aggregate, but they repressed OmpK expression, therefore reducing their susceptibility to the phage infection. Thus, it

appears that *V. anguillarum* isolate PF430-3 can employ two different anti-phage strategies, and the choice of strategy depends on QS.
To gain insight on how QS regulates *ompK* at the single cell level, we measure here the expression of a biotin-tagged OmpK receptor on individual cells. As predicted, OmpK levels of wild-type and Δ*vanT* strains were higher than the Δ*vanO* stain. To circumvent QS-regulation, we then expressed *ompK* from an IPTG-inducible plasmid-borne construct in the QS-mutant strains. Surprisingly, the Δ*vanT* Δ*ompK* p*ompK* mutant was still more susceptible to phage KVP40, whereas Δ*vanO* Δ*ompK* p*ompK* mutant was more resistant to phage KVP40, compared to the Δ*ompK* p*ompk* mutant. Using the biotin-tagged OmpK, we confirmed that single cells of the QS-mutants expressing *ompK* from the plasmid contains similar numbers of OmpK receptors per cell. Thus, abolishing QS-regulation of the *ompK* gene is not sufficient to explain the different phage susceptibility of the QS-mutants.
Taken together, our preliminary results indicate that the prevalence of abundant anti-phage defense strategies in the investigated *V. anguillarum*, which allowing flexible and dynamic co-existences of phage and host.

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[P98] PLASMID HOST RANGE (*PERMISSEVENESS*) IN MICROBIAL COMMUNITIES OF ACTIVATED SLUDGE IN WASTEWATER TREATMENT PLANT

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Horizontal gene transfer (HGT), especially conjugal plasmid transfer, is one of the key drivers in global antibiotic resistance transmission. To predict the fate of antibiotic resistance gene (ARG), the transfer and host range of ARG carrying plasmids in relevant microbial communities needs to be understood. Wastewater treatment plants (WWTPs) are a potential conduit of ARG transfer between human intestinal and environmental bacteria, and WWTPs are being examined as potential hot spot of antibiotic resistance dissemination. In this study, a comprehensive assessment of antibiotic resistance transmission was performed in activated sludge (AS) of WWTP. Utilizing the well-established fluorescent reporter system, plasmid permissiveness in AS microbial communities were evaluated by transfer frequency using microscopic image analysis and by host range identification through combining flow-cytometry sorting and 16S rRNA gene amplicon sequencing. Under mimic sewer conditions (e.g., synthetic wastewater as growth medium), we challenged the sampled AS communities (Danish WWTP Mølleaværket, Lyngby-Taarbæk) with model plasmids from three subclades in IncP-1 compatibility group (pKJK5 (ε), pB10 (β-1) and RP4 (α)) which were harbored by two different host strains - *Escherichia coli* MG1655 and *Pseudomonas putida* KT2440. The results showed that different donor-plasmid combinations had distinct transfer frequencies in the AS microbial communities, ranging from 3.39×10^{-5} to 5.05×10^{-4} T/R (transconjugant/recipient) (0.3 to 5 T per 10,000 R), with the most efficient transfer realized in *E. coli* (pKJK5). Unexpected broad host range across plasmid-host pairs was revealed in phylogenetic profile of transconjugant communities with total 308 exact sequence variants distributed over 13 phyla, including major

group *Proteobacteria* (mainly by *Enterobacteriales* and *Pseudomonadales* in *Gammaproteobacteria*) and a few rare phyla in Gram-positive groups (e.g., *Actinobacteria* and *Firmicutes*), indicating that 'long-distance' transfer across phylogenies and Gram-positive/negative might be frequent under environmental conditions.

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