Horisontal transfer of antimicrobial resistance in meat

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The Danish Microbiological Society Annual Congress 2016

Programme & Abstracts

Monday, 14 November 2015
Eigtved's Pakhus
Copenhagen

DMS Congress 2016 - 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	**Development of the microbiome in early life and the risk of childhood disorders** Jakob Stokholm, *Herlev and Gentofte Hospital, University of Copenhagen*	***Roseobacter* – stars of the ocean** Lone Gram, *Dept. of Biotechnology and Biomedicine, Technical University of Denmark*
10:45	**Coffee and exhibition**	*Ground, first & second floor*

	PARALLEL SESSIONS			**SATELLITE SYMPOSIUM**
	ROOM II *Second floor*	**ROOM III** *Second floor*	**ROOM IV** *Second floor*	**SALON F** *First floor*
	Phage therapy use of bacteriophages in pathogen control Chair: Mathias Middelboe, *Dept. of Biology, Marine Biological Sect., University of Copenhagen*	**Microbial food and feed ingredients** Chair: Egon Bech Hansen, *National Food Institute, Technical University of Denmark*	**Bacterial responses to the host environment & antimicrobial agents** Chair: Birgitte H. Kallipolitis, *Dept. of Biochemistry and Molecular Biology, University of Southern Denmark*	**Simple Open Automatic pipetting Platform – Discover the possibilities of automated liquid handling** Michael Kerrn-Jespersen *Eppendorf Nordic A/S* **Organised by** eppendorf
11:00	Chair introduction	Chair introduction	Chair introduction	
11:05	**Coevolution of phage-host interactions in the fish pathogen *Flavobacterium columnare*** Lotta-Riina Sundberg, *Dept. of Biological and Environmental Science and Nanoscience Center, University of Jyväskylä*	**CRISPR-mediated immunity in bacteria: discovery and applications** Philippe Horvath, *DuPont Nutrition & Health, DuPont France*	***Escherichia coli* shape shifting during urinary tract infection** Jakob Møller-Jensen, *Dept. Biochemistry and Molecular Biology, University of Southern Denmark*	
11:30	**Diversity of *salmonella* and their phages: implications for phage therapy** Lone Brøndsted, *Sect. Food Safety and Zoonoses, University of Copenhagen*	**Development of microbial enzymes for food and feed applications** Christel Thea Jørgensen, *Novozymes*	**Combined treatment of Staphylococci with antibiotics and helper drugs** Janne Kudsk Klitgaard, *Dept. Clinical Research & Dept. Biochemistry and Molecular Biology, University of Southern Denmark*	
11:45	**Anti-phage defense mechanisms in fish pathogens** Mathias Middelboe	**Systematic screening approaches to enable accelerated commercial development of next generation microbes. Driving innovation from the lab to global production** Adam Baker, *Chr Hansen*	**A family of small RNAs in *Listeria monocytogenes*: when, where and how do they act?** Birgitte H. Kallipolitis	

12:00	**Flash poster presentations***	**Flash poster Presentations***	**Flash poster presentations***	
12:15	**LUNCH**		*SALON C, ground floor*	
12:15	**POSTERS**		*Ground & first floor*	
12:15	**EXHIBITION**		*First & second floor*	
12:15	**GENERAL ASSEMBLY** Det Danske Pasteur Selskab		*ROOM IV*	
	PARALLEL SESSIONS			**SATELLITE SYMPOSIUM**
	ROOM II Second floor	**ROOM III** *Second floor*	**ROOM IV** Second floor	**SALON F** *First floor*
	Atmospheric microbiology Chair: Tina Šantl-Temkiv, *Dept. of Bioscience, Aarhus University*	**New microbes and diagnostics*** Chair: Hans Linde Nielsen, *Clinical Microbiology, Aalborg University Hospital*	**Hygiene of surfaces** Chair: Brian Kristensen, *Microbiology & Infection Control, Statens Serum Institut*	**From data to discovery with the QIAGEN Microbial Genomics Pro Suite** Arne Materna, Director Microbial Genomics and Metagenomics Holger Karas, Senior Field Application Scientist **Guest talk:** Challenges of Bioinformatics in an industrial context Mads Bennedsen, *Chr. Hansen* **Organised by** QIAGEN
14:15	Chair introduction	Chair introduction	Chair introduction	
14:20	**Friends or foes?: When plant pathogens make rain** Cindy E Morris, *Plant Pathology, INRA*	**Cultivation and whole genome sequencing of *Campylobacter concisus*** Karina Frahm Kirk, *Dept of Clinical Medicine Aalborg University Hospital*	**The use of wipes in healthcare settings: challenges and efficacy** Jean-Yves Maillard, *Cardiff University*	
14:45	**Ice nucleation activity in soil and air of *Fusarium*** Jan F. Scheel, *Max Planck Institute for Chemistry*	**Implant-related infections: diagnostic challenges and insights from an animal model** Lone Heimann Larsen, *Clinical Microbiology, Aalborg University Hospital*	**The structure and absorption capacity of non-woven fabrics influences the disinfection efficacy of commercially manufactured disinfection wipes** Jesper Heeno Andersen, *Wet Wipe A/S*	
15:00	**General and ice-nucleation activity of airborne bacteria in the Arctic** Tina Šantl-Temkiv	**Routine use of clinical microbiome diagnostics** Henrik Vedel Nielsen,, *Microbiology & Infection Control, Statens Serum Institut*	**Virus inactivation by mechanical action: Efficacy of Wipes in the 4-field test against Viruses** Jochen Steinmann, *Dr. Brill + Partner GmbH Institut für Hygiene und Mikrobiologie*	
15:15	**Flash poster presentations***	**Flash poster presentations***	**Flash poster Presentations***	
15:30	Coffee and exhibition		*Ground, first & second floor*	
16:00	Travel grant ceremony		*ROOM III, Second floor*	
16:15	**Nitrification revisited with single cell tools** Michael Wagner, *Div. of Microbial Ecology, University of Vienna*		*ROOM III, Second floor*	
17:15	Reception with fermented beverage		*SALON C, ground floor*	
18:30	Optional congress dinner			

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

Flash poster presentations

	PARALLEL SESSIONS, MORNING		
	ROOM II *Second floor*	**ROOM III** *Second floor*	**ROOM IV** *Second floor*
	Phage therapy use of bacteriophages in pathogen control	**Microbial food and feed ingredients**	**Bacterial responses to the host environment & antimicrobial agents**
12:00	**[P4] Genome sequences and comparative genomics of bacteriophages infecting Campylobacter jejuni** Martine Sørensen, *University of Copenhagen*	**[P7] Bacillus subtilis as in situ amino acid provider in pigs** Mette Dines Cantor, *Chr. Hansen A/S*	**[P16] Selection of bacterial strategies in the developing infant gut and airway microbiota** Jakob Russel, *University of Copenhagen*
12:05	**[P5]Characterization of a new phage group with a remarkable defense mechanism** Alexander Byth Carstens, *Aarhus University*	**[P74] Consortia based production of biochemical** **Sheila I. Jensen** Technical University of Denmark	**[P17] Time-resolved tracking of resistance mutations during antimicrobial adaptive evolution to single and drug-pairs** Rachel Hickman, *Novo Nordisk Foundation Center for Biosustainability*
12:10	**[P49] Bacterial viruses enable their host to acquire antibiotic resistance genes from neighbouring cells** Jakob Haaber, *University of Copenhagen*	**[P62] Effects of probiotic bacteria against fish pathogens in non-axenic algae and copepod systems** Bastian Barker Rasmussen, *Technical University of Denmark*	**[P31] Within-host evolution of Achromobacter biofilms in cystic fibrosis patients** Signe Maria Nielsen, *Aarhus University*

	PARALLEL SESSIONS, AFTERNOON		
	ROOM II *Second floor*	**ROOM III** *Second floor*	**ROOM IV** *Second floor*
	Atmospheric microbiology	**New microbes and diagnostics**	**Hygiene of surfaces**
15:15	**[P39] Using flow cytometry for the analysis of airborne bacteria in environmental samples collected from a minicipal wastewater treatment plant in Denmark** Jaeyoun Jang, *Aarhus University*	**[P46] Cutaneous propionibacteria, their phylotypes and their association with health and disease** Holger Bruggemann, *Aarhus University*	TBA
15:20	**[P40] Ice-nucleation-active gene in single cells of Pseudomonas syringae and its role in active atmospheric dissemination** Mei Lee Ling, *Aarhus University*	**[P48] Identification of a novel sub-lineage of community-acquired methicillin-resistant Staphylococcus aureus belonging to the CC80 complex** Sofie Edslev, *Statens Serum Institut*	TBA
15:25	**[P44] Assement of microbial load in indoor air of University of Hail, Hail, KSA** Mohd Adnan Kausar, *University of Hail*	**[P52] Genomic epidemiology of the Danish Cluster 2 Mycobacterium tuberculosis outbreak: A retrospective study of a dominating TB lineage from 1992 to 2014** Dorte B. Folkvardsen, *Statens Serum Institut*	TBA

Satellite symposia

11:00-12:15: Satellite symposium organised by Eppendorf Nordic A/S **SALON F**

Simple Open Automatic pipetting Platform – Discover the possibilities of automated liquid handling

Learn how simple it is to automate your manual pipetting routines. Automated pipetting leads to less hands-on time, increased precision and gives consistent results every day. Preparation of Next Generation Libraries and extraction of DNA are applications with a lot of pipetting steps. Both applications can be switched to an automated pipetting instrument and best-case examples will be demonstrated during the presentation.

Michael Kerrn-Jespersen
Eppendorf Nordic A/S

14:15-15:30: Satellite symposium organised by Qiagen **SALON F**

From data to discovery with the QIAGEN Microbial Genomics Pro Suite

The expanding field of metagenomics leaves researchers struggling to convert data generated with a wide range of open source tools into meaningful insights. The Microbial Genomics Pro Suite integrates a comprehensive set of tools for metagenomics into a scalable and user friendly platform.

Metagenomics applications are supported with workflows for the analysis of 16S-, 18S rRNA, or other amplicon data. Further included are workflows for whole metagenome assembly, and the functional analysis of metagenomic data. The recent launch of the CosmosID plugin completes the range of metagenomics applications. The plugin offers best in class taxonomic profiling and pathogen identification and can handle shotgun metagenomics data as input.
To convert microbiome data into biologically meaningful insights, all metagenomics applications are completed with workflows for the statistical analysis of differential abundance in the context of metadata. The user can explore changes in composition or function associated for instance with health or dysbiosis.
A package of workflows for the analysis of outbreaks based on isolate genome data extends the utility of the Microbial Genomics Pro Suite for researchers focusing on epidemiology, food safety and public health.

Invited speaker from Chr. Hansen, Mads Bennedsen will give a talk about ”Challenges of Bioinformatics in an industrial context”

How to scale from manual assembly and analysis of single genomes to an automated analysis pipeline with the capability of assembling, decontaminating, & analyzing thousands of bacterial genomes every year using QIAGEN’s CLC Genomics Workbench. The CLC analysis pipeline perform species identification, gene-finding & annotation, strain typing by NGS-MLST, Pangenome generation & safety analysis for presence of transferable antibiotic resistance genes. Data transfer to databases and distribution of output-files to destination folders are also integrated in the pipeline.

Join the satellite symposium and learn more about the QIAGEN Microbial Genomics Pro Suite and see a live demo that will cover the supported metagenomics applications.

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Microbial genomics and metagenomics

From data to discovery with the QIAGEN Microbial Genomics Pro Suite

A lack of integrated analytics leaves organizations with the burden of integrating and maintaining all the bioinformatics-, statistics- and visualization tools required for their microbial research. The QIAGEN Microbial Genomics Pro Suite overcomes the challenges by:

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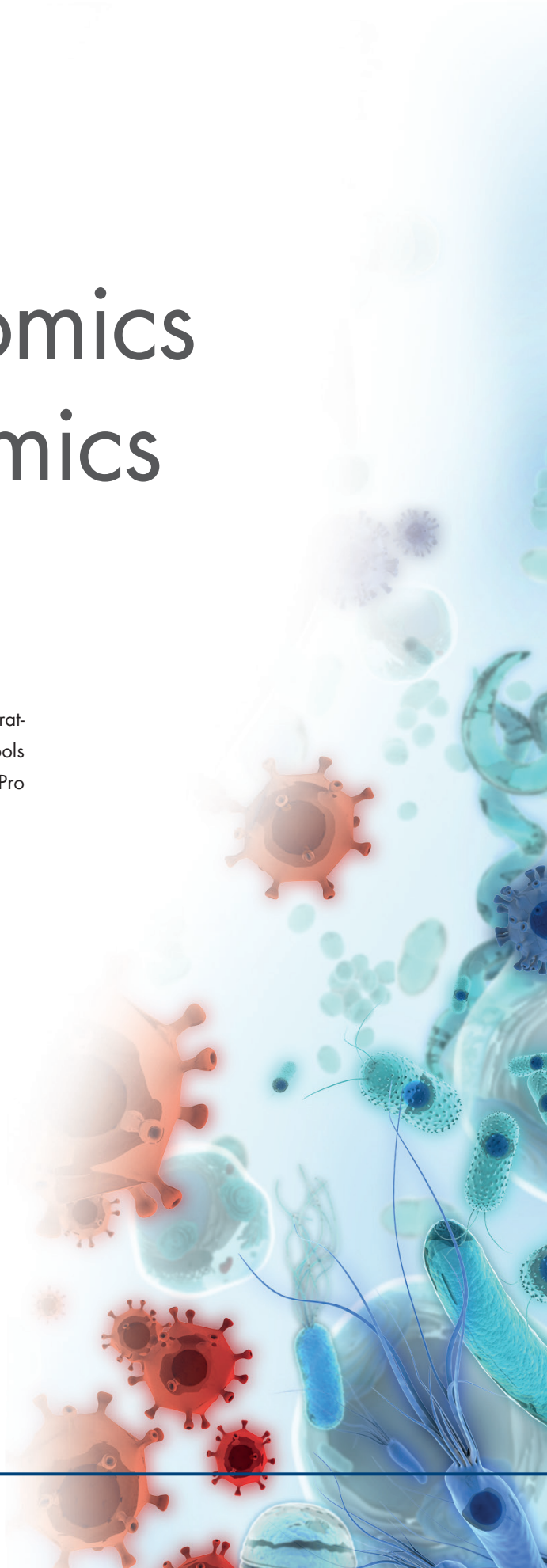

Sample to Insight

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.

Being a member of DMS, you are part of the advancement of microbiology in Denmark. Additionally, as a member of DMS, you are entitled to discounts at FEMS (Federation of European Microbiological Societies) meetings and for FEMS journals.

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- Mette Burmølle (Secretary), Department of Biology, University of Copenhagen
- Marie Allesen-Holm, Coloplast, Wound Care Innovation
- Lars Bogø Jensen, DTU, National Food Institute
- 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
- Stephen Wessels, formerly DHI.

About the keynote session
'Nitrification revisited with single cell tools' by Michael Wagner, Div. of Microbial Ecology, University of Vienna

Nitrification plays a key role in Earth's natural nitrogen cycle and in agriculture. This process comprises two sequential steps, and for more than 100 years experts have assumed these steps to be carried out by different microorganisms. Michael Wagner and coworkers have used innovative single cell tools to discover microbes that perform complete nitrification on their own: A result contrasting textbook knowledge and a milestone of microbiology.

The DMS 2016 Congress is supported by the American Society for Microbiology (ASM).
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Invited talks

[O1] DEVELOPMENT OF THE MICROBIOME IN EARLY LIFE AND THE RISK OF CHILDHOOD DISORDERS

Jakob Stokholm[1]
[1]*Herlev and Gentofte Hospital, University of Copenhagen*

The vast majority of bacteria colonizing humans are found in the gastrointestinal tract, where they provide essential stimulation of the child's developing immune system. From birth, the child is continuously subjected to multiple exposures that influence microbiome ecology. The composition of the gut microbiome matures within the first years of life and the microbiome may have the ability to affect host immune maturation; perturbing homeostasis during this critical period of development could lead to asthma, allergy, and other immunologic disorders. Thus, the microbiome may be an intermediary player in the interaction between the host and its environment in the extrinsic mechanisms that determine the transition from health to chronic disease.

The neonatal microbial colonization patterns are greatly affected by mode of delivery as well as use of intrapartum antibiotics, which can lead to long term microbial derangements. Birth by caesarean section is a recognized risk factor for asthma, as well as for other immune-mediated diseases in childhood and antibiotic exposure in the first year of life has also been associated with increased asthma risk, further pointing to microbe-mediated mechanisms involved.

The objective of this study was to analyze the nature of gut colonization patterns during the first year of life, and associations with the risk of asthma among 700 children from the Copenhagen Prospective Studies on Asthma in $Childhood_{2010}$ ($COPSAC_{2010}$) birth cohort. We hypothesized that compositional differences in the gut microbiome could initiate a trajectory toward asthma development.

[O2] *ROSEOBACTER* – STARS OF THE OCEAN

Lone Gram[1]
[1]*Department of Biotechnology and Biomedicine, Technical University of Denmark*

Bacteria belonging to the *Roseobacter*-clade are some of the most abundant organisms in upper ocean waters and important players in the C- and S-biogeochemical cycles. They are typically associated with algae and during algal blooms they may constitute as much as 30-40% of the bacteria present. DNA from *Roseobacter*-clade related bacteria have been isolated from almost all ocean environments. Many roseobacters produce bioactive secondary metabolites and genome analyses indicate that even more than found hitherto by

chemical analyses can be produced. One of our prime interests is the bioactivity and potential use of roseobacters in biotechnology, however, we are also interested in finding their natural niches and determining the natural role of their bioactivity, in part to rationalise future bioprospecting strategies. Several *Roseobacter* species produce a potent antibacterial compound, tropodithietic acid (TDA) and we have on the Galathea3 cruise isolated one TDA-*Roseobacter* species, *Ruegeria mobilis*, from almost all ocean environments. Genome analyses demonstrated a remarkable homogeneity but also occurrence of sub-clusters. Whilst *R. mobilis* seems to be planktonic, another TDA-producer, *Phaeobacter inhibens*, is an excellent biofilm former, and we have repeatedly isolated this species from marine biofilms. Both of these species are also common in algal cultures used in marine aquaculture as live feed and, due to production of TDA, they antagonise fish pathogenic bacteria. TDA, which acts as an anti-porter is the main molecule responsible for the probiotic effect of roseobacters in marine fish larvae. In model infection trials, vibrio-caused mortality of fish larvae can be completely prevented by TDA-producing roseobacters. Some roseobacters can also produce potent algicidal compounds, roseobacticides, that likely are involved in their natural interaction with algae. However, this could potentially limit their use in aquaculture. Therefore, unravelling the secondary metabolism of roseobacters is key to their potential use in biotechnology and will likely also point to key features determining their role and lifestyle in oceanic environments.

[O3] PHAGE-HOST INTERACTIONS AND PHAGE THERAPY IN THE FISH PATHOGEN *FLAVOBACTERIUM COLUMNARE*

Lotta-Riina Sundberg[1]

[1]*Centre of Excellence in Biological Interactions, Department of Biological and Environmental Science and Nanoscience Centre, University of Jyväskylä*

Importance and volume of aquaculture is growing steadily, but the productivity of the industry is threatened by a wide range of bacterial diseases. Especially Flavobacterial infections cause persistent infections and high mortality in the salmonid fry production. We have isolated phages infecting the fish pathogen *Flavobacterium columnare*, and studied their suitability for phage therapy. In trials with rainbow trout and zebra fish, the survival of both fish species was significantly higher in the presence of the phage. Hundred percent of the zebrafish and 50% of the rainbow trout survived in the phage treatment (survival without phage 0 and 8.3%, respectively). Most importantly, the rainbow trout population was rescued from infection by a single addition of the phage into the water in a flow-through fish tank system. As the phage resistance is considered the major obstacle for phage therapy use, we have also explored both constitutive and adaptive (CRISPR) resistance mechanisms. Under high phage pressure, elicited phage resistance is frequently associated with loss of virulence, whereas CRISPR immunity is not as costly. These results warrant for further studies in phage-host interactions in *F. columnare* and development of phage therapy applications for aquaculture.

[O4] DIVERSITY OF *SALMONELLA* AND THEIR PHAGES: IMPLICATIONS FOR PHAGE THERAPY

Y. Emre Gencay[1], Tessa From Püssing[1], Stephen Ahern[1], Lone Brøndsted[1]
[1]*Department of Veterinary Disease Biology, Frederiksberg, University of Copenhagen*

Salmonella enterica subsp. enterica is highly diverse and consists of more than 2400 serotypes, related to diverse O-antigen attached to the conserved core sugars of lipopolysaccharide. We isolated 50 phages from environmental sources using ten Salmonella serotypes. By using knockout mutants of S. Typhimurium LT2, we showed that nine phages were dependent on BtuB and 30 phages were dependent on O-antigen for infection. Furthermore, three phages were blocked by O-antigen, suggesting core sugars to be the receptor. Host range analysis showed that BtuB-dependent phages have the broadest host range, suggesting conserved phage binding domains in BtuB across serotypes. While most O-antigen-dependent phages showed narrow host ranges, three phages demonstrated broader host ranges, suggesting absorption to common features of O-antigen. Finally, analysis of phage genomes identified different strategies used by LPS-dependent phages to target receptors. In conclusion, phage therapy could be designed according to the diversity of relevant Salmonella.

[O5] POTENTIAL AND CHALLENGES OF USING PHAGES FOR PATHOGEN CONTROL IN AQUACULTURE

Mathias Middelboe[1]
[1]*Department of Biology, Marine Biological Section, University of Copenhagen*

Flavobacterium psychrophilum and *Vibrio anguillarum* are important fish pathogens in salmonid aquaculture worldwide. Due to increased antibiotic resistance, pathogen control using bacteriophages has been explored as a possible alternative treatment. Preliminary tests have shown promising effects of phage addition on the survival of fish larvae and fry in challenge trials with these pathogens. Further, efficient delivery of phages to target organs via phage-coated fish feed pellets have demonstrated that phages can reach the target organs in larger fish and proliferate there in the presence of the pathogen. However, development of phage resistance in the pathogens constitutes a challenge for the use of phages in the control of these pathogens, and the phage-driven physiological and behavioral changes associated with phage-defense mechanisms potentially have large implications for the impact of the pathogen in aquaculture. The presentation provides an overview of the potential of phage-based control of *Flavobacterium psychrophilum* and *Vibrio anguillarum* and discusses implications of the different phage-resistant mechanisms described in these pathogens.

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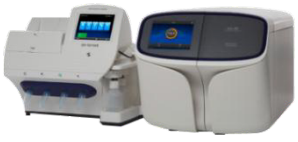

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[O6] CRISPR-MEDIATED IMMUNITY IN BACTERIA: DISCOVERY AND APPLICATIONS

Philippe Horvath[1]
[1]*DuPont Nutrition & Health, Danisco France SAS*

CRISPR-Cas is an adaptive immunity system in bacteria which is directed against nucleic acids, notably viral DNA. In this system, the immunological memory is built through the acquisition of short viral DNA sequences into the chromosome of the bacterial host, within particular regions called CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats). In the interference stage, these sequences are transcribed and processed into small RNA molecules named CRISPR RNAs (crRNAs), that are used by Cas (CRISPR-associated) proteins to recognize and inactivate any foreign DNA showing sequence complementarity to the crRNAs. The ability of certain Cas proteins, notably Cas9, to be guided by a short RNA molecule towards a DNA target and to cleave it at a precise position has been leveraged and diverted into a simple and efficient tool for genome editing. In the last three years, the Cas9/guide-RNA tool has been applied successfully to genome modification of numerous organisms, including microorganisms, plants, animals, and humans.
This presentation will focus on the milestone and fundamental discoveries that established CRISPR-Cas as an immunity system, and on some of its applications in the domain of microbiology.

[O7] DEVELOPMENT OF MICROBIAL ENZYMES FOR FOOD AND FEED APPLICATIONS

Christel Thea Jørgensen[1]
[1]*Novozymes*

Microbial enzymes are widely used by the food and feed industries to improve efficiency, quality and sustainability of food production and although applied in many different applications, the huge potential of these natural catalysts still remain to be unlocked. Recent technology developments in biology allow new enzyme functionality to be made available faster than ever before enabling more specific and local needs of food and feed producers to be met.

[O8] SYSTEMATIC SCREENING APPROACHES TO ENABLE ACCELERATED COMMERCIAL DEVELOPMENT OF NEXT GENERATION MICROBES. DRIVING INNOVATION FROM THE LAB TO GLOBAL PRODUCTION

Adam Baker[1]
[1]*Chr. Hansen*

We will describe our screening of next generation probiotics, the development of technical *in vitro* and *in vivo* platforms, and building a strong portfolio of scientific and clinical data.
In particular we are focused on the gastro intestinal environment and molecular understanding of the interactions between bacteria and the host microbiome at a molecular and clinical level. We leverage a strong core research program and combine it with innovative production related initiatives to deliver rapid product development.

[O9] BACTERIAL SHAPESHIFTING: *ESCHERICHIA COLI* DIFFERENTIATION DURING URINARY TRACT INFECTION

Jakob Møller-Jensen[1]
[1]*Dept. Biochemistry and Molecular Biology, University of Southern Denmark*

The ability to change cell morphology is an advantageous characteristic adopted by multiple pathogenic bacteria in order to evade host immune detection and assault during infection. Uropathogenic *Escherichia coli* (UPEC) exhibits such cellular dynamics and has been shown to transition through a series of distinct morphological phenotypes during a urinary tract infection. Initial steps involve bladder invasion by rod-shaped bacteria to establish intracellular bacterial communities consisting of coccoid cells. Subsequently, during exit from infected cells, UPEC regain their rod-shape and in some cases even form large filaments. Using a flow-chamber based tissue culture infection model we have studied UPEC gene expression during the course of infection. We identify *damX* as a mediator of reversible filamentation during UTI. DamX-mediated filamentation represents a novel pathway for bacterial cell shape control that is independent of the SOS response.

[O10] COMBINED TREATMENT OF STAPHYLOCOCCI WITH ANTIBIOTICS AND HELPER DRUGS

Janne Kudsk Klitgaard[1]

[1] *Dept. Clinical Research & Dept. Biochemistry and Molecular Biology, University of Southern Denmark*

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a growing problem in healthcare settings, resulting in an urgent demand for new and effective treatments. Thioridazine (TDZ) is a potential candidate drug with a bactericidal effect at high concentrations and a synergistic effect in combination with β-lactam antibiotics at lower concentrations. We have showed that TDZ causes its effect by intercalating in the cytoplasmic membrane, thereby disturbing membrane- and cell wall related processes (1). The precise target of TDZ though, is still unknown. In vivo experiments in *C. elegans*, mice, and pigs suggest a potential for usage of TDZ combined with antibiotics for local treatment of MRSA and not for systemic use (2-4).

We have investigated which genetic changes occur when *S. aureus* becomes TDZ resistant and whether resistance towards TDZ leads to a loss of synergy between TDZ and β-lactam antibiotics. 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. Our results indicate that TDZ-resistance leads to a loss of synergy between TDZ and β-lactam antibiotics. Through WGS, 11 mutations in nine different genes were identified, where of most were either cell-wall or cytoplasmic membrane related. So far, a knockout mutant of the cardiolipin synthase gene exhibited reduced susceptibility towards TDZ, indicating that cardiolipin may play a major role in the bactericidal effect of thioridazine.

References:

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[O11] A FAMILY OF SMALL RNAS IN *LISTERIA MONOCYTOGENES*: WHEN, WHERE AND HOW DO THEY ACT?

Birgitte H. Kallipolitis[1]
[1]*Dept. of Biochemistry and Molecular Biology, University of Southern Denmark*

Small non-coding RNAs (sRNAs) act as regulators of bacterial gene expression. In some cases, two or more highly related sRNAs, termed "sibling sRNAs", are produced in a single bacterium. The foodborne pathogen *Listeria monocytogenes* encodes hundreds of sRNAs and serves as a model for studies of sRNA-mediated control in Gram-positive species. Five homologous sRNAs in *L. monocytogenes*, named LhrC1-5, are highly induced in response to cell envelope stress and contribute to infection in macrophage-like cells. Under inducing conditions, LhrC1-5 down-regulate expression of genes encoding cell envelope-associated proteins, including the adhesin LapB; the oligo-peptide binding protein OppA, and the $CD4^+$ T-cell stimulating antigen TcsA, which are all required for full virulence of *L. monocytogenes*. LhrC1-5 exert their regulatory function at the post-transcriptional level by base pairing with complementary sequences in target mRNAs.
Recent evidence suggests that the LhrC family of sRNAs is even larger than first anticipated. Two novel members of the LhrC family have been revealed, making it the largest multicopy family of sRNAs reported so far. The sibling sRNAs were found to act in a functionally redundant manner, however, other characteristics, such as differential expression profiles under infection-relevant conditions, suggest that the sRNAs might also possess non-overlapping functions. Furthermore, each sibling sRNA encodes multiple CU-rich regions engaged in sRNA-mRNA interactions, providing another layer of complexity. This makes the LhrC family a unique case for studying the purpose of sRNA multiplicity in the context of bacterial virulence.

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[O12] FRIENDS OR FOES?: WHEN PLANT PATHOGENS MAKE RAIN

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Plant pathogens can cause diseases of considerable importance to food crops, forests, ornamentals, etc. But many of the microorganisms that can cause plant disease also are saprophytes and have aspects of their life history of which little is known. Growing interest in environmental microbiology has helped to uncover surprising aspects of life history of plant pathogens leading to new perspectives on the beneficial role that they might be playing for the environment. One example is *Pseudomonas syringae* as a plant-associated bacterium first described over 50 years ago. Our vision of its ecology has moved away from ubiquitous epiphytic plant pathogen to multifaceted bacterium *sans frontières* in fresh water and other ecosystems linked to the water cycle. Discovery of the aquatic facet of its ecology has led to a vision of its life history that integrates spatial and temporal scales spanning billions of years and traversing catchment basins, continents and the planet, and that confronts the implication of roles that are potentially conflicting for agriculture and society at large – as a plant pathogen and as a beneficial actor in processes leading to rain and snowfall. This new ecological perspective has also yielded insight into epidemiological phenomena linked to disease emergence. It sets the stage for the integration of more comprehensive contexts of ecology and evolutionary history into comparative genomic analyses to elucidate how *P. syringae* subverts attack and defense responses of the cohabitants of the diverse environments it occupies. I will present the vision of the evolving story of the ecology and biology of *P. syringae* and the conflicting challenges and opportunities for management of plant health and ecosystem services that ensue for this and other plant pathogens.

[O13] ICE NUCLEATION ACTIVITY IN SOIL AND AIR OF *FUSARIUM*

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The fungal genus *Fusarium* is ubiquitous, and many members of this genus cause important diseases of plants, domestic animals, and humans. Thirty years ago, some strains from the genus *Fusarium* were reported to catalyze freezing of water at relatively warm subfreezing temperatures. The impact of biological ice nuclei (BioIN) in global meanings is under debate

and not really understood, but important processes of our geosphere like the water cycle are highly dependent on effective ice nucleation at temperatures between -2°C - -8°C, a temperature range which is almost exclusively covered by biological IN. The ice nucleation activity of *Fusarium* as well as other fungal genera is assumed to be mediated by proteins or at least to contain a proteinaceous compound, but there information is missing regarding the precise composition of fungal IN, the environmental impact and the abundance within the kingdom fungi.

We screened *Fusarium* species strains from field and laboratory collections for IN activity. Beside high frequencies in strains from species known for IN activity, we report the identification of three strains from three new (previously unreported) *Fusarium* species that were IN active. We also found preservation of IN activity from cell free *Fusarium* IN in liquid for at 6°C for over 12 month and from cold storage for over 24 month, suggesting a huge pool of functional IN in soils originate from fungi. Even more striking, we were able to isolate IN active *Fusarium* strains from the atmosphere from drone sampling missions and from rainfall scavenging studies conducted in Virginia, USA.

[O14] GENERAL AND ICE-NUCLEATION ACTIVITY OF AIRBORNE BACTERIA IN THE ARCTIC

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The remote location and the harsh conditions of the Arctic pose challenges to the study of the Arctic atmosphere. This is why our understanding of the atmospheric processes in the Arctic, and thus Arctic meteorology and climate, is limited. This lack of knowledge is particularly large when it comes to the presence, the types and the activity of bio-aerosols. Airborne microorganisms, which are a fraction of the so-called bio-aerosols, have properties that enable them to interact with the formation and development of clouds, rain, and snow. Studies in temperate regions have provided first insights leading to the conclusion that bio-aerosols impact both weather and climate, as they may be involved in the control of the lifetime and extent of clouds. This means that bio-aerosols might also be an important missing link for predicting Arctic radiation budgets in climate models.

In order to improve our understanding of the role of bio-aerosols on climate in the Arctic quantified airborne microorganisms and aimed at determining their general and ice-nucleation activity in air. A series of air samples was collected at different locations and over four years, using high-volume impinger samplers and filters. In addition to air samples, we collected potential source and sink samples, *e.g*. snow, seawater, and terrestrial surfaces. We used a combination of molecular and cultivation based techniques to quantify and identify total and active bacterial cells and ice nuclei in the atmosphere.

Comparable to studies in temperate regions we found between 460 and 80 000 bacterial cells per m^3 of air at different locations in west Greenland and at the Villum research station. At the Villum research station the surface snow samples contained 100-1000 cells per mL, which originated from wet and dry deposition. We found that biological ice nuclei (IN), which most likely were deposited after long-range transport, were both present in air and in snow. Using the heat-sensitivity approach, during which biological structures lost their functionality, we could demonstrate that some samples contained a large proportion of biological IN that were of proteinaceous nature. We postulate that these peptides originate from airborne microorganisms. However, when microorganisms that were isolated from the same samples were examined only a single isolate was ice-nucleation active (INA). We propose that INA proteins originated from two sources: (i) They were excreted to the environment by microorganisms; (ii) They were associated with dead or not cultivable cells. We studied the sources and activity potential of airborne bacterial cells collected in the atmosphere close to Nuuk and found that the communities were assembled from cells aerosolized in local terrestrial environments in addition to long-range transported cells originating from marine, glaciated, and terrestrial surfaces. These cells displayed a high activity potential, reflected in the high 16S rRNA copy number (590±300 rRNA/cell) that correlated with water availability in the air. Bacterial clades differed in their potential for a fast metabolic response. A high activity potential found in members of the class Rubrobacteridae and the order Clostridiales. Of those bacterial families that harbor known ice-nucleation active species, cells with a high activity potential were rare in air, but were enriched in rain. Overall, the results obtained by our studies will improve our understanding of (i) the role of short- and long-range dispersal in the assembly of airborne microbial communities and (ii) the activity of airborne microbial cells and their role in the Arctic atmosphere and ultimately contribute to improved local and global climate modles.

[O15] CULTIVATION AND WHOLE GENOME SEQUENCING OF *CAMPYLOBACTER CONCISUS*

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Background: *Campylobacter concisus* is an oral bacterium that has been associated to enteric disease such as Barrett's esophagus, prolonged diarrhea and inflammatory bowel disease (IBD). Previous studies have found a higher prevalence of *C. concisus* DNA in samples from mucosal biopsies and faecal samples of patients with IBD, compared to healthy controls (HC). *C. concisus* is genetically diverse, and *in-vitro* studies have shown that certain strain subtypes harbor virulence factors capable of disassembling epithelial cells. The aim of our study was to collect multiple strains of *C. concisus* from different locations in IBD patients and HC for genetic comparison.

Methods: Gut mucosal biopsies, saliva and faecal samples were collected from 78 persons (52 IBD, 26 HC) using a filter method in microaerobic (80% N_2, 10% CO_2, 5% H_2, 6% O_2) and anaerobic (80% N_2, 10% CO_2, 10% H_2) atmospheres and assessed by PCR using the primers *ConcisusF* and *ConcisusR* targeting the 16S rRNA gene. Whole genome sequencing of strains from different locations and different clinical presentations was conducted and used for phylogenetic comparison and characterization of core and accessory genes.

Results: In all IBD patients, a total of 52/245 (21%) biopsies were culture positive for *C. concisus*, while 121/245 (49%) were PCR positive ($p<0.001$) For healthy controls, the numbers were 23/182 (13%) and 66/182 (36%), respectively ($p<0.001$). From stool samples, 12/48 (25%) IBD patients were culture positive, 19/48 (40%) were positive by PCR detection. For healthy controls, the numbers were 3/25 (12%) and 5/25 (20%) respectively. As expected, *C. concisus* was abundant in saliva samples from both IBD patients and healthy controls. Analysis of whole genome sequencing results is currently ongoing, but preliminary comparisons confirm genetic diversity of the species.

Conclusions: *Campylobacter concisus* is viable and abundant in both IBD and healthy controls from all tested locations of the gastrointestinal tract. There was a higher prevalence in mucosal and fecal samples from IBD patients compared to healthy controls, possibly indicating a higher abundance in active inflammation. Whole genome sequencing confirms a high genetic diversity of the species, and can possibly be used to differentiate genes in isolates originating from different locations and different disease presentations.

[O16] IMPLANT-RELATED INFECTIONS: DIAGNOSTIC CHALLENGES AND INSIGHTS FROM AN ANIMAL MODEL

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More people are living with a medical implant. In Denmark ~18,000 people have a new hip or knee prosthesis annually, with risk of infection of 0.6-2%. The infection is often located on the implant or close to the implant as a biofilm. Especially, biofilm infections can be difficult to diagnose due to the adherence of bacteria to the implant, and surgical intervention is often necessary to obtain specimens from relevant sites for diagnostic. The purpose of this Ph.D. project was to evaluate diagnostic methods of prosthetic joint infection (PJI), the contribution of different specimens to diagnosis and study the gene expression profile of Staphylococcus aureus in vivo under various conditions. The specimen types were obtained in parallel and evaluated by culturing and 16S rRNA sequencing. The optimal specimen set was culturing a combination of joint fluid, sonication fluid from the prosthesis component, and soft tissue biopsies. Additionally, insight from an in vivo biofilm implant infection model revealed gene expression profiles of S. aureus, which were remarkably similar to each other despite different courses of infection. The gene expression profiles also concorded well with a human PJI infection with the same strain. As a conclusion, the improvement of the diagnosis and treatment of PJI depends of the recognition of the microorganism/s. Only by using a specimen collection the entire spectrum of PJI patients is covered. Secondly, new treatment strategies are warranted, and an improved understanding of how microorganisms behave in biofilm infections might contribute in that respect.

[O17] ROUTINE USE OF CLINICAL MICROBIOME DIAGNOSTICS

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By use of a newly developed platform that simultaneously annotates bacteria, fungi, and parasites, which include a novel annotation software, human clinical routine and project samples have been analyzed at SSI.
The platform is based on PCR amplification by four sets of primers, one targeting the V3-4 region of 16S and three the V3-4 of the 18S gene. Followed by sequencing on the Illumina MiSeq desktop sequencer by use of a 2x250nt setup. Species annotation performed by the newly developed software, BION, by use of the combination of public available databases (RDP and SILVA) and an in house DB.
Till date > 3500 samples have been analyzed with this pipeline, including app. 1000 non-fecal clinical samples. Our obtained experience and data will be presented at the meeting.

[O18] THE USE OF WIPES IN HEALTHCARE SETTINGS: CHALLENGES AND EFFICACY

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High touch surfaces and other healthcare surfaces are routinely clean or decontaminated with wipe/cloth/material following the application of a biocidal product or detergent as a liquid, foam or spray. Alternatively pre-wetted wipes are being used with an increased frequency. Detergent and antimicrobial wipes are becoming more popular because of their ease of use (no need to prepare a biocidal product prior usage) and for some products, their low cost. The use of wipe/cloth as a pre-wetted product or following the application of a biocidal product involves a mechanical action, which is crucial for the efficacy of the product to kill or remove microbial contaminants from a surface. Yet the wiping action is often not appropriately considered in most of the current international and European efficacy tests. In addition the application on surfaces of pre-wetted wipes, and one would argue most of the biocidal products, is often short lasting seconds rather than minutes. Such extremely short contact time is seldom considered in standard efficacy tests. The development of the 3-stage test, which in part became the new ASTM E2967-15, enabled to measure the ability of wipe-based products to remove microbial burden from surfaces and to understand the risks associated with using the same wipe on multiple surfaces, a common practice in healthcare. With the increasing number of pre-wetted wipe products on the market, sometimes with antimicrobial claims that may be difficult to defend, it is important that end users but also manufacturers provide comprehensive efficacy and importantly practical data about their products.

[O19] THE STRUCTURE AND ABSORPTION CAPACITY OF NON-WOVEN FABRICS INFLUENCES THE DISINFECTION EFFICACY OF COMMERCIALLY MANUFACTURED DISINFECTION WIPES

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Ethanol is traditionally used in Danish health care for disinfection of smaller surfaces. Ethanol is preferred due to a prompt action and because disinfectant residues are undesirable. However, blood, body fluids or secretions inactivate ethanol. Thus, when using ethanol for disinfection, a two-step procedure is required, where the surface is washed with detergent followed by disinfection with ethanol applied with a trigger-spray and paper towel or with thin wipes containing ethanol. Other disinfectants, which are water-based, are applied to surfaces in amounts that require subsequent rinsing with water and/or wiping.

Alternative solutions to the traditionally used disinfection products have evolved during recent years, where damp/moist pre-impregnated disinfection wipes have become widely used, as they leave no significant amount of biocidal residues. The design of these wipes is much closer to a traditional cleaning cloth than a typical wipe containing alcohol; wipes are made from non-woven fabrics that have a weight/area of 50-70 gram/m^2 and contain a mixture of viscose and polyester fibres.

Studies conducted by Wet Wipe on the structure and absorption capacity of non-woven fabrics have shown that the impregnation degree and the folding of the wipes, in combination with the structure and absorption capacity of the non-woven fabrics, are responsible for the amount of disinfection liquid released during wiping of a surface.

During the last 3 years, we have developed a dip-slide-based method for efficacy testing of disinfection wipes (the CEI method), which will be published by the Danish EPA. The method is already implemented at the Danish Technological Institute, and Wet Wipe's results from testing disinfection wipes using the CEI method are similar to results from using the ISO EN 16615-standard. The overall conclusion from testing non-woven wipes impregnated with different ethanol concentrations is that ethanol wipes will not remove all bacterial cells and leave approximately 100 $\log_{10}$ cfu/cm^2 on a wiped surface, as measured by the CEI method. This results in a less than 3-$\log_{10}$ reduction of test bacteria, as measured by ISO EN 16615. Some water-based disinfection wipes do perform better than ethanol wipes, but this depends of the type of biocide, the concentration of the biocide and especially the degree of impregnation of the non-woven wipes, which must be above a certain critical level for the disinfection wipe to pass the requirements of both test methods.

[O20] VIRUS INACTIVATION BY MECHANICAL ACTION: EFFICACY OF WIPES IN THE 4-FIELD TEST AGAINST VIRUSES

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Introduction

Surface disinfection is one of the most important measures for interrupting microbial transmission in the hospital. Therefore, it is important to include virus inactivation by appropriate chemical formulations. The virucidal activity of surface disinfectants can be measured in Europe by a quantitative suspension test (EN 14476) and a test that simulates practical conditions (carrier test), like the prEN 16777 V2 or the Guideline (Leitlinie) of Deutsche Vereinigung zur Bekämpfung der Viruskrankheiten e.V (DVV). Both standard methods are not based on any mechanical action. Due to the increased use of wipes in the

hospital and the description of the 4-field test for bacteria (prEN 16615), we studied the inactivation of potential test viruses with this method with chosen wipes from the market.

On a large PVC carrier the impregnated wipe will be applied with a standardized procedure to simulate the disinfection of small surfaces. Surface disinfectants can be tested with a defined standard wipe or a different wipe which is recommended by the manufacturer. The name 4-field test comes from the fact that in this test assay not only the disinfecting efficacy on a contaminated surface is tested, but also the spread to other parts of the carrier. This will be tested on the remaining 3 fields, which have not been contaminated before.

Methods

Four commercially available wipes based on peracetic acid (PAA), quaternary ammonium compounds and 2-propanol as active ingredients and the corresponding fluids were the chosen surface disinfectants. The virus-inactivating properties of these formulations were examined against adenovirus (AdV), murine norovirus (MNV) and polyomavirus SV40 under clean conditions. Tests followed the prEN 16777 using PVC carriers (20 x 50 cm) and the unitary weight of 2.3-2.5 kg. Exposure time was fixed at 5 minutes. Controls like the water control and the cytotoxicity were included. The Tork Premium Special Wipe served as reference. After recovery at the end of exposure time, the virus titres on all 4 fields were determined as $TCID_{50}$/ml in permissive cells.

Results

Following our results all chosen viruses can be used as test viruses in a future European standard. The PAA-based wipe achieved the greatest virus inactivation (≥ 4 log_{10} steps) on the surface with no residual virus in the wipes, whereas in contrast a 2-propanol-based wipe failed to inactivate all 3 test viruses by 4 log_{10} steps. AdV and MNV were more stable than SV40 in the chosen formulations, whereas the polyomavirus exhibited a greater stability during the drying process.

Conclusion

These finding show that the 4-field test is also suited for testing virucidal activity of wipes in a test simulating practical conditions. In addition, an enveloped virus like Modified vaccinia virus Ankara (MVA) should be included as a test virus. In the future, test parameters have to be defined in more detail, and the required claims of this method have to be fixed.

[O21] NITRIFICATION REVISITED WITH SINGLE CELL TOOLS

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Nitrification, the aerobic oxidation of ammonia via nitrite to nitrate, is a key step in the global biogeochemical nitrogen cycle and of major importance for the health of our planet. Nitrification forms most of the nitrate in the world's oceans, impairs the efficiency of nitrogen fertilization in agriculture causing severe eutrophication and dead zones in natural waters, is a crucial step of biological wastewater treatment, and produces significant amounts of N2O as a by-product, which is a highly potent greenhouse gas. For more than a century, it was a central dogma of nitrification research that this process is catalyzed by two groups of chemolithoautotrophic microbes – the ammonia- and nitrite-oxidizers – that mutualistically interact with each other. In this talk, I will report on the discovery and physiological characterization of a complete ammonia oxidizer (Comammox) that catalyzes both steps and that is found widespread in aquatic and terrestrial environments. Furthermore, ammonia and urea have always been considered to be the only substrates that support the aerobic growth of ammonia-oxidizers, but we have recently discovered that some ammonia-oxidizers can grow on cyanate as only substrate. In addition, the classical nitrification process starts with the activity of ammonia oxidizers, which produce nitrite and thus feed the nitrite oxidizers. However, we have recently discovered that reciprocal feeding is common among nitrifiers and that nitrite oxidizers can produce ammonium from urea or cyanate and initiate the nitrification process by feeding the ammonia oxidizers that in turn produce nitrite for the nitrite oxidizers. Taken together, nitrifiers are much more versatile than previously thought and likely many important features of nitrifiers have not yet been discovered. To facilitate nitrification research, we have developed a new methodological pipeline that allows targeted sorting of nitrifiers (or members of any other functional microbial group) from complex ecosystems like soils for genomic analyses or cultivation. For this purpose, we combine a novel labeling strategy with heavy water with Raman microspectroscopy and optical tweezing. Results obtained with this pipeline including the discovery of putative novel nitrite-oxidizers will be presented.

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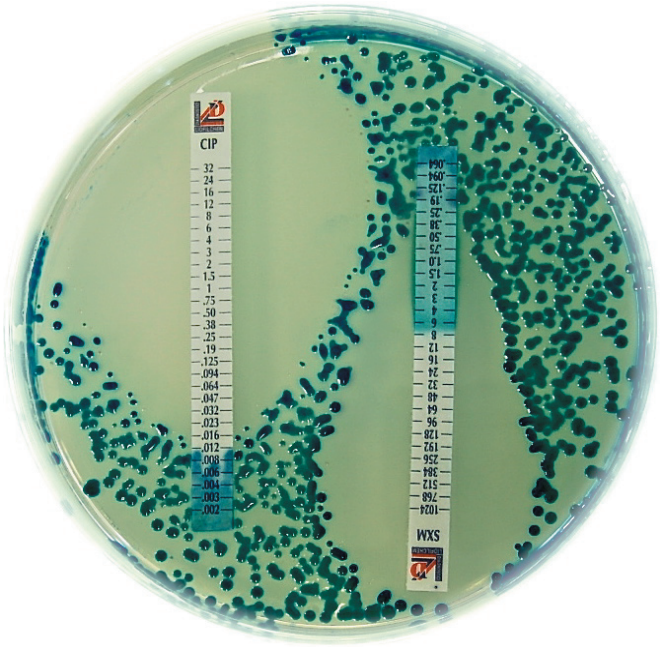

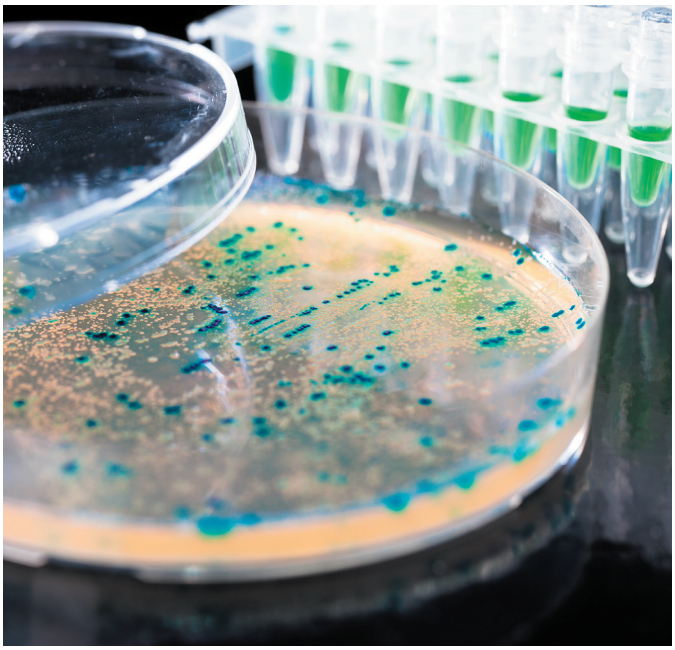

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Poster abstracts

Phage therapy - use of bacteriophages in pathogen control

[P1] DIVERSITY OF LPS-DEPENDENT SALMONELLA PHAGE

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Salmonella enterica subsp. *enterica* consists of more than 2400 serotypes. O-antigen (O-Ag) attached to the conserved core sugars of lipopolysaccharide (LPS) is one of the major contributors of diversity. To tackle this vast surface diversity, *Salmonella* phages target conserved or serotype specific receptors. While O-Ag may be the receptor or mask conserved core sugars, outer membrane proteins are often readily accessible for adsorption. Establishing a diverse *Salmonella* phage collection, we isolated 42 phages using ten prevalent *Salmonella* serotypes. By defined knockout mutants of *S.* Typhimurium LT2, we showed that 9 were dependent on the B_{12} vitamin transporter, BtuB. Another 30 phages were dependent on O-Ag for infection. Furthermore, we showed that three phages that were blocked by the O-Ag, nevertheless infected other serotypes, suggesting that the receptor may be core sugars masked by the LT2 O-Ag. Host range analysis on 71 prevalent serotypes showed that BtuB-dependent phages have the broadest host range, suggesting conserved surface domains in BtuB across serotypes. Most O-Ag-dependent phages showed narrower host ranges, except three that were significantly broader. Interestingly, same three phages could infect many serotypes with similar O-Ag structures, indicating that they target common features of O-Ag. Currently, analysis of phage genome sequences identifies the strategies for LPS-dependent phages can use to target specific receptors. Also genomic comparison allows us to determine host specific determinants of O-Ag dependent phages. In conclusion, both LPS and BtuB-dependent *Salmonella* phages are prevalent in the environment and LPS-dependent phages represent a highly diverse phage group.

[P2] UNDERSTANDING HIGH PHAGE SENSITIVITY OF E. COLI ECOR4

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Escherichia coli is a highly versatile and adaptable species, exhibiting high degree of diversity and consisting of both harmless commensals and pathogenic variants. In fact, only 20% of the genes of the *E. coli* genome are shared among all strains, and there are over 200 serotypes based on O, K, F and H antigens. The *E. coli* surface diversity has fostered diverse phages using many different receptors and infection strategies and most phages only infect a subset of *E. coli*. Yet, our understanding of *E. coli* phage sensitivity is far from complete.
We aim to identify broad phage sensitive *E. coli*. The *E. coli* Reference Collection (ECOR) is a set of 72 reference strains isolated from a variety of hosts and geographical locations representing most of the genetic diversity of *E. coli*. Using the ECOR as host panel, we isolated 128 phages from a variety of environmental samples. When screening environmental samples for phages, some ECOR strains were positive for only a few samples, however plaques could be observed from all samples on *E. coli* ECOR4. Interestingly, in a subsequent host range analysis we found that ECOR4 was also sensitive to 53 (41%) of the isolated phages that otherwise had different host ranges. To identify common genetic elements of the 53 phages we are currently performing whole genome sequencing using MiSeq, and initial results indicate that different phage types are able to infect ECOR4. To elucidate the underlying mechanisms of ECOR4 broad phage sensitivity, we have set out to identify phage receptors and other genes required for infection by constructing Tn5 transposon libraries in ECOR4 and testing for phage sensitivity. Due to its unusual sensitivity to many diverse phages, ECOR4 can serve as a model for analyzing phage sensitivity in *E. coli*.

[P3] THE IMPORTANCE OF PHAGE-HOST INTERACTIONS DURING PHAGE TREATMENT AGAINST CAMPYLOBACTER JEJUNI IN FOOD

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Campylobacter jejuni is the major cause of foodborne human enteritis, with contaminated poultry meat as the main source. Post-harvest phage treatment is promising, since meat storage conditions do not allow *C. jejuni* to grow, thus preventing phage resistant variants to emerge. The aim of this study was to identify phages able to reduce *C. jejuni* counts at chilled temperature. The efficiencies of nineteen individual lytic phages, being either dependent on capsular polysaccharide (CPS) or flagella for infection, were tested against *C. jejuni* NCTC12662 *in vitro* and *in situ* at 5˚C under anaerobic conditions. CPS phages showed varying effectiveness against *C. jejuni*, ranging from no significant to a maximal 0.55 log reduction. In contrast, flagellotropic phages did not significantly reduce bacterial counts.

Based on adsorption assays at 5˚C, it was demonstrated that flagellotropic phages bind reversibly and less efficiently to *C. jejuni* than CPS phages, which may explain their lower killing efficiency. All of the tested CPS phages showed similar binding capacities. Thus, the varying effectiveness of CPS phages to reduce *C. jejuni* may be attributed to differences in other stages of the phage life cycle. Finally, we evaluated a cocktail consisting of our two most effective CPS phages (F356 with 0.49 and F357 with 0.55 log reductions, respectively) on artificially contaminated chicken skin at 5˚C. Application of this phage cocktail led to 0.73 log reduction. Our data suggest that poly-phage therapy may be more effective in combating *C. jejuni* compared to single phage application. A thorough understanding of interactions in *Campylobacter* phages-host systems is prerequisite to further develop an optimal phage treatment against *C. jejun*i in food.

[P4] GENOME SEQUENCES AND COMPARATIVE GENOMICS OF BACTERIOPHAGES INFECTING CAMPYLOBACTER JEJUNI

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Bacteriophages infecting *Campylobacter jejuni* are divided into 2 genera, the Cp220likevirus and the Cp8unalikevirus. Comparative genomics have shown that phages within each genus are highly conserved. However, only few *Campylobacter* phage genome sequences are publicly available and these phages originate from very distinct locations all over the world.
Here we aim to determine the genetic relationship of phages isolated from the same country and on the same farms. Whole genome sequencing and comparative sequence analyses were performed on phages isolated from three different free-range chicken farms in Denmark during the summer of 2011. All phages infect *C. jejuni* strain RM1221 and belong to the Cp220likevirus genus. Sequencing was performed using the Illumina MiSeq technology with sequencing libraries constructed from either a single plaque or purified genomic DNA. The overall similarities of the phages were high, except in regions where putative mobile elements were identified, indicating that acquisition of novel genetic content is associated with such elements. All phage genomes were organized in conserved modules flanked by very large direct or inverted repeats that prevented assembly into one contig. Thus, subsequent PCR's were performed to close the genomes, which interestingly demonstrated rearrangement of conserved modules in phages isolated from the same farm. We are currently using PacBio technology to further verify the assembly of contigs. Furthermore, we observed duplication of regions associated with the repeats, which has previously not been described for this group of phages. In conclusion, we found that phages isolated from the same farm are more genetically related than phages isolated from different farms.

[P5] CHARACTERIZATION OF A NEW PHAGE GROUP WITH A REMARKABLE DEFENSE MECHANISM

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We here present four new *Escherichia coli* phages belonging to two distinct but related genera. These four phages as well as the rest of the phages in these two genera contain an operon with genes that has a high degree of similarity to Queuosine biosynthesis genes. Queuosine is a modified nucleoside derivative of guanosine, which modifies cognate tRNAs by replacing guanine with queuine in tRNAs. The bacterial host cells usually carry their own version of at least some of the queuosine-biosynthesis genes indicating that phage queuosine biosynthesis genes are functionally distinct from their bacterial counterparts.
We will here present preliminary data indicating that this family of phages contains a novel DNA modification system that inserts a Queuosine precursor into the phage DNA.
In order to describe this remarkable trait, we have used Illumina MiSeq, direct plaque sequencing, RNA Seq, restriction endonuclease analysis, and PacBio epigenetics to show a remarkable resistance to several different restriction enzymes and a more detailed picture of the use of alternative bases in the DNA of these molecular parasites.

[P6] INTERACTION OF LYTIC BACTERIOPHAGES WITH *PSEUDOMONAS AERUGINOSA* BIOFILMS

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P. aeruginosa is an opportunistic bacterium causing biofilm-associated infections that are difficult to treat: chronic urinary tract infection on catheters, chronic wounds, ventilated-associated pneumonia in intubated patients, chronic pulmonary disease in patients with cystic fibrosis or chronic obstructive lung disease. The pathogen establishes complex biofilms which protect against both the hosts immune system and antibiotic treatment. The use of bacteriophages has been suggested as an alternative treatment to control *P. aeruginosa* biofilm development. The aim of this study is to investigate in an *in vitro* flow-cell biofilm system the effects of exposing 1 h, 24 h and 72 h old *P. aeruginosa* biofilms to a cocktail consisting of three lytic bacteriophages. The study was performed with a green-fluorescent tagged PAO1 strain using the phages: ATCC 12175-B1, ATCC 14203-B1 and ATCC 14205-B1.

Preliminary results suggest that while the treatment is most effective on the youngest biofilm, even the treatment of the 72 hours old biofilm eliminates most of the bacteria in the biofilm, leaving clusters of aggregated cells which are resistant to phage infection. These results suggest bacteriophage exposure as a possible strategy to treat *P. aeruginosa* biofilms.

Microbial food and feed ingredients

[P7] *BACILLUS SUBTILIS* AS IN SITU AMINO ACID PROVIDER IN PIGS

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Bacillus subtilis has been used in animal feed for many years as a probiotic bacterium and shown previously to be able to over produce amino acids in vitro. In this work a novel concept was investigated - to generate *B. subtilis* strains able to over produce tryptophan in situ. Mutagenesis by UV-irradiation was combined with selection on tryptophan and purine analogues in an iterative process to obtain tryptophan producing *B. subtilis* strains. Two different strains were obtained. Mutant 1 (M1) produced 1 ppm tryptophan and mutant 2 (M2) 14 ppm tryptophan, respectively. Genome sequencing showed that M1 had 3 single nuclear polymorphisms (SNP's) and M2 had 2 SNP's compared to the respective wild type strains. Both strains had one modification in genes regulating tryptophan synthesis. The mtrB gene encoding the TRAP protein, regulating tryptophan synthesis, had an abolished stop codon in M1, whereas in M2 there was a modification up-stream of the *trpS* gene. Reverse transcription PCR was performed to investigate the gene expression of the tryptophan synthesis genes. They were up-regulated in both mutants, but more in M2 than in M1, which might reflect the higher in vitro tryptophan production in M2 compared to M1. This study shows interesting results for a new way of supplying amino acids to animal feed, but further studies are needed.

[P8] DEVELOPING LACTIC ACID BACTERIA FOR THE CONVERSION OF BROWN MACROALGAE INTO GREEN CHEMICALS AND FUELS

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Microbial conversion of biomass plays a major role in establishing a bio-based economy, which aims at replacing fossil resources with renewable substrates for the production of fuels and chemicals. Current efforts in using non-edible ('second generation') biomass rather than food-derived sugars focus on lignocellulosic materials such as crop residues and non-edible plants. However, lignin is often toxic to the production organism and hard to eliminate, and economically feasible conversion of cellulose and hemicellulose is still challenging. An attractive alternative includes brown macroalgae or sea weed, which do not contain lignin, do not require fresh water, are not a major food source, and contain a higher

sugar fraction. The main sugars are mannitol, laminarin (glucose) and alginate (guluronate and mannuronate). We will use metabolic engineering and laboratory evolution of Lactic Acid Bacteria (LAB) for the conversion of brown macroalgae into green chemicals and fuels. To select the best-suited production platform, we are screening *Lactobacillus* and *Pediococcus* strains for traits like genetic accessibility, substrate utilization and several stress tolerances. Most microorganisms, including LAB, do not naturally utilize alginates and hence the introduction of these pathways will be the first step in engineering the selected strain, after which further efforts will focus on co-utilization of the different sugar fractions and establishment of product pathways.

[P9] CHARACTERIZATION OF LACTIC ACID BACTERIA IN SPONTANEOUSLY FERMENTED CAMEL MILK AND SELECTION OF STRAINS FOR FERMENTATION OF CAMEL MILK

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This study has characterized lactic acid bacteria in spontaneously fermented camel milk, with the goal of selecting starter cultures for fermentation of camel milk. This was accomplished through whole community profiling by sequencing of the V3 region of the 16S rRNA gene as well as full length 16S rRNA gene sequencing of selected isolates. The fermented camel milk microbiota was dominated either by Lactobacillales or Enterobacteriaceae, depending on incubation temperature and the provider of the milk. Strains of species with a potential use as starter cultures such as *Pediococcus acidilactici, Lactococcus lactis, Lactobacillus plantarum, Lactobacillus fermentum* and *Lactobacillus salivarius* were isolated. Acidification curves were generated in camel and cow milk and strains within the species *Streptococcus lutetiensis, Streptococcus infantarius*, *Lactococcus lactis, Pediococcus acidilactici* and *Lactobacillus fermentum* showed a good acidification activity in camel milk as well as in cow milk. A high frequency of Gram-negative and potential pathogenic microorganisms was also found in spontaneously fermented camel milk, indicating the need for improved hygiene practices in Ethiopian camel farms. This study has profiled the microbiota of spontaneously fermented camel milk, isolated and characterized novel LAB strains with great potential as starter cultures in camel milk. This will be a significant contribution towards improving food safety and food security in dry regions depending on camel milk production.

[P10] PROTEOLYSIS OF CAMEL MILK BY LACTIC ACID BACTERIA

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Half of the world's camel population lives in the North East Africa. There is a great potential for developing a camel dairy industry. Available starter cultures have showed stunted growth in pure camel milk, why there is a need to identify and characterize new potential starter cultures. Twenty-seven strains of *Lactococcus lactis* were inoculated in camel milk and bovine milk. The strains were a mix of well characterized strains, commercial starter cultures and newly isolated in camel milk. The acidification was monitored and the strains were divided in four acidification groups dependent on their ability to ferment the milk. The milk derived peptides after hydrolysis in camel milk and bovine milk were identified by HPLC-MS and through bioinformatics linked to the relevant milk proteins. The main part of the peptides came from caseins, mainly β-casein. Most of the camel milk isolated strains contained a P_{III}-type cell envelope proteinase (CEP) and acidified bovine milk better than camel milk. Two *Lc. lactis* strains isolated in camel milk showed really good acidification of camel milk, and were not able to acidify bovine milk. Their cleavage specificity of ß-casein was similar to a P_I-type CEP, but since they could not ferment bovine milk, it was assumed that a new CEP type was discovered.

[P11] REDUCTION OF CHEDDAR CHEESE RIPENING TIME THROUGH THE ADDITION OF GLUCOSE

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Ripening of cheese consists of complex microbial interactions between starter lactic acid bacteria (SLAB) and non-starter lactic acid bacteria (NSLAB). One of the key microbial interactions is growth of NSLAB by utilization of metabolites (various sugars) released from SLAB during cell death. The establishment of a high NSLAB level in cheese is slow, taking several months, but it is a prerequisite for high quality. Therefore, significant cost saving would be achieved if a high level of NSLAB could be established faster. This study, using broth models and cheese trials, was performed to optimize and accelerate the SLAB cell death-NSLAB growth interaction. Cheddar cheese was manufactured using a commercial SLAB containing *Lactococcus lactis* subsp. *cremoris* and subsp. *lactis* with fast cell lysis properties. Furthermore, at the curd milling, glucose was added to increase the growth rate

of NSLAB. One third of the added glucose was retained in the curd; however, 1 week post-manufacture, no detectable glucose was present in the cheese. As NSLAB levels were still under the detection limit (10^2 CFU/g of cheese) after 1 week post-manufacture, it is concluded that SLAB were responsible for the glucose depletion. Surprisingly, the broth models showed that at 5 % salt-in-moisture SLAB were unable to utilize glucose. These results indicate that in order to accelerate the cheese ripening by supplementing with sugars, it is necessary to screen for sugars exclusively utilized by the NSLAB.

Bacterial responses to the host environment & antimicrobial agents

[P12] PERTURBATION OF NEONATAL MICROBIAL GUT COMMUNITY BY PERIPARTUM ANTIBIOTICS IN WISTAR RATS LEAD TO DECREASED WEIGHT GAIN

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In the developed world a significant rise of immune mediated diseases as well as of obesity and auto immune diseases in children has been documented. The initial colonization of the gut is often disrupted by oral antibiotics administered to either mothers or children, and this has adverse effects on the commensal gut microbial community.

We hypothesized that modulation of community composition and function induced by peripartum antibiotics affects intestinal microbial composition and general health of the offspring. To address this, 33 pregnant Wistar rats were dosed by oral gavage with either amoxicillin (AMX), vancomycin (VAN) or water (CON) daily from 8 days before delivery until weaning of the offspring. Significantly lower weightgain of the offspring of antibiotic treated dams compared to the control were observed. The antibiotic treated dams had a number of significantly larger organs than control animals. Offspring were dissected at different time points and significant changes between groups were measured. Composition of the gut microbiota, alpha diversity, caecum short chain fatty acid levels, caloric contents of faeces, bile salt levels, acute phase protein haptoglobin in blood, social and locomotive behavior as well as gene expression of tight junction proteins are currently being analyzed.

[P13] STABILITY AND UNFOLDING PATTERNS OF A MULTI DRUG RESISTANT *MYCOBACTERIUM TUBERCULOSIS* DRUG TARGET

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Tuberculosis is the most common cause of infectious disease–related mortality worldwide. As per WHO 5% of globally reported cases of tuberculosis were found to have Multidrug-Resistant (MDR). The WHO report states that “If all notified TB patients (6.3 million) had

been tested for drug resistance; an estimated 300000 cases of MDRTB would have been detected". Asparte-beta-semialdehyde dehydrogenase (ASADH) is the key enzymes of Diaminopimelic acid (DAP) biosynthetic pathway in *Mycobacterium tuberculosis*. DAP is one of the intermediates for synthesis of lysine and also an essential constituent of many bacterial cell walls. Absence of DAP makes the cell wall so fragile that *Mycobacterium* cells lyse almost immediately. The requirement of DAP by *Mycobacterium tuberculosis* along with non-requirement as well as non-production of this compound by humans makes this enzyme an excellent drug target. Here we have studied the guanidinium chloride (GdmCl) directed unfolding of free and NADP-bound forms of ASADH, using multiple spectroscopic techniques, and size exclusion chromatography. The equilibrium unfolding of NADP-free enzyme was found to be a non-cooperative process where no stabilization of any partially folded intermediate of protein is observed. In comparison, the unfolding of NADP-bound enzyme by GdmCl was found to be a cooperative process. The presence of NADP shows a stabilizing effect on the tryptophan environment as well on the native NADP-bound enzyme. This study provides vital information regarding stability of ASADH that is necessary for determining potent leads against this increasingly drug resistant target.

[P14] ABSTRACT WITHDRAWN

[P15] PHYLOGENOMICS ANALYSIS SHOWS DISSEMINATION OF BETA LACTAMASE GENES ACROSS 1638 PSEUDOMONAS AERUGINOSA GENOMES

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Understanding dissemination and evolution of antibiotic resistance is one of the major challenges within clinical microbiology today. As the number of whole genome sequences of bacterial genomes increases, studies of gene dissemination can be done on entire populations. The present study investigates spread of beta lactamase genes in the *Pseudomonas aeruginosa* population. A curated database of beta lactamases comprising 768 genes was constructed and an analysis of 1638 *P. aeruginosa* genomes against the beta lactamase database was performed. A maximum likelihood phylogeny was constructed based on SNPs in the core genome of the 1638 *P. aeruginosa* isolates. The metadata from the beta lactamase analysis as well as *in silico* serotyping data was visualized on the tree. The population-wide phylogenomics analysis showed a clear separation of groups with many different beta lactamase genes present and others with almost none. The method was verified via identification of the core beta lactamase gene *ampC*. Only very few isolates did not harbor the gene, and the entire branch harboring the O12 isolates contained a divergent

ampC gene which again highlights their relatedness and difference from the remaining *P. aeruginosa* population. This study illustrates that there are barriers to the horizontal transfer of resistance genes such as beta lactamases. Identifying these barriers can aid in stopping transfer of resistance genes between isolates in the future.

[P16] SELECTION OF BACTERIAL STRATEGIES IN THE DEVELOPING INFANT GUT AND AIRWAY MICROBIOTA

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Focus on the human microbiotas in early life has increased steadily in the recent years, as they have been suggested to impact wellbeing later in life. Here we decipher universal developmental patterns of infant gut and airway microbiota. We first view the development through a community ecology lens, then with focus on bacterial trophic strategies, and finally we combine the two approaches. We apply this to 16S rRNA amplicon sequences from 2978 fecal and tracheal samples from the $COPSAC_{2010}$ cohort of 700 infants. For the first approach we use deviations from a neutral model to infer selection of bacteria across human hosts. We find that the role of selection increases during gut development, but decreases during airway development. Despite these disparate trends of selection, we observe a shift, invariant with body site, from fast-growing to slow-growing bacteria during development. As this trend is also prevalent in non-host environments, it suggests that selection of trophic strategies is not mediated by host development. Furthermore, it reveals a potential conflict in early life gut communities; here we see selection for both fast-growing bacteria in general and for Bifidobacteriales – an order characterized by slow growing bacteria. Delineating these developmental dynamics is crucial for understanding how our microbiotas are shaped, and ultimately how they affect our health and wellbeing.

[P17] TIME-RESOLVED TRACKING OF RESISTANCE MUTATIONS DURING ANTIMICROBIAL ADAPTIVE EVOLUTION TO SINGLE AND DRUG-PAIRS

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Understanding the evolutionary processes of antibiotic resistance adaption can help us to optimize antibiotic treatment. Often, end-point isolates from adaption evolution experiments are whole genome sequenced. Consequently, this only permits limited information about the dynamics of resistance mutations in the bacterial population. Here we use targeted population sequencing to examine the dynamics of the resistance alleles during adaptation, by time-tracking 5 drug adapted *E. coli* populations done in triplicate. Our antibiotic conditions were single drug evolved conditions (amikacin, chloramphenicol and ciprofloxacin) and antibiotic combinations (amikacin+chloramphenicol and chloramphenicol+ciprofloxacin). Our results show that the drug combination amikacin+chloramphenicol significantly reduced appearance of specific resistance mutations compared to its single drug evolved counterpart. We ascribe this to, the collateral sensitivity interaction between amikacin and chloramphenicol. We identified three different types of allele dynamics in the examined populations: discordant (alleles never occurring together), overlapping (alleles always existing together), or accumulating interactions (alleles accumulating over time). To explore how these dynamics where linked to the phenotypic effects of specific resistance mutations, we re-introduce mutations in to the naïve ancestral wild type. Each strain was tested on relative fitness from ancestral wild type and IC90, to establish why specific mutations were selected or counter-selected in the adapting bacterial populations.

[P18] ENHANCED KILLING OF HYPOXIC P. AERUGINOSA BIOFILM BY CIPROFLOXACIN WITH HYPERBARIC OXYGEN TREATMENT

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Chronic *Pseudomonas aeruginosa* lung infection is the most severe complication in cystic fibrosis patients. It is characterized by antibiotic-tolerant biofilms in the endobronchial mucus with zones of O_2 depletion. While the exact mechanisms affecting antibiotic effectiveness on biofilms remain unclear, accumulating evidence suggests that the efficiency of several bactericidal antibiotics, such as ciprofloxacin, is enhanced by stimulation of the aerobic respiration of pathogens and that lack of O_2 increases their tolerance. The bactericidal effect of several antibiotics require bacterial metabolic activity and depend on the formation of reactive O_2 radicals (ROS). Our aim is to sensitize anoxic biofilms of wildtype *P. aeruginosa* to ciprofloxacin by hyperbaric oxygen treatment (HBOT).

We tested re-oxygenation by HBOT of an O_2-depleted biofilm model with *P. aeruginosa* embedded in agarose to enhance aerobic respiration during ciprofloxacin treatment. 3-day-old anoxic biofilms were treated with 0-2 mg/L ciprofloxacin. The biofilms were further incubated for 90 min ± HBOT (100 % O_2, 2.8 bar).

We demonstrated enhanced bactericidal activity of ciprofloxacin in *P. aeruginosa* agarose-biofilm during 90 min of HBOT ($P<0.05$). In fact, the maximum enhancement of killing by HBOT exceeded 2 log using 0.25 and 0.5 mg/L of ciprofloxacin. Furthermore, we demonstrated increased bacterial growth and production of ROS during HBOT.

This study demonstrates that re-oxygenation by HBOT can significantly enhance the bactericidal activity of ciprofloxacin on *P. aeruginosa* biofilm during 90 min of incubation. Combining ciprofloxacin treatment with HBOT thus clearly has potential to improve the treatment of *P. aeruginosa* biofilm infections.

[P19] MODULATION OF S. AUREUS QUORUM SENSING BY P. AERUGINOSA

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S. aureus and *P.aeruginosa* are frequently found together in CF airway polymicrobial infections and we have determined the genetic basis of adaptation in the transmissible, CF-adapted *P. aeruginosa* DK2 lineage (PADK2). Here, we investigate whether and how the genetic changes in PADK2 have re-modeled its ability to interact with other CF-airway microorganisms such as *S. aureus*.

Infecting bacteria can grow as aggregates on airway surfaces, therefore we examined the *in vitro* transcriptional pattern of SA and PADK2 grown on a solid surface for 24 hours as monoculture or coculture spots. RNA-seq was performed and a pairwise analysis was conducted for each strain. RNA-seq data was validated by RT-qPCR and transcriptional *lacZ* fusions.

In coculture, SA showed a general tendency of downregulation of gene expression, including reduced expression of many genes involved in virulence and regulated by the Agr Quorum Sensing system such as proteases, leukocidins, leukotoxins and haemolysins. Interestingly, the *agrD* gene encoding the precursor of the autoinducing peptide – AIP– regulating QS, was also downregulated in coculture, pointing to inhibition of the Agr QS system. Consistent with this model, protein A was upregulated in these conditions. Conversely, PADK2 showed upregulation of the *pqsD* gene – involved in biosynthesis of the QS molecule HHQ – and a general pattern of enhanced gene expression.

Our *in vitro* study shows that microbe-microbe interactions can modulate expression of important virulence genes which – in the case of *S. aureus* – results in a phenotype compatible with a persistent infection and enhanced survival in the host. *P. aeruginosa* DK2 modulates *S. aureus* gene expression via the Agr QS system by a yet to be identified mechanism.

[P20] GUT MICROBIOTA DYNAMICS IN INDIVIDUALS UNDERGOING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Allogeneic hematopoietic stem cell transplantation (HSCT) is a curative therapeutic approach for various hematologic malignancies. Infectious diseases and intestinal mucositis are among its most severe side effects. Here, we examine longitudinal dynamics of the human gut microbiota of a pilot set of 29 pediatric patients (1 - 15 years) undergoing HSCT, by utilizing 16S rRNA sequencing. In-depth analysis of the patients' gut microbial composition revealed four distinct community state types (CSTs) and longitudinal transitions of patients' CSTs were profiled. Interestingly, the majority of non-survivors exhibited the same CST between week 3 and 4 post HSCT and showed more transitions between CSTs compared to survivors who either persisted in the same cluster or underwent only one transition, suggesting a potential beneficial effect of a stable gut microbial composition. CSTs were mainly discriminated by dominating operational taxonomic units (OTUs) of the genera *Enterococcus*, *Lactobacillus* and *Streptococcus* which were partitioned into highly resolved taxa based on high-information nucleotide positions. Subsequent network analyses revealed high inter-individual variability and reinforced the CST clustering on a finer scale. Gut microbial compositional state, its stability and diversity might therefore be associated with survival and other clinical endpoints and might provide potential novel diagnostics or therapeutic targets to improve survival and convalescence of allogeneic HSCT patients.

[P21] ROSEOBACTER GARDENING OF MICROALGAE: PHYLOGENETIC DISTRIBUTION OF ROSEOBACTICIDES AND THEIR EFFECT ON MICROALGAE

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The *Roseobacter*-clade species *Phaeobacter inhibens* produces both an antibacterial compound, tropodithietic acid (TDA), and algicidal molecules, roseobacticides. In *P. inhibens* DSM 17395, the biosyntheses of TDA and roseobacticides are linked. The purpose of this study was to investigate the production of roseobacticides in TDA-producing *Roseobacter*-clade bacteria and to compare the effect of producers and non-producers on microalgae. Of the 32 *Roseobacter*-clade strains analyzed, roseobacticide production was a unique feature of TDA-producing *P. inhibens*, *P. gallaeciencis* and "*Candidatus* Phaeobacter chimaera" strains. One TDA-producing *Phaeobacter* strain, 27-4, was unable to produce roseobacticides, possibly due to the insertion of a transposable element. TDA-producing *Ruegeria mobilis* and *Pseudovibrio* did not produce roseobacticides. Growth of several microalgae *was* affected by roseobacticide-containing bacterial extracts, while the tested chlorophyte grew normally. Co-cultivation with a roseobacticide producer initially caused improved growth of the haptophyte *E. huxleyi*, but enhanced algal decline, while the non-producer 27-4 had no effect. TDA-producing roseobacters have potential as probiotics in marine larvi-culture where they antagonize fish pathogens in live feed cultures. Thus, it is promising for this application that a non-producing *Phaeobacter* strain was identified and the chlorophyte used as feed was unaffected by the roseobacticides.

[P22] LINKING GENOMIC POTENCY TO ACTUAL GENE EXPRESSION IN HUMAN LUNGS: A PREMIER ANALYSIS OF PSEUDOMONAS AERUGINOSA TRANSCRIPTOME IN SPUTUM SAMPLES

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Cystic fibrosis is a life-threatening disease connected with genetic mutations affecting the activity of the epithelial chloride transporter CFTR. The shortcomings in ion transport determine the formation of a viscous mucus layer that covers epithelial tissues undermining the normal physiology of several organs, in particular, lungs; here, the thick mucus layer creates an optimal environment for bacterial growth leading to a rapid development of

chronic infections, severely reducing life quality and expectancy of the patients. *P. aeruginosa* is the predominant pathogen in these airways infections, mostly due to its ability to easily survive and evolve in CF lungs.
Recently, by sequencing 500 genomes of bacteria isolated from young CF patients we identified key mutations accumulating during early stages of *P. aeruginosa* evolution in airways, gaining an unprecedented insight into the adaptive process. Although our approach uncovered the genomic potency that *P. aeruginosa* acquired during the years of infection, it cannot predict the resulting behaviour in the host. To overcome this limitation, we developed a complementary approach based on transcriptome profiling of *P. aeruginosa* directly in the CF sputum. Preliminary results derived from a few chronically infected patients suggest that in late stages of infection the transcriptional landscape in fully adapted populations of *P. aeruginosa* is dominated by genes involved in transcription, translation, and energy metabolism, just as in exponentially growing cells. Moreover, although under antibiotic treatment, no particular stress response signature is observed, underlining the adaptive capabilities of this bacterium when fully associated with the host.

[P23] METABOLIC REARRANGEMENTS IN PSEUDOMONAS AERUGINOSA STRAINS ISOLATED FROM HUMAN AIRWAY INFECTIONS

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

repertoire in addition to a more efficient use of the resources, resulting in increased fitness and robustness during persistence in the host.

[P24] HORISONTAL TRANSFER OF ANTIMICROBIAL RESISTANCE IN MEAT

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Vertical transmissions of non-pathogenic antimicrobial resistance clones of bacteria from production animals to humans have only been documented in few cases. Contradictory to this identical genes are found in the two reservoirs. So, how is antimicrobial resistance transmitted between the two reservoirs through the production chain if vertical transmission is not the main course? By experimental setup using the gfp-marked plasmid pKJK10 (a derivate of pKJK5) we have studied horizontal transmission of tetracycline resistance in minced meat to the indigenous bacterial flora at different temperatures and by 16S sequencing of green fluorescent colonies identified the recipients of the pKJK10 plasmid. Results will be presented and the consequences of these discussed.

[P25] EFFECT OF ADMINISTRATION OF ANTIBIOTICS PERIPARTUM TO WISTAR RATS ON BILE ACID PROFILES IN OFFSPRING

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Vertical transmission of the maternal microbiota is assumed to be crucial for the offspring's development. A disrupted microbiota composition leading to an altered metabolic activity of the microbiota can affect bile acid profiles, which are known to influence host metabolism. Here, we examined whether perturbation of the maternal gut microbiota during pregnancy, induced by administration of either amoxicillin or vancomycin to pregnant rats, influenced bile acid profiles in the offspring. The dams were treated with antibiotics from 8 days before the dams gave birth and continued until weaning (4 weeks later). Blood samples were collected from offspring at ages 2, 4 and 14 weeks, and from dams at the end of treatment. From these blood samples, bile acids were extracted and 22 bile acids were quantified by targeted liquid chromatography mass spectrometry. Comparing the serum bile acid profiles of antibiotic-treated rat dams with non-treated dams, we found that the antibiotic

treatments significantly changed the bile acid profiles. However, no effect was seen in the offspring of the antibiotic-treated dams at any age. The bile acid profiles of the offspring did however change significantly with age, where the largest amounts of bile acids were found in the 4-weeks old pups. Future work will involve integrating the bile acid data with physiology and microbiota data of both pups and dams.

[P26] UNION MAKES THEM STRONGER BUT NOT RESISTANT: EFFECT OF A DELETION IN L6 TO RIBOSOMAL SUBUNIT JOINING

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The ribosome is one of the most important targets for antibiotics that discriminate between bacterial pathogens and higher organisms. Ribosomes can be targeted by adaptive mutations, which result in fitness increasing phenotypic changes. Several ribosomal mutations have been identified by genome analysis of *Pseudomonas aeruginosa* isolates from airways of cystic fibrosis patients.
We have investigated a 4-amino acid deletion in the large ribosomal subunit protein, L6. Clinical isolates carrying this mutation are resistant to tobramycin and gentamicin, and their growth rate is reduced. *In patient*, tobramycin treatment promoted the maintenance of the mutant in the population of bacteria, but when interrupted the strain was outcompeted, possibly due to its reduced growth rate.
By complementation with wild-type L6, both growth rate and antibiotic susceptibility were restored to wild-type values. These results clearly suggest that the observed phenotypes are directly associated with the deletion in L6. Now we are in the process of elucidating the mechanism of resistance caused by this mutation at the structural level. Preliminary results indicate that an impaired joining of the 50S and 30S ribosomal subunits may be responsible for the described phenotypes.
Clarification of the effects of specific mutations in ribosomal proteins is important for our understanding of biological evolution, and will have impacts on the design of new treatment strategies to combat microbial infections.

[P27] ANTIMICROBIAL POTENTIAL OF THE FUNGUS-GROWING TERMITE SYMBIOSIS

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Termites of the Macrotermitinae subfamily maintain a monoculture of an obligate mutualistic *Termitomyces* fungus as their main food source. The termites forage for dead plant material and mix this with *Termitomyces* spores in their gut to create a substrate – the fungus comb – on which the fungus grows. The plant material is degraded by *Termitomyces*, which produces nodules filled with asexual spores that are mixed with new incoming forage. Through this process, the plant material is completely decomposed for fungal and termite nutrition. Despite a diversity of putative antagonistic fungi in the forage material, fungus combs are virtually free of any fungi other than *Termitomyces*, suggesting the active suppression of potential antagonists. We investigated whether antimicrobial metabolites are produced in the termite gut and fungal comb environments using a combination of metagenomics, metabolomics and antimicrobial assays of termite-associated bacteria against ecologically and medically relevant strains. RNAseq of the gut microbiota of one colony for each of two termite genera (*Macrotermes* and *Odontotermes*) revealed the expression of a number of PKS and NRPS gene clusters putatively coding for antimicrobials, and liquid chromatography-mass spectrometry (LCMS) analysis of both guts and combs suggests they harbor an array of structurally diverse metabolites. Taken together, these initial results suggest that bacterial metabolites may play a role in maintaining clean fungus combs.

[P28] CLONING AND EXPRESSION OF CHITINASES FROM MARINE BACTERIA WITH ANTIFUNGAL ACTIVITIES

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Filamentous fungi cause damage in many areas, e.g. agriculture and indoor climate. Current control practices involve the use of fungicides and therefore there is a high demand for safe bio-pesticides. Chitin is a component of fungal cell walls and the dominant polymer of the marine environment. Since the marine environment is still an under-explored niche, we tested eleven marine bacteria for antifungal activity against seven different fungi and used

whole-genome sequencing to evaluate the potential for chitin degradation. We subsequently cloned and expressed chitinase genes and tested transformants and extracts for antifungal activity. All bacteria were antifungal, and three strains showed pronounced antifungal effect, however with different fungal targets. One strain *Pseudoalteromonas piscicida* S2040*,* had pronounced antifungal effect towards all fungi. Genome mining revealed two to six chitinase genes per bacterial strain. Two strains, *Photobacterium galatheae* S2753 and *P. piscicida* S2724 were superior in chitin degradation on plates, and we therefore cloned seven chitinase genes from these strains. Two ChiA chitinases were secreted and actively degrading chitin. We tested extracts of the chitinase clones for antifungal activity, but they were not antifungal in this setting. In conclusion, we successfully cloned and expressed two chitinases. Furthermore, we found three strains with pronounced antifungal effect, which could be potential candidates for future bio-pesticides.

[P29] ANTIBACTERIAL AND IMMUNOMODULATORY ACTIVITIES OF *CYMBOPOGON MARTINII* ESSENTIAL OIL AND GERANIOL

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Cymbopogon martinii essential oil (CMEO) has been widely used in skin treatments due its antibacterial and anti-inflammatory activities. *Propionibacterium acnes* is a skin bacterium that is associated with the pathogenesis of acne vulgaris and other diseases. Here, we evaluated the antibacterial activity of CMEO and its major substance, geraniol, against *P. acnes in vitro* by determining minimum inhibitory concentrations (MICs). Results show that distinct subtypes of *P. acnes* were differently sensitive to the compounds, as MICs ranged between 0.7 and 1.5 mg/mL. In addition, we tested the immunomodulatory activity and cytotoxicity of CMEO and geraniol in keratinocytes in the absence or presence of *P. acnes*. The compounds were not cytotoxic; IL-8 production in keratinocytes pretreated with the compounds was significantly reduced in *P. acnes* type I infected cells, compared to non-treated cells. This indicates that CMEO/geraniol has immunomodulatory effects that can partially neutralize the pro-inflammatory activity of *P. acnes* type I

[P30] STAYIN ALIVE-PERSISTENCE IN *PSEUDOMONAS AERUGINOSA* IN PATIENTS WITH CYSTIC FIBROSIS

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Pseudomonas aeruginosa is responsible for the majority of chronic lung infections in patients suffering from cystic fibrosis (CF). Even though intensive antibiotic treatment is given to the patients in order to eliminate *P. aeruginosa,* the infection often persists. Persisters are drug tolerant population of bacteria that are able to survive antibiotic treatment without becoming genetically resistant to the drug. Persistence is a problem in clinical settings, as persisters are believed to contribute to the difficulties in the treatment of many infections. However very little is known about the specific mechanism affecting the formation of persisters.Using the *P. aeruginosa* lung infection in CF patients as a model to study persister phenomenon, our objective is to understand why antibiotic treatment fails. We have looked into the early stages of infection where antibiotics fail to fully eradicate the bacteria but no antibiotic resistance development is seen. We have sequenced the genomes of 500 *P. aeruginosa* isolates from children with CF. Infection clone types and their phenotypic behavior has been analyzed in multiple settings. We have screened 354 isolates for persister phenotypes and found that approximately 25% are tolerant to ciprofloxacin. The quantitative characterization of the persister phenotype together with genetic analysis of the most frequently mutated genes in our isolates are providing insight into the failure of treatment of *P. aeruginosa* infections.

[P31] WITHIN-HOST EVOLUTION OF ACHROMOBACTER BIOFILMS IN CYSTIC FIBROSIS PATIENTS

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Achromobacter has the capacity to form biofilm, which is crucial to persisting lung infections in CF patients. Upon transition from primary to chronic infection, the bacteria undergo evolutionary adaptations to the environment in the CF lung and to a biofilm lifestyle, where it is protected from antibiotics and the host immune system. The aim of this project is to study gene regulation as an adaptation to chronic biofilm infection the CF lung.

Whole-genome transcriptome analysis was performed on RNA extracted from planktonic cultures and biofilms from clinical isolates of *Achromobacter xylosoxidans*, collected from the same patient over a number of years.
Genes related to virulence, motility, metabolism and extracellular compounds were differentially expressed in sequential isolates. Changes in biofilm formation and antimicrobial resistance in isolates from primary to chronic infections may be caused by altered gene expression rather than loss or gain of new genetic material. Differentially expressed genes were also identified in biofilms as opposed to planktonic cultures in stationary growth phase. Knock-out of selected genes will be performed to evaluate their function in *Achromobacter* biofilm formation.
Gained knowledge on genes specifically involved in biofilm formation may provide insight into understanding the underlying mechanisms involved in *Achromobacter* biofilm formation and persistence in chronic infections.

[P32] ABSTRACT WITHDRAWN

[P33] MUTATIONS IN STAPHYLOCOCCUS AUREUS RRNA CAUSING LINEZOLID RESISTANCE INCREASE SUSCEPTIBILITY TO OTHER ANTIBIOTICS

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Antibiotic resistance can develop spontaneously by single nucleotide mutations in bacteria during selective pressure, as in long term treatment of persistent infections. The antibiotic linezolid is a last line antibiotic for treatment of e.g. multi-resistant *S. aureus* infections. Although the mutation rate to this antibiotic is known to be very low, cases of clinical resistant mutants have been reported worldwide.
Altering between growth on solid agar with a linezolid gradient (selecting for higher resistance) and growth in liquid medium with a sub-inhibitory level of linezolid (selecting for higher growth rate), we have developed mutants with high resistance (32-64 x MIC of WT) to linezolid. Their mutations were determined by sequencing to be one of the most common clinically occurring mutation; the G2576T mutation in the domain V region of 23S rRNA, known from both *Staphylococcus* and *Enterococcus* spp.
Antibiotic susceptibility testing was performed by disk diffusion to elucidate any collateral resistance effects in the mutants, and we found markedly higher susceptibility to penicillin, gentamicin and tetracycline, compared to the WT strain. This finding suggests that these resistance mutations result in secondary sensitivities that can be used to propose new treatment strategies or treatment alternation to combat persistent, multi-resistant *S. aureus* infections.

[P34] MINIMUM BACTERIOCIDAL CONCENTRATIONS (MBC) OF NISIN AGAINST BACILLUS AND CLOSTRIDIUM SPP. OF RELEVANCE FOR THE SAFETY AND SPOILAGE OF DAIRY PRODUCTS

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Species of the genera *Clostridium* and *Bacillus* are common contaminants of milk with the potential to compromise the safety or shelf-life of products that have not been subjected to processes targeting the inactivation or removal of spores. Nisin, an antimicrobial peptide produced by *Lactococcus lactis*, is recommended for the control of Gram+ bacteria including spores, when the formulation or storage conditions of dairy could potentially allow their growth. The goal of this study was to determine the minimum concentrations of nisin necessary to inactivate different species of pathogenic and spoilage sporeformers of relevance to dairy.
Minimum Bacteriocidal Concentrations (MBC) were estimated for 21 strains of nine *Bacillus* spp. and 11 strains of two *Clostridium* spp. using a dilution method protocol. The effect of nisin was studied using inoculums of 1-4.5 log CFU/ml in Mueller-Hinton Broth or Wilkins Chalgren Anaerobe broth in 96 well plates containing serial dilutions of nisin. Plates were incubated at 30^{o}C for 24h and the contents of non-turbid wells were plated in suitable media to confirm the MBC.
MBC values as high as 12.5 ppm may be needed for the inactivation of vegetative forms of some of the studied species. For inoculum levels up to 3 log CFU/g, measured MBC values were generally much higher for *Bacillus* spp. in comparison to *Clostridium* spp. which were found to be more susceptible to nisin. For inoculum levels greater than 3 log CFU/g, no significant differences were observed between the studied species. In general, lower inoculums of the same strain resulted in lower MBC values but this was not the case for two strains of *B. licheniformis* and three strains of *B. cereus* that exhibited MBC values of 12.5 ppm for low inoculum levels.

[P35] METAGENOMIC RECONSTRUCTION OF BACTERIAL GENOMES FROM MARIANA TRENCH SEDIMENTS

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The Mariana Trench is the deepest spot on earth located in the pacific ocean. The deepest part, the challenger deep, was measured to be 10.900m in 1951. The pressure exerted by the water column on the deepest site is 109mpa. This cold and high-pressure environment seems likely to harbor organisms with interesting adaptations to the thermodynamic challenges they face.
DNA extracted from 7 sediment core samples have been sequenced on the Illumina Hi-Seq obtaining ~30m reads. After sequence cleaning and assembly metagenomic bins were constructed with CONCOCT and manually refined with A'nvio, resulting in 65 bins. These genomes have been validated against a database of known single copy core genes, and 18 were found to be more than 75% complete and less than 10% redundant**,** indicating a high-quality genome. Taxonomic identification of the bins suggest strains from well-known genera, except for one for which assignment was low confidence.
For comparison a database of 37 high-quality genomes from the NCBI refseq was constructed, encompassing psychrofiles, barophiles, aquatic and terrestial organisms.
This work is an exploratory attempt at comparing the metabolic and stress response capabilities of known organisms of diverse lifestyles with organisms recovered from the Mariana Trench.

[P36] PREDICTING MUTATION IMPACT ON PHENOTYPIC CONVERGENCE IN CHRONIC INFECTIONS

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Characterizing the convergent phenotype(s) of evolving pathogens will improve treatment efficacy and inhibit drug resistance. Here, we use the chronic lung infections by *Pseudomonas aeruginosa* (PA) in cystic fibrosis (CF) patients as a model of evolution, evaluating 457 longitudinal PA isolates from 34 CF patients (Marvig et al. 2015.). Mutations occur in 62.5% of metabolic genes predicted active in cystic fibrosis sputum versus a rate of 55.7% over the whole genome. To predict the functional impact of these metabolic mutations on relevant 'tasks' that contribute to an ideal CF phenotype, we built 457 isolate-specific models using novel mutation-based constraints applied to a genome-scale metabolic

network reconstruction. Using predicted growth capacity, virulence factor production, and flux patterns, we identified substantial metabolic adaptation in 24 out of 36 clone types. Growth was impacted in 57% of isolates due partly to 66 mutations in the 123 genes essential to growth in *in silico* CF sputum. By iteratively reversing single and paired mutations within an isolate-specific mutation set, we identified 24 pairs of co-occurring mutated genes and 8 single genes that account for all instances of major growth inhibition. These mutations serve as potential biomarkers that we can combine with *in vitro* data and patient history to link mutational trajectories with bacterial phenotypes and associated outcomes for each patient.

Atmospheric microbiology

[P37] AIRBORNE ALTERNARIA AND CLADOSPORIUM FUNGAL SPORES. SOURCES IN DENMARK AND EFFECT ON ASTHMA

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Background:
Alternaria and *Cladosporium* are ubiquitous in the air and important aeroallergens (1; 2), however little is known about their sources and their effect on asthma. There is a link between *Alternaria* concentrations and density of agricultural areas (3;4), but the sources of *Cladosporium* are still to be identified.

Aim:
To establish the most relevant local sources of *Cladosporium* and *Alternaria* in Denmark with a specific attention to crop harvesting and its effect on asthma exacerbations.

Hypotheses:
a) Daily fungal spore counts and harvesting periods are associated with asthma exacerbations; b) Higher loads of *Alternaria* in western *vs.* eastern Denmark; c) The major portion of airborne *Cladosporium* is emitted from sources other than grain crops.

Methods and plans:
Two epidemiological studies based on health registers. Obtaining data on spore air concentrations through microscopic analysis of archived samples. Identification of airborne *Cladosporium* source areas by use of back trajectories. Evaluation of *Cladosporium vs. Alternaria* colonization by field measurements during grain crops harvesting.

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[P38] AERIAL SPREAD OF MICROORGANISMS FROM FARMS

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Animal husbandry is most likely an important source for microorganisms occurring in the air, including important pathogens, allergens as well as multi-resistant bacteria. Methods for collecting, quantifying and characterizing microorganisms from outdoors air have been developed and used to study their spreading from a pig farm. Simultaneously, the background concentration of microorganisms in rural air, not directly influenced by husbandry, was determined. Results from this study will be presented and discussed in relation the dispersion of microorganisms from farms, effects on humans and whether it is possible to identify indicator organisms for the spreading of microorganisms in general from animal husbandry.

[P39] USING FLOW CYTOMETRY FOR THE ANALYSIS OF AIRBORNE BACTERIA IN ENVIRONMENTAL SAMPLES COLLECTED FROM A MINICIPAL WASTEWATER TREATMENT PLANT IN DENMARK

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The microbial load in aerosols present in the atmosphere has been central topics in current environmental research especially from the substantial public health concerns. However, biological characterization of air samples presents a significant challenge due to their dilute nature and methodological issues in a sample treatment process. Flow cytometry has been utilized to analyze microbial communities mainly in aquatic ecology studies with reduced labor and rapid analysis rate, however, little has been published on the application of it to airborne bacteria retain in environmental samples.

The objective of this study is to detect and enumerate populations of bacteria in air samples collected from a municipal wastewater treatment plant in Denmark by both flow cytometry and DNA-based methods, and to analyze the relationship among parameters from applied methods, and to validate the utility of flow cytometry for bioaerosol monitoring. The present study is ongoing and preliminary results will be discussed.

[P40] ICE-NUCLEATION-ACTIVE GENE IN SINGLE CELLS OF PSEUDOMONAS SYRINGAE AND ITS ROLE IN ACTIVE ATMOSPHERIC DISSEMINATION

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Ice nucleation active (INA) bacteria are proposed to affect Earth's climate. It was proposed that INA producing bacteria might actively induce their ground deposition by stimulating precipitation through ice formation. Situated on the cell-surface, INA proteins are facilitating the formation of ice by acting as templates.

We investigated the presence of INA proteins and characterized the gene expression with our model bacterium *Pseudomonas syringae* R10.79 that was isolated from a rain sample. *Ina*-gene expression and protein localization was studied by combining RT-qPCR, flow cytometry and immunofluorescence assays. Using flow cytometry analysis, we detected three subpopulations of INA positive *P. syringae* cells based on their level of INA protein content. We observed that only 4% of the cells expressed INA genes and that the production of INA proteins within the bacteria population was growth phase-dependent.

The metabolic potential of strain R10.79 that could support expression of the INA gene in the atmosphere was studied by combining substrate utilization assays with genome analysis. We demonstrated the strain's copiotrophic nature as it utilized 44% of all carbon sources including abundant atmospheric trace gasses and possesses 93 different catabolic pathways in its genome.

Our results showed that the INA proteins were localized all over the cells' surface and that INA gene expression patterns varied with culture age and induction temperature for INA protein production.

[P41] DESIGNING AN IMPROVED SAMPLING DEVICE FOR BACTERIA IN AIR

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Background
A new model of a high-volume-flow impinger is under development for health and environmental applications. A prototype was tested with good results (Zweifel et al 2012).

Inside a sophisticated mixing chamber, the bacteria contained in the air are being stripped into an aqueous liquid. In the transition from air to water, they are concentrated by several orders of magnitude and preserved for later analysis. The efficiency of the prototype was characterized recently (Santl Temkiv et al 2016). As the prototype originally was not designed for microbiology, limitations persist in terms of usability, sterility, size and weight.
The improved model will overcome these limitations. It will be produced from autoclavable materials to facilitate sterile sampling conditions. It will include a sealed lid and connection ports for easy cleaning and sample exchange. Other design constraints include minimized airflow resistance, minimized loss of water and maximized retention of particles in the water.

Objectives and research questions

Currently we are analyzing the flow in the prototype:

- How does the air and water flow inside the mixing chamber?
- Where are trouble spots located in the flow?
- If the flow is turbulent, how can it be modelled?

With this knowledge, improvements will be designed in terms of usability and accuracy compared to the prototype.

Methodology

The prototype is being translated in CAD software. Then the CAD model will be exported into flow analysis software. With the data provided from the analysis, a product development phase will be started. Once we have arrived at an optimized model, it will be manufactured by 3D printing and tested under "real" conditions.

[P42] THE VIABILITY STATE OF AIRBORN BACTERIA

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Introduction

Reproducible aerosolization of bacteria is a prerequisite of controlled laboratory studies of airborne bacterial viability. This was recently achieved [1] .

Goals

We aim to investigate the role of water activity for aerosolized bacteria by assessing:

- Viability of aerosolized bacteria at varying relative humidity.
- Water uptake of airborne bacteria at varying relative humidity.

Methods

The Humidified Tandem Differential Mobility Analyzer (HTDMA) measures the size of airborne particles at well-defined relative humidity (RH). Measurements of aerosol particles up to 2 µm at up to 93% RH can be carried out [1] , enabling us to quantify bacterial water film thickness. Also, we plan to expose a bioaerosol to fixed low RH with subsequent sampling and assessment of bacterial viability. In a different experiment, we plan to measure bioaerosol water uptake at various RH.

Perspective

Hereby we seek to increase the understanding of how humidification state influences airborne bacterial viability. This could be beneficial for research in the fields of airborne diseases, microbial biogeography and bioaerosol atmospheric impacts.

References:

1. Löndahl et al., manuscript in prep.
2. Lopes-Yglesias et al., AS&T, 48, 969 – 980, 2014

[P43] AIRBORNE MICROALGAE DISPERSAL: DIVERSITY, RESISTANCE TO ATMOSPHERIC CONSTRAINS AND IMPACT ON THE ENVIRONMENT

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Aeolian transport is a largely overlooked route of microalgae dispersal. Microalgae can be emitted to the atmosphere, transported over large geographic scales, deposited to ground by atmospheric currents, and grown in natural and artificial environments. However, little is know about the diversity of dispersed airborne microalgae, the mechanisms that promote their dispersal success, and their interaction with atmospheric processes. We here present the methodology and preliminary results of a study aiming at investigating the capacity of both aquatic and airborne microalgae at coping with atmospheric stressors (low temperature and desiccation) and at inducing ice formation between -12°C and -24°C. Their taxonomy is being assessed by 18S rDNA sequencing and compared to available data on the diversity of airborne microalgae. In future, a combination of metagenomics and atmospheric modeling (e.g. backward and forward trajectory analysis) will help identifying emission sources and sinks of dispersing airborne microalgae and thus highlighting the route of their future expansion, promoted by climate change.

[P44] ASSEMENT OF MICROBIAL LOAD IN INDOOR AIR OF UNIVERSITY OF HAIL, HAIL, KSA

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Background:
Indoor air may contain pathogenic and non-pathogenic live or dead bacteria, fungi, viruses, allergens, endotoxins, pollen, etc. Microorganisms are considered significant part of the indoor air as causal agents of respiratory disorder and lung dysfunction in occupational and non-occupational environment. The measurement of microbial load in indoor environment samples is of great importance due to their potential negative impact on occupational safety & health. The present study was undertaken to isolate and identify different bacteria present in the indoor environment of University of Hail, KSA.

Method:
Air samples were collected using Spin Air 5500 (Air sampler, IUL Instruments, Barcelona, Spain) on bacteriological culture media (Nutrient gar plate and chocolate agar plate) from the different location College of Medicine, University of Hail, Hail, KSA. Media plates were incubated at 37^{o}C overnight. For identification of microbes, microbial colonies obtained from air samples were sent to Molecular Diagnostics and Personalized Therapeutics Unit (MPTU), College of Applied Medical Sciences, University of Hail. Hail, KSA.

Results:
By using MALDI-TOF Biotyper, we identified the following microbes in the air samples of different hospitals of Hail: *Bacillus pumilus, Staphylococcus hominis, Exiguabacterium aurantiacum, Acinetobacter baumannii, Enterobacter cloacae, Staphyloccus haemolyticus, Lactococcus lactis* and *Escherichia coli.*

Conclusions:
Both pathogenic and nonpathogenic bacteria were present in the indoor air of the hospitals. Patients, staff and doctors in hospitals are exposed to different airborne bacteria, which may lead to variety of infections including respiratory diseases.

[P45] EVALUATION OF THE EXPRESSION OF *INA* GENE IN SINGLE CELLS OF *PSEUDOMONAS SYRINGAE* R10.79 AND ITS SURVIVAL UNDER SIMULATED ATMOSPHERIC CONDITIONS

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The Earth's atmosphere can be considered a gaseous ocean in which biological particles, the so-called bioaerosols, are suspended. A fraction of these bioaerosols serve as ice nucleation and cloud condensation agents. Some ice-nucleation active (INA) bioaerosols have been identified as bacteria that live on plant-surfaces, such as the species *Pseudomonas syringae*. The cells of *P. syringae* are INA due to the production of highly specialized INA proteins, which serve as a template for ice formation. We investigated a model INA strain *P. syringae* R10.79 (i)for the effect of aerosolization on cell viability, (ii)the impact of cold induction and growth phase on *ina* gene expression, and (iii)the distribution of INA proteins on surfaces of single cells. We designed a bioaerosol experimental set-up and found that 34-46% of cells remained viable after aerosolization. By using immunofluorescence assay and flow cytometry, we quantified the proportion of cells expressing *ina* genes. The highest density of INA cells were found in the mid-late exponential phase. Cold induction was not observed to have an enhancing effect on the IN activity. Finally, we performed confocal microscopy on the sorted INA cells and observed that the INA protein was distributed over the entire cell surface. Understanding the effects of aerosolization on bacterial survival and *ina* gene expression will ultimately provide insights into the role of bioaerosols in Earth`s hydrological cycle.

New microbes and diagnostics

[P46] CUTANEOUS PROPIONIBACTERIA, THEIR PHYLOTYPES AND THEIR ASSOCIATION WITH HEALTH AND DISEASE

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Cutaneous propionibacteria such as *Propionibacterium acnes* dominate the skin microbiota of the face and upper part of the human body. *P. acnes* has several conflicting properties, some of which are mutualistic and others are potentially harmful for the host. Analysis of the population structure of *P. acnes* highlighted its multiphyletic composition. Together with comparative genomics data, which revealed phylotype differences within the species, the hypothesis arose that certain lineages of *P. acnes* are health-beneficial while others are drivers of disease.
We investigated the *P. acnes* phylotype distribution in healthy individuals and in patients suffering from skin disorders, postoperative infections or prostate cancer, respectively, using an unbiased, culture-independent next-generation-sequencing approach employing a single-locus-sequence-typing scheme.
Substantial differences were uncovered: The *P. acnes* population of disease-affected body sites showed a largely diminished complexity as compared to the composition of skin-located populations in healthy individuals. A few subtypes of *P. acnes* were found to be associated with disease, suggesting that a dysbiosis of the *P. acnes* population might play a role in disease formation. Analyses of phylotype properties and differences are ongoing; such analyses could shed light on the pathogenic potential and the evolutionary history of cutaneous propionibacteria and their host-interacting strategies.

[P47] DETECTION OF HIDDEN LOW-GRADE INFECTION BY CULTURE AND 16S AMPLICON SEQUENCING

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Orthopedic implants play an important role in maintaining health and mobility. However, these implants sometimes fail owning to a variety of mechanisms including bacterial

infection and aseptic loosening. Often, failure cannot be clearly diagnosed because of the inability to isolate the causative organisms which often exist as biofilms on the surface of the implant. Molecular methods have several advantages for detecting biofilm organisms compared to culture.
The aim of this study was to detect infections from orthopedic implants using 16S amplicon sequencing (NGS) and prolonged culture. We analyzed both biopsies and sonicates of the explanted devices.
We included 70 prosthetic joint patients who were assumed to have aseptic loosening based on negative joint fluid culture prior to revision surgery. Five patients were found to be infected: One solely by culture (*Staphylococcus aureus*), one solely by NGS (Staphylococcus sp.) and three with concordant data by both methods.
Additionally we included a patient with pedicle screw loosening after spine surgery. Clinically there were no signs of local or general infection. Routine microbial culturing was negative. However, long-term cultures detected *Propionibacterium acnes*. Molecular analysis revealed presence of *Corynebacterium* species.
The data emphasize the importance of thorough microbiological analysis and illustrates the value of both culture and NGS to detect hidden low-grade infection.

[P48] IDENTIFICATION OF A NOVEL SUB-LINEAGE OF COMMUNITY-ACQUIRED METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS BELONGING TO THE CC80 COMPLEX

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Community-acquired (CA)-MRSA) has during the last two decades emerged globally, with specific genetic lineages predominating in distinct geographical regions. The CA-MRSA isolates belonging to clonal complex 80 (CC80) is known as the European CA-MRSA clone. We recently reconstructed the evolutionary history of this lineage, which revealed a Sub-Saharan origin with following expansion to Northern African, the Middle East and Europe. The prevailing CC80 MRSA clone expresses Panton-Valentin leukocidin (PVL) encoded by *lukS*/*F*-PV integrated in prophage ΦSa2 and contains a type IVc staphylococcal cassette chromosome *mec* (SCC*mec*), which confers resistance to broad-spectrum beta-lactams. Also, strains are very often resistant to tetracycline, fusidic acid, and kanamycin/amikacin. In the last few years, an significant increase in the appearance of PVL-negative CC80 CA-MRSA strains have been observed in Denmark, which encouraged us to conduct a second, and more extensive study with an emphasis on these novel variants. This analysis included 217 CC80 isolates (23 MSSA and 194 MRSA), and revealed the existence of three distinct clades

in the CC80 complex: i) a basal MSSA clade encompassing Sub-Saharan African isolates, ii) a derived clade encompassing MRSA-SCC*mec*-IVc isolates, and iii) a novel identified clade encompassing MRSA-SCC*mec*-IVa isolates. In addition to carrying another SCC*mec* subtype, the isolates clustering in the novel clade distinguishes from the predominating European CA-MSRA by being PVL-negative and susceptible to fusidic acid and kanamycin/amikacin. This study show that the CC80 CA-MRSA lineage is more diverse than previously assumed and provides new insight into the general emergence and spread of CA-MRSA.

[P49] BACTERIAL VIRUSES ENABLE THEIR HOST TO ACQUIRE ANTIBIOTIC RESISTANCE GENES FROM NEIGHBOURING CELLS

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Prophages are quiescent viruses located in the chromosomes of bacteria. In the human pathogen, *Staphylococcus aureus,* prophages are omnipresent and believed to be responsible for the spread of some antibiotic resistance genes. Here, we demonstrate that release of phages from a subpopulation of *S. aureus* cells enables the intact, prophage-containing population to acquire beneficial genes from competing, phage-susceptible strains present in the same environment. Phage infection kills competitor cells, and bits of their DNA are occasionally captured in viral transducing particles. Return of such particles to the prophage-containing population can drive the transfer of genes encoding potentially useful traits such as antibiotic resistance. This process, which can be viewed as 'auto-transduction', allows *S. aureus* to efficiently acquire antibiotic resistance both *in vitro* and in an *in vivo* virulence model (wax moth larvae) and enables it to proliferate under strong antibiotic selection pressure. Our results may help to explain the rapid exchange of antibiotic resistance genes observed in *S. aureus*.

[P50] GENOMIC CHARACTERIZATION OF AVIAN PATHOGENIC ESCHERICHIA COLI ISOLATES FROM NORDIC BROILER PRODUCTION REVEALS A MAJOR ST117 O78:H4 LINEAGE

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Background:
Colibacillosis is caused by avian pathogenic *Escherichia coli* (APEC) which decreases the animal welfare and poultry production worldwide. During 2014 to 2016, a remarkable increase in colibacillosis cases on Nordic poultry farms was reported. The majority of poultry farmers in the Nordic countries import Swedish broiler breeders that are part of a breeding pyramid. In the present study, we investigated the genetic diversity among *E. coli* isolates primarily collected from chickens with colibacillosis, using whole genome sequence analyses

Methods:
Hundred and fourteen bacterial isolates were primarily collected from broilers and broiler breeders with colibacillosis in the Nordic countries and selected for whole genome sequencing. Subsequently, identification of virulence and resistance genes, *in silico* typing and phylogenetic single nucleotide polymorphism analysis was performed.

Results:
The phylogenetic analyses showed a major clade of 47 closely related ST117 O78:H4 APEC isolates. The isolates in this clade were collected from both broiler chickens and breeders with colibacillosis in different Nordic countries.

Conclusions:
This study revealed a ST117 O78:H4 lineage of APEC isolates collected from diseased broilers and breeders on poultry farms in multiple Nordic countries. The data indicate that the ST117 O78:H4 clone was transferred vertically through the broiler breeding pyramid into distantly located poultry farms across the Nordic countries.

[P51] OPTICAL HYDROGEN PEROXIDE SENSORS FOR STUDYING IMMUNE RESPONSES AND BACTERIAL INFECTIONS

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H_2O_2 is involved in vital and deadly processes in cells and is a key compound in the immune response to bacterial and fungal infection; this makes it an important analyte for clinical diagnostics. Due to its reactivity, quantitative measurements in biological samples are very challenging and most optical measurement principles are based on irreversible reactions, which do not enable continuous sensing of H_2O_2 dynamics. In the present PhD project, we aim to alleviate these methodological limitations by developing novel fiber-optic H_2O_2 sensors.

A quasi-reversible optical sensing scheme for H_2O_2 is based on the Prussian Blue (PB) / Prussian White (PW) redox pair[1]. PW is oxidized to PB by H_2O_2, which causes a color-change. By immobilizing the PB pigment on the tip of an optical fiber the concentration-dependent color change can be monitored. The system is coupled to a luminescent crystal which results in a coupled change of the read-out fluorescence intensity and the amount of PB; and therefore H_2O_2. The sensor tip can be recharged by moving it into agar containing ascorbic acid, which reduces PB back to PW. By replacing this crystal with a particle containing different luminophores, higher signal intensities could be achieved and it was possible to apply the sensor system in blood and in simulated conditions, monitoring the response of bacteria to antibiotics in culture conditions.

References:

1- K. Koren, P. Ø. Jensen and M. Kühl, *Analyst.* 2016, **141**, 4332-4339.

[P52] GENOMIC EPIDEMIOLOGY OF THE DANISH CLUSTER 2 MYCOBACTERIUM TUBERCULOSIS OUTBREAK: A RETROSPECTIVE STUDY OF A DOMINATING TB LINEAGE FROM 1992 TO 2014

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Despite its status as a tuberculosis low-burden country, Denmark has experienced sustained, active transmission of the disease. The Danish *Mycobacterium tuberculosis (Mtb)* strain collection at the International Reference Laboratory of Mycobacteriology includes specific outbreak strains spreading in the population. The most prominent example is the so-called "C2/1112-15" cluster, which is a collection of isolates displaying identical genotypes based on their IS6110 RFLP/MIRU-VNTR patterns. The cluster was first identified in the beginning of the 1990'ies in only a few patients. Since then, it has caused disease in more than 1000 individuals, making it the predominant lineage in Scandinavia. In 2001, the cluster was found in Greenland.

In this preliminary study, we have conducted WGS on 114 representative isolates from the C2/1112-15 cluster. These isolates were mainly collected in the Greater Copenhagen area and span the years 1992-2014. Using phylogenetic analysis, we found that all isolates are confined to the same *Mtb* sub-lineage, commonly known as lineage 4.8. By comparing the 114 genomes to publically available genomes belonging to the same lineage, we observe that C2/1112-15 constitutes a monophyletic clade clearly distinct from other outbreaks publicly available. We observe a major and a minor lineage within C2/1112-15 with a most common recent ancestor dating back to 1959. Using molecular clock analysis, we calculated an overall mutation rate of the cluster to be 0.24 SNPs/genome/year. Using a median-joining network approach we also determined the existence of seven discrete transmission chains within the major lineage that all originate from a clonal group of isolates, the earliest of which was collected in 1993.

[P53] RICKETTSIA INFECTION IN DENMARK

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Background

Rickettsia spp. are small, gram-negative, obligate intracellular organisms that can cause a wide range of human disease, from the mild and self-limiting African tick bite fever to more severe manifestations such as Rocky mountain spotted fever and Mediterranean spotted fever. Another species of rickettsia, *Rickettsia helvetica*, has been found in 4.7% of Danish forest ticks, making it one of the most common tick-borne pathogens found in Denmark. Despite this, its clinical significance is still relatively unknown and the association between *Rickettsia helvetica* and disease in humans has not been fully elucidated. The prevalence of rickettsia infections in Denmark has not been established.

Patients and methods

The National Patient Register (LPR) was searched for patients diagnosed with rickettsiosis (laboratory confirmed and clinical diagnosis) in the years 2010-2015 at Rigshospitalet, Hvidovre Hospital and Odense University Hospital. The medical records of these patients were subsequently reviewed for relevant exposure, clinical manifestations and biochemical results at the time of diagnosis. The aim of the study is to study the prevalence of imported and endemic rickettsiosis in Denmark. Furthermore, it will provide an overview of the diagnosis, clinical manifestations, recent travel history and biochemical results of patients diagnosed with rickettsiosis in Denmark in the years 2010-2015.

[P54] WIDESPREAD PRESENCE OF MRSA CC398 IN THE DANISH PRODUCTION OF FARMED MINK (*NEOVISON VISON*)

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Animal reservoirs of MRSA have been raised as an issue regarding human health. MRSA CC398 is widespread in the Danish pig production with levels increasing to around 70 % (2014) since 2008, illustrating MRSA CC398 as a successful clone. Outside Denmark, MRSA CC398 has spread to other livestock animals (e.g. calves and horses). The first cases of

infections in Danish mink farm workers were reported in 2009 and a cross sectional study of MRSA in farmed mink was initiated in 2014.

Three different sample categories were investigated **1)** feed samples, **2)** paws and pharyngeal swabs from healthy animals collected at pelting and **3)** samples from five different body sites of mink carcasses submitted to the National Veterinary Institute for clinical reasons not related to MRSA.

MRSA was found in both healthy and diseased mink with 40 % and 34 % positive samples, respectively. In clinical submissions, paws and pharynx samples were most frequently found positive, 32 % and 17 %, respectively. Nasal and intestinal samples were rarely positive for MRSA (8% and 8%, respectively) and no perianal swabs were found positive. In healthy mink, paws and pharynx were found positive in 28 % and 16 %, respectively. Feed samples were found positive in 13 % of samples collected from feed factories.

The *spa*-type was primarily t034 followed by t011 (CC398) equivalent to the dominant *spa*-types found in the Danish pig production. The predominant presence of MRSA on paws and in pharynx, combined with these livestock-associated MRSA related *spa*-types supports feed, containing fresh slaughter offal from the pig production, as the likely source of introduction.

This study clearly indicates that MRSA CC398 is widespread in farmed mink and that it most likely is introduced by contaminated food.

Hygiene of surfaces

[P55] WHERE DOES SALMONELLA HIDE AFTER GRINDING OF MEAT?

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Salmonella transfer, from one piece of meat onto a meat grinder and back from the grinder to the next piece of meat being ground, has previously been examined. After grinding of five *Salmonella* contaminated pieces of meat the transfer to a sequential number of 40 clean meat pieces have been examined. The transfer of *Salmonella* decreased rapidly during grinding of the first pieces after which the decrease became markedly slower resulting in a so-called tail. This was explained by assuming a bimodular transfer from two distinct environmental loci inside the meat grinder. In one locus, *Salmonella* is hypothesized to be loosely attached supporting a fast transfer. In another locus, *Salmonella* is hypothesized to be tightly attached making the transfer slow. The objective of the present study was to verify this hypothesis. This was done by identifying loci inside the meat grinder where *Salmonella* Typhimurium DT 104 was either loosely or tightly attached. Using the previous study design 17 loci, from various sections of the grinder, were swabbed with rayon tipped swabs. The number of *Salmonella* attached to these loci was measured. Results indicated that the 17 loci separated into two distinct groups, with different transfer from infected meat to meat grinder and different transfer rates from the meat grinder back to the clean meat. The difference seemed to be caused by the design of the meat grinder. By culturing it was not possible to account for all *Salmonella* added to the meat and, subsequently, it was investigated whether inactivation of *S.* Typhimurium DT 104 occurred in specific loci during grinding. Swabbing methods applying a physical rinse with a mild detergent were used to detach live and dead bacteria.

[P56] STAPHYLOCOCCUS SPECIES IN SEDIMENTED AND AIRBORNE DUST IN DANISH HOMES

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Staphylococcus aureus is an opportunistic human pathogen which can be antibiotic resistant (methicillin-resistant *S. aureus* called MRSA). Other *Staphylococcus* species can also be opportunistic pathogens and may play a role in horizontal transfer of genes for antimicrobial resistance. This study aimed to identify and quantify *Staphylococcus* species in sedimented

and airborne dust from living rooms in Danish homes. Dust samples collected from 69 homes were analysed. MRSA was found in one home. In total, 17 different staphylococci species were found. Dominating *Staphylococcus* species in terms of concentrations and in terms of number of homes with the species were *S. capitis, S. hominis, S. epidermidis, S. saprophyticus* and *S. warneri*. *Staphylococcus* was the second most common identified genus after *Micrococcus*. In conclusion, *Staphylococcus* species constituted a considerable proportion of the dust and airborne bacteria in Danish homes, however, *S. aureus* and MRSA only had low incidences.

[P57] SURVIVAL OF STAPHYLOCOCCUS AUREUS IN STABLE DUST

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Methicillin-resistant *Staphylococcus aureus* (MRSA) are multiresistant bacteria, which can cause serious infections. The clone MRSA CC398 has adapted to livestock, and is widespread in Danish pig farms. Transmission of MRSA CC398 has been shown to occur from pigs to humans and even between humans. People working in pig stables are exposed to MRSA CC398 directly by contact with the pigs, but also indirectly via the environment. Stable dust has been shown to contain MRSA CC398, and hence dust can be a vector for transmission both through aerosolized particles and through particles settled on surfaces.
The purpose of this study was to determine the rate of decay for *Staphylococcus aureus* and MRSA in dust. Electrostatic dust fall collectors (EDCs) were used for passive sampling of settling airborne dust in 11 barn sections from 6 different pig farms. Extraction and enumeration of cultivable *S. aureus* and MRSA from the EDCs were performed for a period of 0-30 days post sampling.
A total of 138 quantitative measurements of *S. aureus* survival in dust from all farms were used to estimate the exponential decay constant λ according to a model for exponential decay; $N(t) = N_0 \times e^{-\lambda t}$. The data fitted well to the model ($\lambda = 0.13$, $R^2 = 0.84$) despite a large difference in initial concentrations of *S. aureus* between farms (N_0 differed by more than 2-log). Time significantly reduced loads of *S. aureus* in the dust, and the mean half-life for the samples was 5 days.

Other

[P58] EKSTRA CHROMOSOMAL CIRCULAR DNAS ARE MAINTAINED IN YEAST POPULATIONS OVER SEVERAL GENERATIONS - PROVIDING BASE FOR GENETIC VARIATION

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Copy-number variations (CNVs) create much of the genetic variation underlying evolution. Yet, detecting chromosomal CNVs before they reach establishment in large cell populations is a major challenge. By screening for a potential precursor of amplifications, the so-called extrachromosomal circular DNA (eccDNA), we reasoned that we might elucidate some of the early ongoing processes in genomic rearrangements and CNVs. To explore the existence of circular DNA in eukaryotes we have developed a highly-sensitive eccDNA purification method, Circle-Seq, that detects as few as one eccDNA in 2500 *Saccharomyces cerevisiae* genomes. We have already revealed that eccDNAs are common in yeast cells. More than a thousand different eccDNAs larger than 1 kb were recorded in the S288c strain background, covering 23 % of the genome (Møller *et al*., 2015). To investigate if eccDNAs are maintained in growing populations of yeast cells, we measured eccDNA species in populations of young cells and the same populations that had divided for < 20 generations by using the Mother Enrichment Program (Lindstrom and Gottschling, 2009) in combination with the Circle-Seq method. We found that populations of old cells contained between 53.3 % and 69.7 % of eccDNAs present in the young populations, suggesting that these eccDNAs were maintained in the populations as they age. The sizes were in average 11485 bp, they contained several essential genes + the core consensus sequence of replication origins and one of the eccDNAs furthermore contained a centromere. The fact that these eccDNAs are kept in the population over several generations enhance the chance that they can reintegrate into the chromosomes and possibly cause rearrangement in the genome.

[P59] BACTERIOPHAGES FOR THE WINE INDUSTRY. SPEAK, FRIEND, AND ENTER!

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Vitis sp. diseases are a constant threat to wine production and quality. At the same time consumers' demand for wine of enhanced organolepticattractiveness that should result from sustainablepractices during all stages of wine making is increasing the challenges for the wine industry. Thus, the development of efficient biological treatmentsis more than

urgent and relevant, so as to control notorious grapevine pathogens and pests. In light of these, the aim of our ongoing study is to elucidate the role of bacteriophages in promoting or impeding bacteria important for the grapevine health and wine production. For this reason, special focus is given on finding bacteriophages against the fastidious, xylem-limited bacterium *Xylella fastidiosa* that causes Pierce's disease of grapevine. Furthermore, theexistence ofpotential bacteriophage foes of *Lactobacillus plantarum* -a bacterium participating in the malolactic fermentation of wine-is investigated. The aforementioned tests are being conducted using a wide range of samples. In this poster, our first results are presented.

[P60] ASSOCIATION BETWEEN EXPOSURE TO AIRBORNE NOROVIRUSES AND GASTROENTERITIS AMONG WASTEWATER WORKERS

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An increased incidence of acute gastroenteritis (AGE) has been reported among workers at wastewater treatment plants (WWTPs). The cause is unknown but the symptoms are consistent with AGE caused by norovirus (NoV) infection. The objective of present study was to investigate if exposure to airborne NoVs is the cause of the increased incidence of AGE reported among WWTP workers.
Personal exposure to airborne enteric viruses was examined monthly among 14 WWTP workers during a one-year study period. Air sampling was performed throughout a working day using personal air samplers mounted in the inhalation zone of the workers. Gastrointestinal symptoms were reported by workers and their stool samples screened for NoV Genogroup (G)I and GII, sapovirus, adenovirus 40/41, astrovirus, rotavirus, enteric bacterial pathogens and protozoa.
NoV genomes were detected in 47% of all personal air samples (n=106), albeit mostly in low concentrations. A higher percentage of air samples were found positive for NoV in winter/spring than in summer/fall. Asymptomatic infection with NoV GI was observed in one WWTP worker in January and February. NoV GI and GII were detected in the personal air sample from this worker in February, but not in January. In addition, *G. intestinalis* was

detected in the stool of this worker in August. No other enteric pathogens were detected in the stools from the WWTP workers.
Although the majority of WWTP workers were exposed to airborne NoV on several occasions, exposure seldom correlated with gastrointestinal symptoms and infections. However, as asymptomatic infection with NoV was found in one worker following exposure to airborne NoV, a possible association between occupational exposure to airborne NoV and AGE might exist.

[P61] EVALUATION OF METHODS FOR THE CONCENTRATION AND EXTRACTION OF VIRUSES FROM SEWAGE WATER IN THE CONTEXT OF METAGENOMIC SEQUENCING

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Viral sewage metagenomics is a novel field of study. In raw sewage human waste is mixed with household, industrial and drainage water, and virus particles are therefore found in low concentrations. This necessitates a step of sample concentration to allow for virus detection. Additionally, viruses harbor a large diversity of both surface and genome structures, which makes universal viral genomic extraction difficult. Current studies have tackled these challenges in different ways employing a wide range of viral concentration and extraction procedures. However, there is limited knowledge of the efficacy and biases associated with these methods in respect to viral sewage metagenomics, hampering the development of this field.
By the use of next generation sequencing this study aimed to evaluate the efficiency of four commonly applied viral concentrations techniques (precipitation with polyethylene glycol, organic flocculation with skim milk, monolithic adsorption filtration and glass wool filtration) and extraction methods (Nucleospin RNA XS, QIAamp Viral RNA Mini Kit, NucliSENS® miniMAG®, or PowerViral® Environmental RNA/DNA Isolation Kit) to determine the viriome in a sewage sample. We found a significant influence of concentration and extraction protocols on viral richness, viral specificity, viral pathogen detection, and viral community composition, advising against comparing results or conducting meta-studies before carefully checking the methodology employed.

[P62] EFFECTS OF PROBIOTIC BACTERIA AGAINST FISH PATHOGENS IN NON-AXENIC ALGAE AND COPEPOD SYSTEMS

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Aquaculture provides half of all seafood produced worldwide, and is the main source of protein for approx. three billion people. Bacterial diseases are a major bottleneck in the rearing of some finfish, especially at the larval stages. Antibiotics are still used and sustainable alternatives are sought after. We have shown that tropodithietic acid (TDA) producing bacteria of the *Roseobacter* clade have probiotic properties and are able to protect live feed such as rotifers and *Artemia* as well as turbot and cod larvae against pathogenic vibrios. New methods in the breeding of copepods, which is thought to be the "natural" live feed, have made them relevant in aquaculture. Thus, the purpose of this study was to investigate if the TDA-producing roseobacters could inhibit *Vibrio anguillarum* in non-axenic algae and copepod systems. Preliminary data show that GFP-tagged *V. anguillarum* colonized the outer surface and gut of the copepods indicating that they could act as potential vectors for the pathogens. The *Roseobacter*-clade bacterium, *Phaeobacter inhibens* inhibited the growth of *V. anguillarum* in the non-axenic *Rhodomonas salina* cultures used as copepod feed. However, in a first series of experiments, the pathogens did, unexpectedly, not grow in the copepod cultures as it does in other live feed cultures. On-going experiments are addressing this issue and the potential of *P. inhibens* as an inhibitor of *V. anguillarum* in copepod cultures.

[P63] THE INS AND OUTS OF SULFATE TRANSPORT IN SULFATE REDUCING PROKARYOTES

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Dissimilatory sulfate reduction is a key process in the remineralization of organic matter in most anoxic environments. It is catalyzed by sulfate reducing prokaryotes (SRPs), which encompass a diverse group of organisms spanning several phylogenetic lineages. SRPs use sulfate as terminal electron acceptor for the oxidation of organic electron donors and hydrogen. The first step in the sulfate reduction pathway is the transport of sulfate across the cell membrane. This uptake has a major effect on sulfate reduction rates as it controls the rate of sulfate accumulation in the cell.
Much of the information on sulfate transport was obtained by studies on assimilatory sulfate reduction. Despite our growing knowledge on the physiology of SRPs there are no studies identifying the proteins involved in the transport of sulfate in SRPs. In order to identify

sulfate transporters in SRP we used *in silico* analysis to compare the complete genomes of 44 SRPs and map the taxonomic distribution and genetic neighborhood of genes encoding putative sulfate transporters.
We identified members of five major families of putative sulfate transporters across the analyzed SRP genomes. None of these families were however consistently present among the different phylogenetic groups and none showed a consistent genomic co-localization with other genes involved in sulfate reduction. Our study offers a comprehensive overview of sulfate transport genes in SRPs and provides a roadmap for molecular approaches to improve our understanding of the mechanistics of sulfate transport.

[P64] IN SILICO AND IN VITRO CHARACTERIZATION OF GLYCOSYLTRANSFERASES OF PSEUDOMONAS AERUGINOSA PAO1

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Introduction:
Pseudomonas aeruginosa is an opportunistic pathogen that possesses a huge arsenal of virulence factors reflected in it genome size featuring approximately 5500 genes. A well-characterized virulence-factor of *P. aeruginosa* is lipopolysaccharide (LPS) playing the dual role as a protective barrier and a potent endotoxin. The LPS biosynthesis is rather complex involving many glycosyltransferases (GT) sequentially transferring nucleotide activated sugars onto glycosyl acceptors. Even though it was previously regarded exclusive to eukaryotes research have revealed that GTs moonlight as players in post-translational modification (PTM).

Methods:
As the roles of GTs in PTM are rather unexplored the GT machinery of *P. aeruginosa* PAO1 was investigated by bioinformatics analysis and phenotypic characterization. 34 knock-out GT mutants were obtained from the PAO1 two-allele transposon mutant library and phenotypic assays were performed.

Results:
The combined bioinformatics and phenotypic screen revealed a particular region of the PAO1 genome containing a high density of hypothetical uncharacterized GT genes. It became clear that this region is either found in its entirety or absent when investigating different *P. aeruginosa* genomes. It appears to be involved in exopolysaccharide production and the GC

content of this region is markedly lower compared to the rest of the PAO1 genome. Results of knock-out mutants will be discussed.

Conclusions:
A broad phenotypic screen and bioinformatics analysis of the GT machinery of *P. aeruginosa* PAO1 revealed an uncharacterized GT-rich region which might be the result of a horizontal gene transfer event. The functions of this region should be investigated further.

[P65] FUNGAL SPECIES PRESENT ON AND AEROSOLOSED FROM WET AND DRY GYPSUM BOARDS

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Elevated exposures to fungi in buildings are associated with headache, fatigue, concentration difficulties, asthma and allergies. It is of importance to investigate the effect of fungal growth within the building envelope on indoor air.
Over a period of 6 weeks, fungi were grown on pieces of sterilized gypsum boards. The fungi used to inoculate the boards were collected from the indoor air in a moisture damaged building, and therefore represent a real life sample of a fungal community in moisture damaged buildings. Half of the gypsum boards were allowed to dry after growth was detected, simulating fault alleviation in a moisture damaged building. Samples of scrapings from the surface of both wet and dry gypsum boards were analysed by use of MALDI-TOF-MS. Likewise filter samples of aerosolised spores from both wet and dry boards were analysed in order to get an indication of fungal species growing on the surface vs. species aerosolised to the indoor air.
Species identified from the samples of the surface scrapings were notably different from species found in aerosol samples. In general, there is a larger diversity of species in the scrapings than in the aerosols from both the wet and dry boards. This indicates that a sample of a mould infected surface in a building is not a sufficient measure for what might be found in the indoor air and vice versa.

[P66] ABSTRACT WITHDRAWN

[P67] NEW TYPE OF *STREPTOCOCCUS THERMOPHILUS* BACTERIOPHAGES - PROBLEMATIC EVOLUTION IN DAIRY PLANT

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*B*acteriophages are the main cause of fermentation failures in dairy plants. *Streptococcus thermophilus* belongs to thermophilic starter cultures commonly used for cheese and yogurt production. Therefore, investigating streptococcal phages is necessary for preventing and controlling phage attacks during dairy fermentations.

Screening of a large number of *S. thermophilus* phages from the Chr. Hansen A/S collection revealed two phages differing from typical representatives of the group. Morphology analysis indicated that phages CHPC577 and CHPC926 held shorter tails and unusual baseplates, when compared to the traditional *S. thermophilus* phages. DNA sequencing showed their close homology to a subgroup of *Lactococcus lactis* phages P335. By testing adsorption of the homologous streptococcal and lactococcal phages to the surface of various S. *thermophilus* and *L. lactis* strains, we revealed the possibility of cross-reactivity with another species.

Our data indicate that the expanding use of thermophilic *S. thermophilus* together with mesophilic *L. lactis* has triggered the recombination between phages infecting different bacterial species, leading to new challenges for combating phage attacks in dairy plants.

[P68] IMPACT OF TROPODITHIETIC ACID-PRODUCING PHAEOBACTER INHIBENS ON EUKARYOTE-ASSOCIATED MICROBIAL COMMUNITIES

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The *Roseobacter* clade is a widely distributed group of marine bacteria exhibiting a versatility of metabolic adaptations and trophic strategies. Some genera are often found in association

with eukaryotes, where bacterial secondary metabolites (e.g. antibiotics) may serve as host protection. Members of the *Phaeobacter* genus can be associated with mollusks, and may also engage in dynamic symbiosis with the microalga *Emiliana huxleyi*. The antibiotic tropodithietic acid (TDA) has been proposed to be involved in the latter interaction and the aim of this study is to investigate how TDA-producing *P. inhibens* affect the microbiota associated with European flat oysters and *E. huxleyi. P. inhibens* was introduced to oysters and *E. huxleyi* and the effect on microbial community composition was assessed using V4 amplicon sequencing. Members of the *Phaeobacter* genus were indigenous to the existing oyster microbiota. Addition of the TDA-producing *P. inhibens* caused significant changes in the microbial community of the oysters allowing certain OTUs (e.g. putative roseobacters and Alteromonadales) to increase in relative abundance, while others (e.g. *Mycoplasma* and *Sulforospirillum*) decreased compared to oysters with no exogenous *P. inhibens*. Data on *E. huxleyi* are currently under way, yet based on changes in the oyster microbiome, it seems that *P. inhibens* is capable of modulating eukaryote associated microbial communities and potentially the overall functionality of the microbiome.

[P69] BACTERIAL COMMUNITIES HITCHING A HIKE - A GUIDE TO THE RIVER SYSTEM OF THE RED RIVER, DISKO ISLAND, WEST GREENLAND

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Glacier melting and altered precipitation patterns influence Arctic freshwater and coastal ecosystems. Arctic rivers are central to Arctic water ecosystems linking glacier meltwaters and precipitation with the ocean through transport of particulate matter and microorganisms. However, the impact of different water sources on the microbial communities in Arctic rivers and estuaries remains unknown. In this study we used 16S rRNA gene amplicon sequencing to assess a small river and its estuary on Disko Island, West Greenland (69°N). We describe the bacterial community through a river into the estuary, including communities originating in a glacier and a proglacial lake. Our results show that water from the glacier and lake transports distinct communities into the river in terms of diversity and community composition. Bacteria of terrestrial origin were among the dominating OTUs in the main river, while the glacier and lake supplied the river with water containing fewer terrestrial organisms and more psychrophilic taxa were found in the dominant community supplied by the lake. At the river mouth, the dominant bacterial communities from the lake and glacier were unnoticeable but became evident again further

into the estuary. This showed, that the correct resolution of samples along a network is crucial for understanding the origin and transport of microbial communities. On average 23% of the estuary community consisted of indicator OTUs from the river. Environmental variables showed only weak correlations with community composition.

[P70] MICROBIAL GRANULATION MANAGEMENT: SIMPLE CHANGES IN REACTOR OPERATION ENABLE CONTROL OF GRANULAR PROPERTIES AND THE ENGINEERING OF MICROBIAL COMMUNITIES IN WASTEWATER APPLICATIONS

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The use of microbial granules in wastewater applications is becoming increasingly popular due to their favorable properties like high settling velocity or their resilience to mechanical and environmental stress. A simple change in operational strategies of lab scale reactors was hypothesized to enable the operators to control the size of the bio-granules. The size of granules determines the fractionation of different redox zones in the biofilm and therefore affects the niche differentiation of the microbial community. With the change of the microbial community various performance parameters like the ammonium or nitrous oxide removal rate vary and are therefore a function of the granule size. Mathematical modelling was applied to support the hypothesis. Granules of two distinct sizes were grown and compared in settling velocity, substrate turnover rates and microbial community composition. The investigation shows that a simple change in process operation is feasible for managing bio-granulation and microbial communities.

[P71] CO-CULTIVATION IN A STRUCTURED ENVIRONMENT FACILITATES INTERSPECIFIC MUTUALISM

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Bacteria interact across species boundaries, resulting in highly complex communities. The knowledge of the underlying molecular and evolutionary mechanisms of interspecies interactions is currently limited and so is their response to long-term co-existence. The data presented here demonstrates co-adaptation of two species in co-culture, resulting in mutually enhanced productivity.
We used two co-isolated soil bacteria, *Xanthomonas retroflexus* and *Paenibacillus amylolyticus*, to study co-evolution. Cultures were grown as mono- and co-cultures over

time with regular cells transfers into new media. We observed the establishment of a wrinkly *X. retroflexus* variant after 8 days of growth, which increased in frequency throughout the experiment when co-cultured with *P. amylolyticus*. Co-cultures of this wrinkly variant together with *P. amylolyticus* were more productive compared to monocultures, assessed by individual cell numbers. This indicates that mutual benefits are gained by both species in the evolved community. Biofilm biomass production was also significantly increased and changed when the wrinkly variant and *P. amylolyticus* were co-cultured.
Genome sequencing of the wrinkly *X. retroflexus* variants revealed consistent mutations in genes that encode sensor proteins involved in the production of second messenger c-di-GMP. We hypothesize that such mutations may lead to variants better suited for the sessile biofilm lifestyle with other cells in close proximity.
The data presented here shows that interspecific interactions are affected by long-term co-cultivation to, in this case, stabilizing a mutualistic association.

[P72] DIRECT IDENTIFICATION OF FUNCTIONAL AMYLOIDS BY LABEL-FREE QUANTITATIVE LC-MS

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Amyloids are highly ordered fibrillar protein polymers used by organisms from all domains of life due to their exceptional properties. We have previously shown that amyloids are widespread among microorganisms in biofilms from various habitats. They are therefore believed to play important roles in biofilm ecology. Despite their common appearance in biofilms, few amyloids have been characterized from biofilm-associated bacteria. However, the few amyloids that have been studied so far have already provided an astonishing demonstration of how the amyloids can be exploited with roles ranging structural components of biofilms, cell envelopes and spore coats to cytotoxins and as reservoirs for quorum-sensing signaling molecules.
The identification of novel functional amyloids is key to understand the many roles of amyloid in biofilms. Isolation of amyloids is unfortunately not a straightforward task. The insolubility and extreme stability of most functional amyloids exclude them from traditional protein analyses. Many functional amyloids are also highly adhesive and therefore bind to pipette tips and other consumables. Pure cultures, large sample volumes and high productivity of amyloids are therefore required for successful purification. We here present a quantitative proteomics technique that allow direct identification of functional amyloid candidates in complex samples based on their structural stability in the presence of increasing concentrations of formic acid.

[P73] MICROSCALE DYNAMICS OF H2, CO2 AND PH DURING H2 SUPPLY TO BIOGAS REACTORS

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Hydrogen produced from periodic excess of electrical energy may be added to biogas reactors where it is converted to methane that can be used in the existing energy grid. It has, however, been described that the process may become CO_2 limited resulting in reactor failure. By use of microsensors we investigated the microscale distribution of the major relevant parameters (H_2, CO_2 and pH) when H_2 was supplied to a ~4-mm layer of biogas reactor content through a silicone membrane.

When a mixture of 75% H_2 and 25% N_2 was supplied, H_2 was readily consumed at a rate of 0.33 nmol cm^{-2} s^{-1} in a 400 μm layer next to the membrane. However, CO_2 was depleted within 10 h, decreasing the H_2 consumption rate to 0.22 nmol cm^{-2} s^{-1}, expanding the H_2 consumption zone to 1700 μm, and resulting in a pH increase from 8.5 to 9.5. After CO_2 depletion, CO_2 was gradually supplied and the highest rate observed at 7% CO_2 with a consumption rate of 0.20 nmol cm^{-2} s^{-1} in a zone of 1500 μm. Hence, partially recovering the hydrogen consumption rate after the CO_2 depletion.

The supply of hydrogen through a silicon membrane resulted in effective and rapid conversion of hydrogen by the community present in the biogas reactor content. Since CO_2 quickly became limiting, increasing the pH, the hydrogen addition should be limited to an amount/rate that does not cause excessive elevations in pH due to CO_2 depletion.

[P74] CONSORTIA BASED PRODUCTION OF BIOCHEMICALS

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One of the great challenges facing society is how to sustainably produce food, chemicals and other commodities required to maintain and develop our current life style. To compete with and ultimately replace existing petrochemical-based manufacturing processes, the development of innovative and effective solutions is needed.

In this project we have explored the possibility of using designed consortiums for the co-valorization of the main carbon sources in lignocellulosic biomass (xylose, glucose, arabinose, and acetic acid). In one study we have used pre-processing simulations, constraint-based modelling, and state-of-the art metabolic engineering tools to develop a consortium of cells capable of efficient valorization of synthetic hemicellulosic hydrolysate. Stable co-existence and effective co-valorization was achieved through niche-differentiation, auxotrophy, and adaptive evolution. In another study stable consortia based "two-in-one-go" fermentation was achieved through niche partitioning, syntrophy (auxotrophy combined with removal of inhibitory side product), and CRISPRi mediated gene silencing. The results achieved demonstrate that consortium based approaches for valorizing complex biomass and waste related carbon sources can be an attractive alternative to the design of a so-called "superbug" and can thereby add significant value to biorefineries.

[P75] O2 AND N2O MICROPROFILING OF PSEUDOMONAS AERUGINOSA IN AN IN VIVO-LIKE BIOFILM MODEL

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The biofilm-forming bacterium *Pseudomonas aeruginosa* (*Pa*) has a highly flexible energy metabolism, enabling it to persist in norm- and anoxic environments. We employed an *in vivo*-like alginate bead biofilm model, in which *Pa* forms small cell aggregates similar to those observed in chronic wounds and lungs of chronically infected cystic fibrosis (CF) patients.

We performed CLSM microscopy as well as O_2 and N_2O microsensor measurements on alginate encapsulated *Pa* grown norm- and anoxic w/wo 10 mM KNO_3 supplement. The O_2 distribution in the beads was also visualized with optical O_2 sensor nano-particles.

CLSM revealed that *Pa* cluster in the periphery of beads. The O_2 measurements showed O_2 depletion in the peripheral 50-100 µm of the beads after 24 hours norm-oxic growth. Furthermore, when KNO_3 was available as alternative electron acceptor, there was a

significant production of N_2O from *Pa* grown for 24 hours under norm- as well as at anoxic conditions, and bacterial growth was observed deeper in the beads.
The alginate bead model enabled us to perform microprofiling of *Pa* grown under different *in vivo*-like conditions. *Pa* has a high O_2 consumption in the periphery of the beads, and is capable of performing denitrification under anoxic conditions in deeper layers of the beads. These measurements are in line with published measurements of O_2 and N_2O in sputum from CF patients with chronic *P*a infections showing strong O_2 depletion and formation of N_2O in deeper anoxic sputum layers.

[P76] SINGLE FILAMENT GENOMICS OF CABLE BACTERIA AND TRANSCRIPTOMICS OF D. ALKALIPHILUS GIVE INSIGHTS INTO THE SULFIDE OXIDATION OF DELTAPROTEOBACTERIA

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Until recently, the ability to grow by chemolithotrophic sulfide oxidation was believed to be restricted to the α, β, γ, and ε classes of the Proteobacteria. The discovery of the sulfide-oxidizing strain MLMS-1 and filamentous Desulfobulbaceae (commonly referred to as cable bacteria), which couple sulfide oxidation with oxygen reduction over centimeter distances, provided the first evidence for this ability in δ-Proteobacteria. The mechanism for sulfide oxidation in δ-Proteobacteria is currently unknown. Strain MLMS-1 and cable bacteria lack typical key genes for sulfide oxidation. Instead, MLMS-1 and cable bacteria feature all genes necessary for sulfate reduction. *Desulfurivibrio alkaliphilus* AHT2, a close relative of cable bacteria and the first member of the δ-Proteobacteria to grow by sulfide oxidation with nitrate, is genomically similar to cable bacteria in that it lacks key sulfide oxidation genes, yet contains the complete sulfate reduction pathway. We therefore used *D. alkaliphilus* as a model organism for δ-proteobacterial sulfide oxidation and analyzed its gene expression via RNA-Seq under sulfide-oxidizing conditions. Surprisingly, all key genes of the sulfate-reduction pathway were highly expressed. These results, combined with our genomic analyses, lead us to propose that sulfide oxidation by δ-proteobacteria, and thereby also cable bacteria, proceeds via a reversal of the sulfate reduction pathway.

[P77] ASSESSING MOTILITY IN ENVIRONMENTAL COMMUNITIES- A NOVEL METHOD

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Very few attempts have been made at assessing motility at the community level. The focus has previously been heavily on pure culture studies, largely neglecting the fact that motility can be influenced by complex multispecies interactions. A crucial factor in dispersal in 'water-unsaturated' habitats such as soil is the thickness of the liquid films surrounding soil particles. The goal was therefore to develop a method that allows, from environmental samples, to:

- Obtain community-level surface motility profiles under controlled hydration conditions
- Describe the diversity of the fastest colonizers
- Easily isolate motile strains

The method is based on the Porous Surface Model (PSM), previously used to quantify the dispersal of fluorescently-tagged bacteria under controlled hydration. In this study, a procedure was developed to allow for its use on complex communities, which also proved effective for isolation of motile bacteria. The procedure was validated using the motile GFP tagged *P. putida* K2440 and the non-motile mutant *P. putida* K2440 dsRed *FliM*$^{-}$. The method used on soil and lake communities revealed clear effects of altered hydration conditions on the speed of colonization. Part of the communities were even able to disperse under low hydration conditions (-3.1kPa), previously proven too dry for *P. putida* motility.

[P78] GREEN-FLUORESCENT PROTEIN LIKE PIGMENTS SCATTER LIGHT AND ENHANCE CORAL HEATING

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Green fluorescent protein (GFP)-like pigments potentially have beneficial effects on coral photobiology. The present study investigated the relationships between green fluorescence, coral heating and tissue optics for the massive coral *Dipsastraea* sp. We used microsensors to measure tissue scalar irradiance and temperature along with imaging of green

fluorescence. Green fluorescence correlated positively with coral heating and scalar irradiance enhancement at the tissue surface. Coral tissue heating saturated for maximal levels of green fluorescence. The action spectrum of coral surface heating revealed that heating was highest under red irradiance while scalar irradiance enhancement in coral tissue was highest when illuminated with blue light. We suggest that GFP-like pigments scatter the incident radiation, which enhances light absorption and heating of the coral. However, heating saturates, because strong light scattering reduces light penetration through the tissue eventually reducing light absorption at high fluorescent pigment density. We conclude that GFP-like pigments have a key role in modulating coral light absorption and heating.

[P79] ILLUMINATING THE DORMANT BIOSPHERE: CULTIVATION-INDEPENDENT IDENTIFICATION OF ENDOSPORES IN A MARINE SEDIMENT

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Endospores are dormant cellular resting stages formed by certain members of the bacterial phylum *Firmicutes*. They occur in high abundances in marine sediments and make up half of the microbial biomass in the subsurface seabed. Endospores are highly resistant to lysis and are thus typically overlooked in molecular surveys of environmental microbial diversity.
We profiled the taxonomic composition of the endospore and vegetative bacterial communities in the sediment and water column of Aarhus Bay, Denmark, using a selective endospore DNA extraction procedure and Illumina sequencing of 16S rRNA gene amplicons.
Firmicutes constituted <1% of the vegetative bacterial community, yet we found evidence for sporulation or germination throughout a 5 m long sediment core, as up to 26% of the species forming the endospore community were also represented in the vegetative bacterial community. Deposition of endospores from the water column also contributed to endospore accumulation in the sediment, as the endospore communities of water column and sediment shared 20% of their species.
Furthermore, a fraction of the endospores species identified in the water column were also present in the vegetative community of the sediment suggesting that endospores depositing from the water column may germinate and grow upon burial in the sediment. Our study highlights the role of endospores in maintaining marine biodiversity as they facilitate dispersal and function as a seedbank in the subsurface seabed.

[P80] ECOLOGICAL PATTERNS OF NITRIFIERS IN THE URBAN WATER CYCLE

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Nitrifiers traditionally divided as ammonia oxidizing (AOBs) and nitrite oxidizing bacteria (NOBs) are key to ammonia removal in both drinking water production (DWP) and waste water treatment (WWT). The maintained functionality of these guilds is likely affected by both their abundance and composition. How substrate fluxes affect these remain unclear. We evaluated the consistency of qPCR and MiSeq based on phylogenetic (16S) and functional genes (*nxrB* and *amoA*) to estimate nitrifiers relative abundance. Then we describe how nitrifiers composition varies between nitrifying systems for low (DWP) and high substrate concentration (WWT)? In four Drinking Water Treatment Plants (DWTPs) sand filters fed with groundwater and in four Waste Water Treatment Plants (WWTPs) nitrifying and anammox reactors were sampled for biomass and full water chemistry characterization. *Nitrospira* was dominant in DWTP (average of 20% of total bacteria); less marked for *nxrB* qPCR. For AOBs we observed high estimates with qPCR (16S), low with *amoA* and intermediate with 16S MiSeq. Caution is required when quantifying β-Proteobacteria AOB with qPCR. Depending on the site (community composition?), very different estimates can be obtained targeting *amoA* or the 16S rDNA gene. *Nitrospira* are much more abundant in DWTPs than in WWTPs, specifically those belonging to Lineage 2 (possible Comammox). Nitrifier distribution at the genus and sub-genus are not random but display clear niches that are driven by substrate concentrations.

[P81] THE DANISH MICROBIAL TERROIR

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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 the edaphic factors, such as pH, mineral composition or climate, can affect the microbiota harbored on the soil surface and in the deeper 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 Danish vineyards. A further aim is to establish a common protocol for further comparison of microbial communities in vineyards worldwide.
We have applied a 16s library amplicon based approach by using NGS technology on soil, grape and leaves coming from the same vineyard; The technical biases due to DNA

extraction, library preparation and sequencing have been estimated using technical and biological replicates for each step. In order to assess the existence of a *unique* Danish microbial *terroir*, we will compare this data with others coming from different vineyards around the world and also with selected datasets from the Earth Microbiome Project. Until now, a "*core*" of microbes that contributes to the community's fingerprint attributable to the Danish microbial *terroir* in a grapevine field has been revealed.

[P82] TIME-SERIES STUDY OF MICROBIAL COMMUNITY STRUCTURE IN SEASONAL STRATIFIED LAKES

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Lakes are some of the most undersampled environments with respect to microbial diversity although they serve important ecological functions. This is not surprising considering the multitude of different types of lakes in existence. Danish lakes often have a high level of anthropogenic influence and a feature regularly observed in them is seasonal stratification with hypoxic or anoxic bottom waters. This results in changes in biogeochemical cycling, causing seasonal variation in microbial diversity.
The aim of our study was to separate general seasonal patterns in microbial lake communities from site-specific factors and identify the driving environmental factors behind them. Four Danish lakes (differing in depth, pH and humus content) were sampled at multiple water depths monthly from April to October and we analyzed nutrient contents, oxygen levels and microbial community structures (16S rRNA gene amplicon sequencing). Three of the lakes experienced stratification and hypoxic/anoxic bottom waters during summer and into the fall. The variation in oxygen and nutrients during the season turned out to be major factors driving diversity with lake specific factors further influencing the microbial community composition. The most abundant OTUs were of the hgcI-clade (*Actinobacteria*), known lake cosmopolitans of *β-proteobacteria* (*Albidiferax*, *Limnohabitans*, *Polynucleobacter*) and *α-proteobacteria* (SAR11). However SAR11 was not abundant in the humus rich lake.

[P83] APTAMERS AS POSSIBLE TARGETING AGENTS FOR DELIVERY OF ANTIBIOTICS TO STAPHYLOCOCCUS AUREUS BIOFILMS

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Treatment of *Staphylococcus aureus* biofilm infections with conventional antibiotic therapy is highly challenged as only sub-lethal doses can be administered. By encapsulating the antibiotics in nanovesicles functionalized with a biofilm-targeting agent, a high concentration of antibiotics can potentially be delivered and released locally in the biofilm. A key challenge to this approach is development of a targeting agent. Aptamers have demonstrated potential as targeting agents for drug delivery. Several aptamers specific for planktonic *S. aureus* are already described. However, their ability to bind *S. aureus* in the biofilm state has not been shown. In this study we investigated if aptamers developed towards planktonic *S. aureus* could also bind to *S. aureus* in biofilm state, and if they could facilitate the interaction of nanovesicles with the biofilm. Nine *S. aureus*-specific aptamers derived from the literature were screened for binding to *S. aureus* biofilm. Only five out of the nine *S. aureus*-specific aptamers bound to *S. aureus* biofilms. CLSM imaging showed that these aptamers penetrated and bound throughout the biofilms. Next, nanovesicles were functionalized with one of the biofilm-binding aptamers. Preliminary results show binding of these targeted nanovesicles to *S. aureus* biofilms. Non-targeted nanovesicles showed no binding. In conclusion, our study presents several aptamers as possible targeting agents for antibiotic delivery to *S. aureus* biofilms.

[P84] PLANT BIOMASS DECOMPOSITION CAPACITIES IN MICROBIAL SYMBIONTS ASSOCIATED WITH FUNGUS-GROWING TERMITES

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The fungus-growing termite-fungus-bacteria symbiosis is the main plant decomposer in the Old World. The termites mix plant substrate and asexual *Termitomyces* fungal spores found in nodules in a first gut passage and inoculate it as fresh comb, within which *Termitomyces* grows. In a second gut passage of the mature comb, the gut microbiota complements *Termitomyces'* enzymatic capacities to ensure near-complete decomposition. Here we seek to better understand this *Termitomyces*-gut microbe complementarity. Based on comprehensive polymer profiling and acid hydrolyses, we initially show that plant polymer content consistently drops from fresh to old comb to termite guts. Analyses of carbohydrate-active enzyme expression, using RNAseq on *Macrotermes natalensis* fungal samples, showed that CAZymes were highly expressed in fresh comb, less so in old comb, and least in nodules. However, enzyme activities were higher in nodules than in fresh and old comb, while guts were similar to nodules in both activity and composition. Our findings suggest that the termites likely transport enzymes present in nodules, and deposit these with the substrate in the fresh comb to facilitate fast decomposition of the recently incorporated plant material. The high expression and enzyme activity of CAZymes and the consumption of plant biomass throughout the decomposition process indicate that the combined efforts of the organisms involved in this tripartite symbiosis assure efficient plant decomposition.

[P85] METATRANSCRIPTOMIC ANALYSIS INDICATES MICROBIAL ACTIVITY IN ACTIVE LAYER PERMAFROST DURING THAWING AND FREEZING

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Permafrost affected soil systems represent 17% of the global land area. The active layer of soil overlaying permafrost in the Arctic is subjected to dramatic annual changes in temperature and soil chemistry, which we hypothesize to affect microbial transcriptional activity. Little is known about the mechanisms employed by soil microbial communities to survive at sub-zero temperatures. Thus, we investigated the microbial transcriptional responses upon thawing and again during freezing of active layer permafrost soil from Svalbard. We incubated active layer soil samples at gradually increasing temperature from -10 to 2 ^{o}C over six days and extracted RNA every second day. Three weeks at 2 ^{o}C were followed by a gradual decrease in temperature over six days to -10 ^{o}C and RNA extraction every second day. Metatranscriptomic data were obtained using HiSeq 150bp PE sequencing. To our surprise, the microbial transcriptomic profile barely changed from -10 to 2 ^{o}C. Thus, transcription of TCA genes remained stable during warming and freezing indicating similar transcriptional activity at -10 and 2 ^{o}C. However, a few transcripts did change. Transcripts related to chaperone DnaK/GroEL significantly increased during warming, indicating heat shock response, while transcripts related to DNA repair decreased during warming and increased again during freezing. Overall, these results indicate that microbial activity takes place at sub-zero temperatures and responds to temperature changes.

[P86] LIVING PHOTONIC CRYSTALS: THE NANO-POROUS SILICATE FRUSTULE OF DIATOMS MEDIATES EFFICIENT PHOTOSYNTHESIS

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Diatoms are productive primary producers in aquatic ecosystems and main contributors to global carbon fixation. The single diatom is enclosed in a porous silicate cell wall called frustule, which is formed by two overlapping valves that fit together like petri dishes. The frustule provides mechanical stability and protection against predation, but its conspicuous architecture with chambers and pores of different sizes in the micro- to nanometer range also affect the path of visible light. We investigated the optical properties of isolated frustules and used a novel spectrally filtered white laser setup to study the spatial distribution of photosynthesis within a single live specimen. We could show that the valve wall allows for the propagation of visible light, which was sufficient to affect photosynthesis all over the diatom cell. Furthermore, the asymmetric construction of the valve wall exhibits an effective photon trapping mechanism leading to strong spectral selection and enhancement of blue-green light inside the frustule, which is strongly absorbed by the diatom photosynthetic apparatus. Scattering of blue wavelengths are due to low effective contrast between silicate wall and luminal space, and furthermore restricted to an acute angle of incidence. We hypothesize that the optical properties of frustules affect photobiological processes in the living cell by effective light capture, distribution of light through the horizontal slab waveguide and scattering of photosynthetically productive blue radiation into the organism.

[P87] ACID AND ALKALI PRODUCTION BY INDIVIDUAL COMMUNITY MEMBERS CAN DRIVE MICROBIAL COMMUNITY SYNERGY THROUGH A PH STABILIZATION OF THE ENVIRONMENT

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On-going research strives to harness microbial communities for industrial purposes such as wastewater treatment, bioremediation, biological control agents and plant growth promotion. Utility of multi-species communities has proven more robust and productive due to potential synergistic interactions than their single species counterparts. However, synthetic assembly of these communities often falls short of expectations, as it is difficult to predict how the inter-species interactions in a multi-species community shape its function and development.
In the present study we describe the drivers behind community synergy in a previously identified synergistic consortium based on four soil isolates: *Microbacterium oxydans*, *Xanthomonas retroflexus*, *Stenotrophomonas rhizophila* and *Paenibacillus amylolyticus*. We found that acid and alkali production from individual community members could drive community synergy by stabilizing the pH in the environment, *in vitro*. Through cultivation of the full consortia environmental acidification by *Paenibacillus amylolyticus* was equalized by the alkalization of *Xanthomonas retroflexus* and *Stenotrophomonas rhizophila* and vice versa, promoting higher community cell counts. Furthermore we were able to show that the acid and alkali production also occurred when the isolates were cultured in soil, suggesting that pH stabilization of the local environment in soil can cause *in vivo* community synergies. Our findings could prove useful for designing new microbial communities for industrial purposes.

[P88] METAGENOMICS OF BACTERIA, FUNGI AND PROTISTS AFFECTED BY BIOCHAR AND EARTHWORMS IN SOIL

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Thermal gasification converts biomass into a combustible gas in an oxygen-poor environment, the bi-product being biochar which can be used as soil amendment to increase pH, sequester carbon and supply phosphate and potassium to crops.

To risk assess the potential effects of biochar amendment to agricultural soils on soil ecosystem services especially biodiversity and carbon sequestration to mitigate climate change. The effects on soil microorganisms and fauna (protists and earthworms) in an agricultural clayey to sandy soil with SOM content of ca. 3% were assessed with activity based assays and NGS. Crops were alternating oil seed rape and winter wheat and biochar was added for 3 years. Earthworms and soil were sampled from field plots either left untreated, or amended with biochar; soil was sampled from bulk soil and earthworm drilosphere.

Earthworms had a priming effect on protist abundance and basal soil respiration, however, in biochar amended soil the protist abundance decreased in the drilosphere. Culturable bacteria and extracellular enzymatic activities were not significantly affected by biochar or earthworms. Only one earthworm species increased in abundance in the biochar amended soil. NGS analysis was more sensitive as metagenomics of bacterial communities (16S rDNA) revealed effects of biochar while metagenomics of fungi/protist communities (18S rDNA) revealed effects of biochar and less priming effects of earthworms. Generally, the addition of biochar according to the plant P demand had limited effect on soil microorganisms and fauna in the tested agricultural soil, and could be a sustainable fertilizer mitigating climate change by increasing soil carbon content.

[P89] THE GUT MYCOBIOME OF ELDERLY DANES

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Aging is associated with changes of the prokaryotic component of the gut microbiota - becoming less diverse, and increasing proinflammatory profile compared to younger adults. These changes have been linked with frailty in several studies. However, our knowledge of the influence of the gut mycobiome on health and disease in elderly remain sparsely investigated. Consequently, the aim of this study was to characterise the feacal mycobiota in relation to host health parameters.

Feacal samples from 99 healthy individuals ranging from 65 to 81 years old were collected, and fungal composition was determined using ITS1-ITS2 amplicon sequencing. The obtained sequences were analysed non-parametrically using the QIIME pipeline to assess fungal taxa composition and diversity based on alpha and beta diversity, respectively. ANOSIM and adonis analyses were performed to assess the significance level between categories associated with the clinical features among individuals.

The elderly gut is home to three main phyla Ascomycota, Basidiomycota and Zygomycota, with genera *Penicillium*, *Candida*, and *Aspergillus* being particularly common. Based on HbA1c-levels, the individuals could be clustered into 3 groups, High, Medium and Healthy. Clusters according to genus abundance co-segregated with glycated glucose levels (HbA1c), carbohydrate intake, and insulin secretion based on C-peptide levels. Interestingly, the dissimilarity matrices of Jaccard distance showed significant ($P<0.05$) variation between clusters with glycated glucose level.

Collectively, these findings suggest that the presences of specific gut mycobiome member is associated with glycemic behaviours among the healthy individuals of the elderly Danes population.

[P90] STRUCTURAL AND FUNTIONAL STUDIES OF *ESCHERICHIA COLI* AGGREGATIVE ADHERENCE FIMBRIAE (AAF/V) REVEAL A DEFICIENCY IN EXTRACELLULAR MATRIX BINDING

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Enteroaggregative *Escherichia coli* (EAEC) is an important cause of acute and persistent diarrhea worldwide. EAEC expresses aggregative adherence fimbriae (AAFs) which play a crucial role in pathogenesis by facilitating the attachment of the organism to the intestinal mucosa. The structure of two AAF variants have previously been published, showing that AAFs assemble into flexible linear polymers that recognize a common receptor, the extracellular matrix protein fibronectin, via clusters of positively-charged amino acid residues along the length of the fimbriae.
Here we addressed the binding properties of the newest member of the AAF family, Agg5A, which shows high prevalence in EAEC strains. We present nuclear magnetic resonance (NMR) structures to provide an atomic structure of the protein made by the novel variant Agg5A. We show that although Agg5A is similar to the two other related AAFs, structural and electrostatic differences renders the protein unable to interact with fibronectin.
Our results provide new insights into the binding mechanisms of AAF, contribute to the understanding of the pathogenesis of EAEC and the development of novel antiadhesive treatments.

[P91] MICROSCALE MEASUREMENTS OF LIGHT AND PHOTOSYNTHESIS DURING CORAL BLEACHING: EVIDENCE FOR THE OPTICAL FEEDBACK LOOP?

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Excess irradiance plays a key role during thermal stress events that trigger coral bleaching. It has been hypothesised that *Symbiodinium* light exposure is accelerated during coral bleaching, leading to a feedback loop of symbiont loss, excess light and photodamage. However, light measurements within coral tissues during a stress event are still needed to confirm this hypothesis. In this study we used light microsensors to investigate the *in* vivo light exposure of *Symbiodinium* in the branching coral *Pocillopora damicornis* and the massive coral *Favites sp.* during a thermal stress experiment on the Great Barrier Reef, Australia. Compared to healthy corals, the *in vivo* light exposure of *Symbiodinium* was up to 5-fold enhanced in bleached corals. Additionally the *Symbiodinium* light exposure within the host *Favites sp.* differed from *P. damicornis,* as low light optical microniches (~25% of incident irradiance) remained in bleached coral tissues, suggesting an important role of coral host tissue optics in photoprotection of the remaining symbionts during

thermal stress. Microscale O_2 measurements show that compared to healthy corals, *Symbiodinium* in bleached corals display enhanced rates of gross O_2 evolution and light respiration, while areal rates of net photosynthesis and the maximum quantum yield of photosystem II decreased. Our data suggests that the strongly accelerated *in vivo* light exposure of *Symbiodinium* upon onset of coral bleaching leads to excess photosynthetic activity that may exaggerate their stress response.

[P92] IDENTITY AND CHEMOTACTIC BEHAVIOR OF BACTERIA AROUND OXYGEN-CONNECTED CABLE BACTERIA SUGGEST THEY ACT AS AN UNIVERSAL ELECTRON ACCEPTOR IN SEDIMENTS

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Cable bacteria are long filamentous bacteria, which transmit electrons over centimetre distances. All cable bacteria described couple sulfide oxidation to the reduction of oxygen, nitrate, or nitrite, and form a monophyletic cluster within the Desulfobulbaceae.
Using enrichments of freshwater cable bacteria, we investigated a coupling in anoxic sediments between aerobic bacteria and cable bacteria. Sequencing of 16S rRNA gene amplicons revealed an enrichment of nitrifiers, iron oxidizers, methylotrophs, and sulphide oxidizers in anoxic sediment with cable bacteria, but not without cable bacteria. To visualise this association, we constructed microscope slides with a central chamber containing cable bacteria and sulphide-rich sediment, separated from oxygen at the rim of the slide by a centimetre-wide space. This mimics sediment conditions, where cable bacteria have to navigate a suboxic zone between oxygen and sulphide.
Cable bacteria filaments positioned themselves stretched out from sulphide to oxygen. Once connected with one end to oxygen, the filament part furthest from oxygen became surrounded by swimming bacteria, which swarmed around individual cable bacteria filaments. Using a laser microdissection microscope, we cut individual cable bacteria in two, terminating the connection to oxygen. Immediately the chemotactic behaviour of the swarming bacteria ceased, and they dispersed in a random way, indicating that they were dependent on actively electron-conducting cable bacteria. FISH suggests the swarming bacteria are not a single symbiont species but rather a wide range of aerobic bacteria. We therefore propose that cable bacteria act as universal electron acceptors for aerobic bacteria in suboxic sediments.

[P93] EVALUATION OF DIRECT LYSIS FOR THE EXTRACTION OF NOROVIRUS RNA FROM RASPBERRIES AND STRAWBERRIES

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Consumption of soft fruits contaminated with noroviruses (NoV) frequently cause disease outbreaks. To ease surveillance and control for viruses in berry productions, current detection methods need increased simplicity and sensitivity.
Towards a reference method A, ISO/TS 15216, comprising viral elusion-concentration-extraction, we evaluated a method B, including only direct lysis, for the efficiency to recover spiked modelvirus, mengovirus (MC_0), while eliminating inhibitors for the subsequent RT-qPCR detection of NoV from frozen samples of raspberries ($n_{methodA}$=8/$n_{methodB}$=9) and strawberries ($n_{methodA}$=24/$n_{methodB}$=9).
The recovery rate/efficiency of MC_0 using method A was 100%/4±3% for sample extracts of raspberries and 83%/18±29% for strawberries, while method B resulted in 100%/8±10% for raspberries and 66%/2±2% for strawberries. For strawberries, this could be increased by testing 10-fold diluted extracts to 100%/58±37% using method A, and to 100%/16±7% using method B. The PCR inhibition during NoV detection for undiluted sample extracts of raspberries/strawberries using method A was 78±16%/81±22%, and 69±21%/99% using method B. A decreased inhibition, to 2±50%/26±20% for method A and to 20±14%/58±30% for method B, was obtained by testing diluted extracts.
Method B showed better recovery and less inhibition in undiluted extracts of raspberries, while method A were best for strawberry and diluted raspberry samples, which may be considered when testing different soft-fruit.

[P94] CHLOROPHYLL *f* DISTRIBUTION AND DYNAMICS IN CYANOBACTERIAL BEACHROCK BIOFILMS

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Chlorophyll (Chl) *f*, the most far-red (720–740nm) absorbing Chl species, was discovered in cyanobacterial isolates from stromatolites and subsequently in other habitats as well. However, the spatial distribution and temporal dynamics of Chl *f* in a natural habitat have so far not been documented. Here, we report the presence of Chl *f* in cyanobacterial beachrock biofilms. Hyperspectral imaging on cross-sections of beachrock from Heron Island (GBR, Australia), showed a strong and widely distributed signature of Chl *f* absorption in an endolithic layer below the dense cyanobacterial surface biofilm that could be localized to aggregates of Chroococcidiopsis-like unicellular cyanobacteria packed within a thick common sheath. High-pressure liquid chromatography-based pigment analyses showed in situ ratios of Chl *f* to Chl *a* of 5% in brown-

pigmented zones of the beachrock, with lower ratios of ~0.5% in the black- and pink-pigmented biofilm zones. Enrichment experiments with black beachrock biofilm showed stimulated synthesis of Chl *f* and Chl *d* when grown under near-infrared radiation (NIR; 740nm), with a Chl *f* to Chl *a* ratio increasing 4-fold to 2%, whereas the Chl *d* to Chl *a* ratio went from 0% to 0.8%. Enrichments grown in white light (400–700nm) produced neither Chl *d* or *f*. Beachrock cyanobacteria thus exhibited characteristics of far-red light photoacclimation, enabling Chl *f* -containing cyanobacteria to thrive in optical niches deprived of visible light when sufficient NIR is prevalent.

[P95] MICROBIOTA IN BIO-GAC FILTERS FOR CLEANING GROUNDWATER POLLUTED WITH CHLORINATED SOLVENTS

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Chlorinated solvents are responsible for polluting groundwater at a large number of sites in Denmark. Apart from spoiling the groundwater, the pollutions are very costly to the municipality seeking to reduce the level of pollution by different cleaning technologies; e.g. by filtering the polluted groundwater through granulated activated carbon (GAC). Besides adsorbing the pollutants, the GAC surface supports growth of bacteria forming biofilm on the surface (bio-GAC). Supposedly, the bacteria in the bio-GAC filters degrade/mineralize cis-dichloroethene (cDCE)/vinyl chloride (VC) as these compounds are only found in trace quantities in the percolated water. However, with time the filters capacity to remove the pollutants declines and the GAC has to be replaced with new material, which is a costly process. At a filter facility in the Copenhagen area, nutrients and oxygen were added to the polluted groundwater entering the bio-GAC. Water and materials were sampled upstream, in the filter, and downstream the filter and analyzed regarding CFUs, extractable DNA, and taxonomic composition (16S rDNA sequencing) of the microbial community in the bio-GAC. The bio-GAC microbial community reacted positively to addition of the substrate and the number of operational taxonomic units increased from approximately 100 to more than 200 at 260 days after onset of substrate addition. Sampled bio-GAC material is presently used for ongoing cDCE/VC degradation lab experiments.

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Surname	First name	Abstract no.
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