Assessment of Methods to Quantify Livestock Associated MRSA in Pig Herds

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The Danish Microbiological Society Annual Congress 2015

Programme & Abstracts

Monday, 9th November 2015
Eigtved's Pakhus
Copenhagen

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	**Microbial life in gradient environments** Niels Peter Revsbech, *Aarhus University*		**Blocking of bacterial signaling as antimicrobial treatment strategies** Michael Givskov, *University of Copenhagen*	
10:45	**Coffee and exhibition**		*First & second floor*	
	PARALLEL SESSIONS			**SATELLITE SYMPOSIUM I**
	ROOM II *Second floor*	**ROOM III** *Second floor*	**ROOM IV** *Second floor*	**SALON F** *First floor*
	Use of whole genome sequencing data for investigation of bacterial spread Chair: Anette M. Hammerum, *Statens Serum Institut*	**The environmental resistome** Chair: Barth F. Smets, *Technical University of Denmark*	**Plant-microbe interactions** Chair: Jan Sørensen, *University of Copenhagen*	
11:00	Chair introduction	Chair introduction	Chair introduction	**Can you avoid traditional culture? - How Molecular Methods improve the microbiology workflow in the lab** Astrid Ferlinz, *Global Market Development in Microbiology and Infectious Disease, ThermoFisher Scientific*** ThermoFisher SCIENTIFIC
11:05	**Distinct plasmid lineages disseminate extended-spectrum beta-lactamase genes between *Escherichia coli* strains from different hosts** Willem van Schaik, *University Medical Center Utrecht*	**Environmental pollution from antibiotic manufacturing creates hotspots for resistance development** Joakim Larsson, *University of Gothenburg*	**Rhizosphere microbiomes and plant health** Irene de Bruijn, *Netherlands Inst. for Ecology (NIOO-KNAW)*	
11:30	**Implementation of whole-genome sequencing for detection and response of foodborne outbreaks** Eva Litrup, *Statens Serum Institut*	**Quantifying and visualizing the transfer of exogenous plasmids to environmental microbial communities** Arnaud Dechesne, *Technical University of Denmark*	***Pseudomonas fluorescens* derived cytokinins determine the biocontrol of *Pseudomonas syringae* infection in *Arabidopsis* - a novel biocontrol concept** Dominik K. Großkinsky, *University of Copenhagen*	
11:45	**Use of whole-genome sequencing data for surveillance of carbapenemase producing bacteria** Anette M. Hammerum, *Statens Serum Institut*	**The origin of acquired antibiotic resistance** Lars Hestbjerg Hansen, *Aarhus University*	**Novel, pseudomonas-derived antifungal lipopeptides from a disease suppressive soil in Greenlandic potato fields** Rosanna C. Hennessy, *University of Copenhagen*	
12:00	**3 flash poster presentations***	**3 flash poster presentations***	**3 flash poster presentations***	
12:15	**LUNCH**		*SALON C, ground floor*	
12:15	**POSTERS**		*First floor*	
12:15	**EXHIBITION**		*First & second floor*	
12:15	**GENERAL ASSEMBLY** Det Danske Pasteur Selskab		*ROOM II*	

	PARALLEL SESSIONS			SATELLITE SYMPOSIUM II
	ROOM II Second floor	**ROOM III** Second floor	**ROOM IV** Second floor	**SALON F** Second floor
	Metabolic engineering Chair: Jochen Förster, *Technical University of Denmark*	**Device associated infections** Chair: Karen Krogfelt, *Statens Serum Institut*	**Novel microbial metabolisms in natural systems** Chair: Bo Thamdrup, *University of Southern Denmark*	
14:15	Chair introduction	Chair introduction	Chair introduction	
14:20	**Metabolic engineering for bio-based chemicals: technical progress and commercial realities** Thomas Grotkjær, *Novozymes*	**Developing dual-action biomaterials for bone regenerative applications.: enhancing cellular growth and preventing bacterial biofilms** Jason Peter Mansell, *University of the West of England*	**Direct interspecies electron transfer (DIET) in methanogenic consortia** Amelia Rotaru, *University of Southern Denmark*	**Microbiome profiling, isolate typing, and reference genome assembly – new solutions for microbiologists on QIAGEN's CLC platform**** 
14:45	**Making yogurt sweet using natural approaches for strain improvement** Ana Rute Neves, *Chr. Hansen*	**Vascular catheter-associated infections caused by Staphylococcus aureus: Pathogenesis and prevention** Thomas Emil Andersen, *Odense University Hospital*	**Discovery and environmental significance of phototrophic gemmatimonadetes bacteria** Yonghui Zeng, *University of Southern Denmark*	
15:00	**Development of an efficient glycerol utilizing *Saccharomyces cerevisiae* strain via adaptive laboratory evolution** Tomas Strucko, *Technical University of Denmark*	**Biofilm-mediated oral diseases** Daniel Belstrøm, *University of Copenhagen*	**Anammox and the "New" nitrogen cycle** Bo Thamdrup, *University of Southern Denmark*	
15:15	**3 flash poster presentations***	**3 flash poster presentations***	**3 flash poster presentations***	
15:30	Coffee and exhibition		*First & second floor*	
16:00	**Germ theory: Medical pioneers in infectious diseases** Robert Gaynes, *Emory University School of Medicine*		*ROOM III, Second floor*	
17:00	Reception with fermented beverage		*SALON C, ground floor*	
18:30	Optional congress dinner		*Spiseloppen, Bådsmandsstræde 43, 1407 København*	

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

*** Please see page 5 for information about the Satellite Symposia*

Flash poster presentations

	PARALLEL SESSIONS, MORNING		
	ROOM II *Second floor*	**ROOM III** *Second floor*	**ROOM IV** *Second floor*
	Use of whole genome sequencing data for investigation of bacterial spread Chair: Anette M. Hammerum	**The environmental resistome** Chair: Barth F. Smets	**Plant-microbe interactions** Chair: Jan Sørensen
12:00	**[P70] Isolation characterization and sequence analysis of *Escherichia coli* phages isolated from animal faecal samples** Witold Kot, *Aarhus University*	**[P2] Adaptive laboratory evolution of *Escherichia coli* reveals arduous resistance development to a combination of three novel antimicrobial compounds and to the short amp p9-4** Linda Citterio, *Technical University of Denmark*	**[P23] Diclorprop mineralization by *Sphingomonas herbicidovorans* MH - dependency of oxygen availability** Anders Johansen, *Aarhus University*
12:05	**[P72] Comparative genome analysis of *Clostridium perfringens* isolates from healthy and necrotic enteritis infected poultry and diseased pigs** Troels Roncom, *National Veterinary Institute*	**[P4] Detection and characterization of methicillin-resistant *Staphylococcus aureus* (MRsA) and methicillin-susceptible *S aureus* (MSSA) from Danish retail meat products** Yuanyue Tang, *University of Copenhagen*	**[P22] A comparative study on adhesion and mineralization capacity of bacterial pesticide-degraders attended for sand filter inoculation** Elin Djurhuus Joensen, *The Geological Survey of Denmark and Greenland (GEUS)*
12:10	**[P26] Substantial molecular evolution in prolonged latent *Mycobacterium tuberculosis* infections in humans** Anders Norman, *Technical University of Denmark*	**[P73] Prediction of plasmid contigs in sphingomonad genomes** Tue Kjærgaard Nielsen, *Aarhus University*	**[P71] Genome sequences for two *Pseudomonas jessenii* from copper contaminated soil in Denmark** Yanan Qin, *University of Copenhagen*

	PARALLEL SESSIONS, AFTERNOON		
	ROOM II *Second floor*	**ROOM III** *Second floor*	**ROOM IV** *Second floor*
	Metabolic engineering Chair: Jochen Förster	**Device associated infections** Chair: Karen Krogfelt	**Novel microbial metabolisms in natural systems** Chair: Bo Thamdrup,
15:15	**[P51] Enzymatic production of human milk oligosaccharides** Morten Jepsen, *University of Copenhagen*	TBA	**[P12] Treatment of hospital wastewater with a combined activated sludge and biofilm process, Hybas™** Alice Christensen, *Danish Technological Institute*
15:20	**[P55] Exploring marine environments to unravel tolerance mechanisms to relevant compounds** Henrique Machado, *Technical University of Denmark*	TBA	**[P43] Regulation of intertidal microphytobenthos photosynthesis over a diel emersion period** Paulo Cartaxana, *University of Copenhagen*
15:25	**An antibiotic selection system for protein over-producing bacteria** Maja Rennig, *Technical University of Denmark*	TBA	**Chitin degradation in marine bacteria** Sara Paulsen, *Technical University of Denmark*

Satellite symposia

11:00-12:15: Satellite symposium organised by Thermo Fisher Scientific **SALON F**

Can you avoid traditional culture? How Molecular Methods improve the microbiology workflow in the lab

In the last 10 years the introduction of a wealth of new technologies for the traditional clinical microbiology lab has helped to make life of microbiologists a bit easier. Automated processes can replace manual manipulation of culture plates and it is possible to identify microorganisms and get their antibiotic susceptibility profile without any human touching the culture plate. Identification of isolates can be done within minutes and in a very cost-effective manner using MALDI-TOF. Molecular technologies such as PCR, Sanger sequencing and Next-Generation sequencing can further simplify, improve or speed up the process for identification, antibiotic resistance profiling and typing.

In this symposium you will learn about how molecular technologies have improved customer workflows in the microbiology lab:

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- Simultaneous detection of multiple respiratory pathogens using TaqMan™ Array Card technology
- Rapid sequencing of whole-genome Ebola virus using Ion Torrent next-generation sequencing in Sierra Leone

We will also introduce the new Ion Torrent NGS systems, Ion S5™ and Ion S5™ XL instruments, the fastest next-generation sequencers on the market.

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

Microbiome profiling, isolate typing, and reference genome assembly – new solutions for microbiologists on QIAGEN's CLC platform

The expansion of NGS technology is accelerating in all directions with an ever increasing variety of applications. The CLC Microbial Genomics Module offers the ability to analyse complex microbial samples as well as typing of cultured bacterial samples. The new version 1.1 includes the following features:

Typing and epidemiology of a cultured bacterial sample

- Template workflows, which can be easily optimized for outbreak- or routine analysis of a specific pathogen.
- Tools for NGS-based Multilocus Sequence Typing (MLST), resistance typing, as well as detection of genus and species information.
- Phylogenetic tree reconstruction tools that enable generation of trees based on single nucleotide polymorphisms (SNPs) or infer K-mer trees from NGS reads and/or genomes.
- A table format that collects typing results and combines with sample metadata such as sample information, geographic origin, treatment outcome, etc.
- Filtering facilities based on typing results and metadata enable selection of relevant subsets of samples and associated files for downstream analysis.
- Typing results and metadata can be visualized in the context of the phylogenetic tree.

Whole genome analysis of a complex microbial sample

- Microbiome analysis via OTU-clustering of 16S rRNA or other amplicon data.
- OTU-clustering and taxonomic annotation using common reference databases such as Greengenes, Silva and UNITE.
- Stacked and area charts, and zoomable sunburst diagrams to explore and compare the taxonomic composition of samples, or sample groups.
- A range of statistical tools to reveal differential abundance of OTUs, estimation of alpha- and beta diversities, or to carry out PERMANOVA analysis. Principal Coordinate Analysis (PCoA) results can be explored in 3D in the context of the sample metadata.
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Microbial genomes and metagenomes of microbial communities are more accessible than ever thanks to modern sequencing technologies. With QIAGEN Bioinformatics, microbial genomics data analysis has become user-friendly and scalable to meet your sample throughput.

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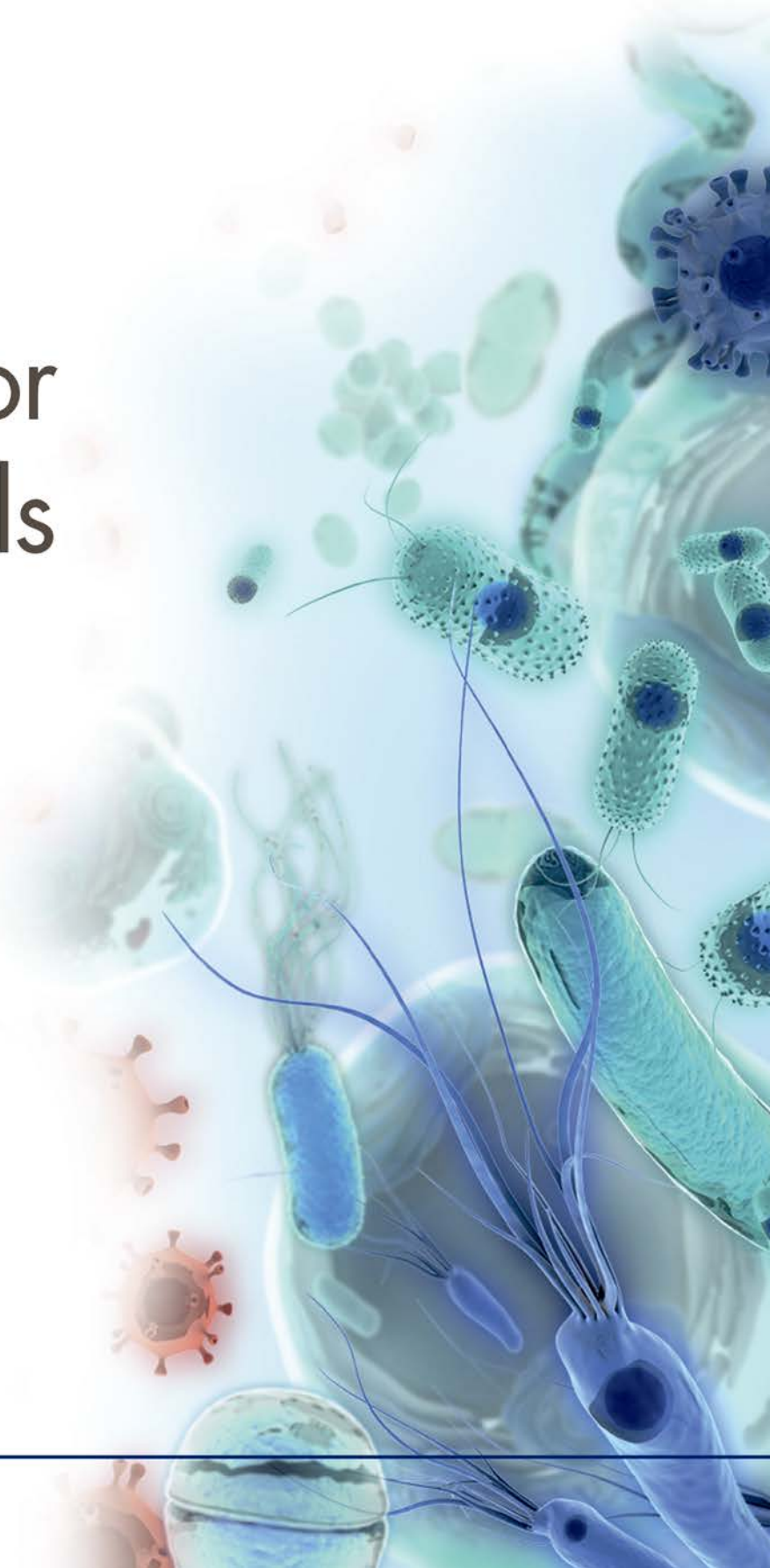

Sample to Insight

Table of contents

ion torrent

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- Identify fusion transcripts in tissue samples.
- Explore SNPs, indels, CNVs and even gene expression levels from 10ng of FFPE DNA or RNA.

Inherited disease research

- Investigate known variants associated with drug metabolism.
- Sequence both small and large gene research panels.
- Discover SNPs, indels and CNVs for research of rare or unknown disorders by whole exome sequencing.

Infectious disease research

- Identify and type microbes through genome sequencing.
- Assess known variants associated with antibiotic resistance.

Aneuploidy research

- High–throughput copy number and aneuploidy assessment.

† Automated analysis time includes aligned BAM files.

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About DMS

The Danish Microbiological Society (DMS) is a professional association in the fields of human and veterinary medical microbiology, general microbiology, food microbiology, environmental microbiology and biotechnology. DMS has existed for 57 years 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.

Contact

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Scientific Committee

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- 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

'Germ Theory: Medical Pioneers in Infectious Diseases' by Professor Robert Gaynes, Emory University School of Medicine, Atlanta, USA.
A historical overview of medical microbiology from the first pioneers to present. Professor Gaynes presents the "inside stories" of these pioneers' struggles to have their work accepted, which can inform strategies for tackling current crises in infectious diseases and motivate and support today's scientists.

The DMS 2015 Congress is supported by the Federation of European Microbiological Societies (FEMS). Read more about FEMS at www.fems-microbiology.org

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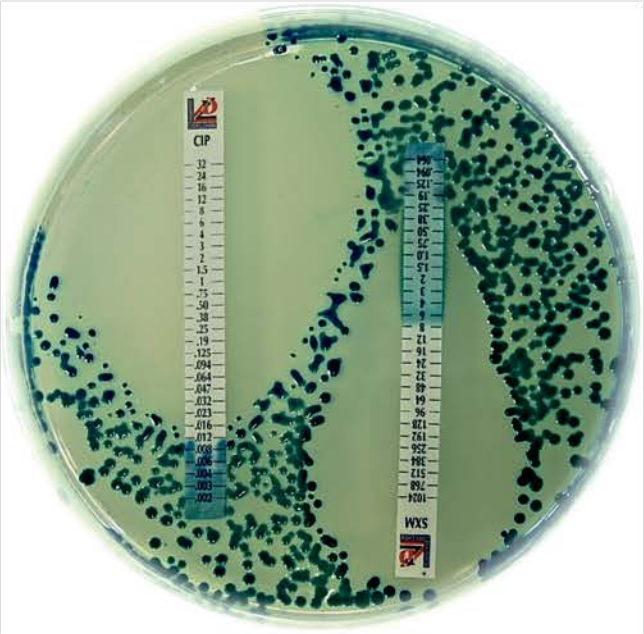

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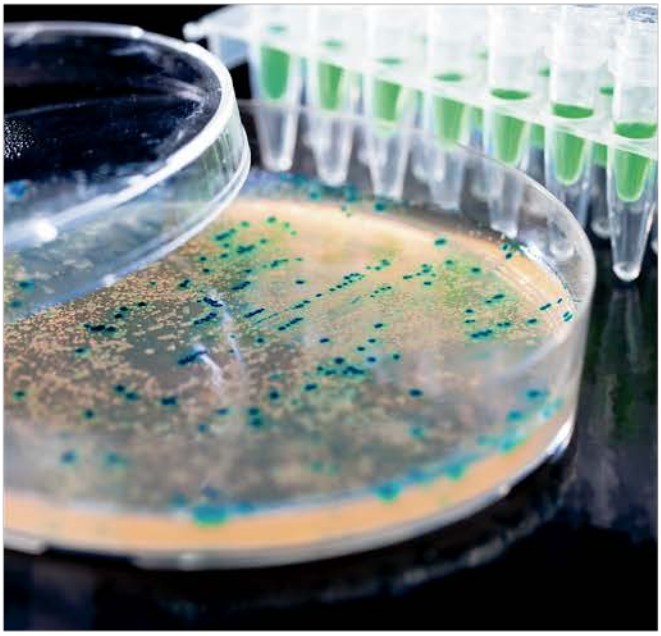

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Invited talks

[O1] MICROBIAL LIFE IN GRADIENT ENVIRONMENTS

Niels Peter Revsbech[1]
[1]*Department of Bioscience, Aarhus University, Denmark*

All environments inhabited by microbes are characterized by gradients in physical and chemical parameters. Such gradients may have extensions of tenths of meters in pelagic limnic or marine environments, or they may be present at a micrometer scale in biofilms or around individual cells. Various methods can be used to study these gradients and the associated microbial metabolism. One powerful approach is the analysis with needle-shaped microsensors and electrodes having tip diameters ranging from 2-100 µm. At present we can analyze a wide array of parameters with such techniques, including the chemical species O_2, CO_2, NO_3^-, NO_2^-, N_2O, H_2S, H_2, and CH_4. The results obtained from our microscale analysis have led to several important new discoveries, such as the finding of denitrifying foraminifera and electrically conducting cable bacteria. The work has also resulted in new methods for quantifying microbial metabolism in gradient environments such as photosynthesis and coupled nitrification/denitrification in biofilms and sediments. During the last decade our expertise in sensor analysis has furthermore resulted in a new understanding of the functioning of oceanic oxygen minimum zones which had to be re-named due to our work.

[O2] BLOCKING OF BACTERIAL SIGNALING AS ANTIMICROBIAL TREATMENT STRATEGIES

Michael Givskov[1, 2]
[1]*Director, Professor Dr. Techn, Costerton Biofilm Center, Department of Immunology and Microbiology University of Copenhagen, Denmark*
[2]*Research Director, Singapore Centre on Environmental Life Sciences Engineering, Nanyang Technological University, Singapore*

Twelve years ago, we delivered proof-of-concept that Quorum Sensing (QS) signaling constitutes a viable target for antimicrobial intervention strategies directed against bacteria in the biofilm mode. Opportunistic pathogens need to establish a biofilm before they can embark on causing disease. QS signaling controls expression of an arsenal of virulence factors and coordinates the temporal event in the complex process of causing disease. Among the virulence factors is the powerful toxin rhamnolipid. When bacteria aggregate in biofilms, the QS system uses extracellular signal molecules to transmit the message to turn on production of virulence and the defense against cellular immunity. Production of virulence factors recruit neutrophils and macrophages, which upon contact with the rhamnolipid containing biofilm matrix lyse, releasing inflammation mediators. This vicious cycle, causes a reduction in clearance, incapacitates cellular immunity, and leads to increased inflammation, ultimately leading to tissue destruction. Furthermore, rhamnolipid driven destruction of neutrophils and macrophages release large quantities of DNA that eliminates the antimicrobial effects of aminoglycosides and antimicrobial peptides. Over the

years, my research teams have patented and published a number of different inhibitors of QS and proven their functionality in rodent models of infection. QS inhibitors function as antimicrobials because they switch off the production of virulence factors and defensive compounds by the biofilm, allowing for the cellular immune system to function properly. Compelling evidence suggests that c-di-GMP is a universal chemical signal that controls essential events of the biofilm lifecycle. High internal levels of c-di-GMP drive bacteria to form biofilms, whereas low internal c-di-GMP levels promote biofilm bacteria to disperse by swimming motility and assume a planktonic mode of life. In pathogens such as *Pseudomonas aeruginosa* and *Escherichia coli*, multiple diguanylate cyclase enzymes catalyze the synthesis of c-di-GMP, whereas multiple phosphodiesterase enzymes break down the molecule. Notably, our recent *in vivo* studies have shown that if bacterial cells are depleted of c-di-GMP (by overexpressing a single c-di-GMP-degrading enzyme) infecting biofilms are dismantled. Combination therapy has a much more pronounced effect on eradicating biofilm infections than single drug treatments. Consequently, we aim at identifying novel chemical compounds that can dismantle biofilms and force bacteria away from the protective biofilm-state to a free-living mode where bacteria become highly susceptible to the action of conventional antimicrobials and host immunity.

[O3] DISTINCT PLASMID LINEAGES DISSEMINATE EXTENDED-SPECTRUM BETA-LACTAMASE GENES BETWEEN *ESCHERICHIA COLI* STRAINS FROM DIFFERENT HOSTS

Willem van Schaik[1]
[1]*Department of Medical Microbiology, University Medical Center Utrecht, The Netherlands*

Third-generation cephalosporins are a class of β-lactam antibiotics that are often used for the treatment of human infections caused by Gram-negative bacteria, especially *Escherichia coli*. Worryingly, the incidence of human infections caused by third-generation cephalosporin-resistant *E. coli* is increasing worldwide. Recent studies have suggested that these *E. coli* strains, and their antibiotic resistance genes, can spread from food-producing animals, via the food-chain, to humans. However, these studies used traditional typing methods, which may not have provided sufficient resolution to reliably assess the relatedness of these strains. We therefore used whole-genome sequencing (WGS) to study the relatedness of cephalosporin-resistant *E. coli* from humans, chicken meat, poultry and pigs. One strain collection included pairs of human and poultry-associated strains that had previously been considered to be identical based on Multi-Locus Sequence Typing, plasmid typing and antibiotic resistance gene sequencing. The second collection included isolates from farmers and their pigs. WGS analysis revealed considerable heterogeneity between human and poultry-associated isolates. The most closely related pairs of strains from both sources carried 1263 Single-Nucleotide Polymorphisms (SNPs) per Mbp core genome. In contrast, epidemiologically linked strains from humans and pigs differed by only 1.8 SNPs per Mbp core genome. WGS-based plasmid reconstructions revealed three distinct plasmid lineages (IncI1- and IncK-type) that carried cephalosporin resistance genes of the Extended-Spectrum Beta-Lactamase (ESBL)- and AmpC-types. The plasmid backbones within each lineage were virtually identical and were shared by genetically unrelated human and animal isolates.

Plasmid reconstructions from short-read sequencing data were validated by long-read DNA sequencing for two strains. Our findings failed to demonstrate evidence for recent clonal transmission of cephalosporin-resistant *E. coli* strains from poultry to humans, as has been suggested based on traditional, low-resolution typing methods. Instead, our data suggest that cephalosporin resistance genes are mainly disseminated in animals and humans via distinct plasmids.

[O4] IMPLEMENTATION OF WHOLE-GENOME SEQUENCING FOR DETECTION AND RESPONSE OF FOODBORNE OUTBREAKS

Eva Litrup[1]
[1]*Statens Serum Institut, Copenhagen, Denmark*

In Denmark, the laboratory-based surveillance of foodborne infections caused by *Listeria monocytogenes*, Salmonella and Verocytotoxin-producing *Escherichia coli* (VTEC) is performed by typing of all isolates from patients to detect clusters and link to potential sources. A number of different laboratory techniques such as serotyping, detection of virulence genes and high-discriminatory molecular typing methods are used for this purpose. The availability of new sequencing techniques has allowed for whole-genome-sequencing (WGS) of bacterial isolates to replace this variety of different methods.
It is our goal to implement WGS for surveillance of foodborne pathogens whenever this is feasible scientifically and economically, i.e. useful output and costs comparable to present methods. We have used retrospective data for the evaluation of expected variability between epidemiologically linked isolates as well as for establishing backward comparability to relevant typing methods. In 2013, we fully implemented WGS for surveillance of Listeria infections in replacement of PFGE. The past two years, the use of WGS has significantly increased the number of confirmed outbreaks of listeriosis and the linking to a specific food source. For VTEC, our focus has been on extracting the virulence profile and the O:H-serotype from the WGS data. Since the beginning of 2015, all clinical VTEC isolates in Denmark have been sequenced real-time, replacing serotyping, PCR and PFGE. The WGS-data was analyzed using an in-house pipeline, giving outputs like MLST, serotype, and virulence profile. The typability of 86 VTEC isolates using WGS-based prediction of serotype was 92% for the O type and 100% for the H type compared to the phenotypic typability of 86% and 57%, respectively. Suspected clusters of isolates belonging to the same MLST were further investigated by SNP-analysis.
It is our experience, that WGS can already be used as a routine method for outbreak detection and response by applying a similar approach for most species. However, there is a need for more tailor-made solutions if backward comparability is wanted for other species.

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[O5] USE OF WHOLE-GENOME SEQUENCING FOR SURVEILLANCE OF CARBAPENEMASE PRODUCING ORGANISMS (CPO)

Anette M. Hammerum[1]
[1]*Statens Serum Institut, Copenhagen, Denmark*

Background: Carbapenems comprise one of the only classes of antimicrobial agents that can be used for treatment of infections with multi-resistant Gram-negatives like *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. Treatment options for infections with carbapenem resistant bacteria are often none or suboptimal. Resistance can be caused by the presence of various carbapenemases of which the most frequently occurring are *K. pneumoniae* carbapenemase (KPC), Oxacillinase (OXA), Verona integron-encoded metallo-β-lactamase (VIM), and New Delhi metallo-β-lactamase (NDM), and Imipenemase (IMP).
In recent years, Danish Departments of Clinical Microbiology (DCM) have on a voluntary basis submitted carbapenem resistant isolates for verification and genotyping to the Antimicrobial Resistance Reference Laboratory at Statens Serum Institut.
Methods: From January 2014, Carbapenemase producing organisms (CPO) sent to SSI has been whole-genome-sequencing (WGS). The ResFinder web server, and MLST web server, from www.genomicepidemiology.org were used to identify acquired antimicrobial resistance genes, and MLST-profiles from the assembled WGS data, respectively. Suspected clusters of isolates belonging to the same MLST were further investigated by SNP-analysis.
Results: During 2014, 55 carbapenemase producing bacteria were detected from 48 patients. More than one isolate from the same patient were included, if the isolates belonged to different bacterial species and/or if the isolates harboured different carbapenemases. Six of the isolates were from bloodstream infections.
Enterobacteriaceae: In 2014, 35 CPE (from 29 patients) were detected compared to 19 in 2013 and 20 CPE during 2008–2012 (Figure 1). Twenty-two of the 35 CPE isolates harboured OXA-48-like genes. Twelve NDM-producing isolates and one KPC-producing isolate were detected
The NDM-1 producing *Citrobacter freundii* outbreak, which started in 2012 in the North Denmark Region, continued in 2013 and 2014. Until the end of 2014, six patients were involved in this outbreak. None of these patients had a prior history of travel noted in their hospital records. The origin of the NDM-1 producing *C. freundii* was unknown.
During 2014, NDM-5 producing *K. pneumoniae* were detected from three patients at a hospital in the Capital Region of Denmark. The isolates had highly similar SNP-profiles, indicating a possible spread between the patients or a common origin. None of the patients had travelled recently, and the origin of the NDM-5 producing *K. pneumoniae* was unknown.
Acinetobacter spp: The number of carbapenemases producing *Acinetobacter* spp was the same level as in 2013. Nine OXA-23 producing *A. baumannii* isolates were detected in 2014. Furthermore, one OXA-40-like producing *A. baumannii*, one OXA-58-like producing *A. baumannii* and one NDM-1 producing *A. Acinetobacter pittii* were detected. NDM-1 producing *A. pittii* are rare and the origin of this isolate was unknown.
P. aeruginosa: In 2014, six VIM-producing *P. aeruginosa* isolates were detected from six patients. Furthermore, two NDM-producing *P. aeruginosa* were detected from two patients.

Conclusion: The occurrence of carbapenemase-producing bacteria in Denmark is increasing, a trend worrisome to patients and clinicians. Especially the spread of CPE is of concern, since *Enterobacteriaceae* can be carried in the intestine for a long time without any symptoms of infections, which makes outbreak control difficult. Use of WGS for surveillance of CPO is very useful and make it easy to compare national data with international data.

[O6] ENVIRONMENTAL POLLUTION FROM ANTIBIOTIC MANUFACTURING CREATES HOTSPOTS FOR RESISTANCE DEVELOPMENT

D.G. Joakim Larsson[1]
[1]*Department of Infectious Diseases, Institute of Biomedicine, the Sahlgrenska Academy at the University of Gothenburg, Sweden*

Introduction: Antibiotic resistant bacteria and resistance genes occur in all environments. That however, does not make all environments equal in terms of risks for recruitment of novel resistance factors from the environmental reservoir to human pathogens. On the contrary, risks will depend on selection pressures, abundances and nature of the resistance factor(s) present, as well as potential for gene transfer and further dissemination to humans. It is important to recall that emergence of resistance in a pathogen in principle only need to arise once, at one site. Thus, managing risks with "hotspots" are crucial regardless of where on earth they may be located. Objective: To characterize known and novel antibiotic resistance factors in various environments polluted with high levels of fluoroquinolones (mg/L) from bulk drug manufacturing in Patancheru, India.
Methods: We have applied classical culturing, shotgun metagenomics, quantitative PCR, functional metagenomics, sequencing of integron cassettes, whole genome sequencing, plasmid capture experiments, and plasmid sequencing.
Results: To the best of our knowledge, the investigated industrial treatment plant harbours bacteria with the most extreme multi-resistance profile described in any environment. Similarly, the occurrence of integrons, analyzed both by community PCR and PCR of isolates, is unprecedented here. River and lake sediments are hosts for bacteria with resistance genes of principally all classes in very high numbers as assessed by metagenomics and quantitative PCR. Functional metagenomics revealed a plethora of novel resistance gene candidates for quinolones, tetracycline, chloramphenicol, and betalactams – including meropenem. Interestingly, plasmids and genes involved in horizontal gene transfer are highly abundant as well. In accordance, we have captured several novel broad-host conjugative resistance plasmids, some of which contain the qnrVC1 gene, previously only known as a chromosomal gene.
Conclusion: These findings show that aquatic environments, exposed to exceedingly high levels of antibiotics for extended times, are incubators for multi-resistant bacteria with apparent risks for gene transfer to human pathogens. Actions are therefore urgently needed to reduce risks at such locations.

[O7] QUANTIFYING AND VISUALIZING THE TRANSFER OF EXOGENOUS PLASMIDS TO ENVIRONMENTAL MICROBIAL COMMUNITIES

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Plasmid transfer is deemed responsible for the rapid spread of antibiotic resistance among microbes. While broad host range plasmids are known to transfer to diverse hosts in pure culture, their transfer potential to complex communities has not been comprehensively studied. The ability of a community to take up exogenous plasmid should, however, be an important element affecting the fate of mobile genetic elements released in the environment.
We have devised a method to evaluate the permissiveness of a bacterial community towards exogenous plasmids, both quantitatively (how many bacteria can take up a model plasmid?) and in term of diversity (what type of bacteria take up the plasmid?). The method takes advantage of fluorescent marker genes, image analysis, flow cytometry and next generation sequencing.
We revealed that an unexpectedly high diversity of soil microbes can take up broad host range plasmids, with common transfer across the Gram 'barrier'. We next looked for factors that modulate permissiveness and, in particular, identified a taxon-specific effect imposed by metals when supplemented in concentrations that cause partial inhibition of the community metabolic activity.
Overall, our findings highlight the high potential for exogenous plasmids to be transferred to soil microbial communities and indicate that community permissiveness – as affected by environmental conditions- needs to be considered to predict the fate of plasmids in the environment.

[O8] THE ORIGIN OF ACQUIRED ANTIBIOTIC RESISTANCE

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[O9] RHIZOSPHERE MICROBIOMES AND PLANT HEALTH

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The impact of beneficial microorganisms on growth and health of humans, animals and plants is becoming more apparent. Many studies have shown that members of the rhizosphere microbiome can have profound effects on seed germination, seedling vigour, plant growth and development, nutrition and plant tolerance to pathogens and abiotic stress[1]. For the vast majority of rhizosphere microorganisms, however, there is limited knowledge on the mechanisms involved in modulation of plant growth and plant health. For the rhizosphere bacteria, results showed that representatives of the y-Proteobacteria protect plants from pathogen infection by the production of chlorinated peptides[2]. By metataxonomic and metatranscriptomic analyses, we studied the dynamics and in situ activities of the microbial communities in disease suppressive soils. Here, an overview will be given on our current knowledge on how rhizosphere bacteria impact the tolerance to soil-borne pathogens.

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[O10] *PSEUDOMONAS FLUORESCENS* DERIVED CYTOKININS DETERMINE THE BIOCONTROL OF *PSEUDOMONAS SYRINGAE* INFECTION IN *ARABIDOPSIS* - A NOVEL BIOCONTROL CONCEPT

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Considering future demands in plant protection and restrictions in the use of classic pesticides, the development of alternative strategies is a major goal in plant sciences. Biological control of plant diseases by beneficial microbes offers therein a high potential for integrated plant disease management. Mechanisms contributing to such biocontrol phenomena comprise direct effects on pathogens or virulence factors and strengthening the plant. Despite the importance of phytohormones as essential plant immunity regulators, recently including also the classical growth-promoting cytokinins[1,2], their microbial production is not yet considered in biocontrol of diseases. We identified cytokinin production of *Pseudomonas fluorescens* G20-18 as a key determinant of biocontrol of *Pseudomonas syringae* infection in *Arabidopsis*. Treatment with this strain strongly suppressed symptom development and spread of the pathogen, thus maintaining tissue integrity, and ultimately biomass yield. While cytokinin deficient loss-of-function mutants were impaired in controlling the infection, complemented mutants (gain-of-function), exhibiting

restored cytokinin production, showed a similar biocontrol effect as the wild-type strain. The efficiency of the biocontrol effect correlated with the cytokinin levels in planta caused by the different bacterial strains. The analyses of *Arabidopsis* mutants impaired in defence pathways revealed the necessity of functional cytokinin perception in combination with other components such as salicylic acid to fully establish this biocontrol effect. The identification of microbial phytohormones to trigger biocontrol effects offers novel options for the development of successful strategies in plant protection which may be combined, based on cytokinins, with other positive effects such as increased abiotic stress tolerance and plant growth.

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[O11] NOVEL, PSEUDOMONAS-DERIVED ANTIFUNGAL LIPOPEPTIDES FROM A DISEASE SUPPRESSIVE SOIL IN GREENLANDIC POTATO FIELDS

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Potato cultivation in southwest Greenland omits the use of pesticides, relies on limited crop rotations, and despite the presence of plant pathogenic fungi in the soil, has not suffered from severe disease outbreaks. In this presentation, the bacterial strain *Pseudomonas* sp. In5 which significantly contributes to the suppressiveness of soil at Inneruulalik in southern Greenland will be explored. A combination of molecular genetics and genomics coupled with matrix-assisted laser desorption ionization – time of flight (MALDI-TOF) imaging mass spectrometry (IMS) identified a large genomic island encoding the two non-ribosomal peptides nunapeptin and nunamycin, which are key components of the antifungal activity of In5. Bacterial-fungal interaction studies uncovered a complex interaction whereby nunamycin appears most active against Rhizoctonia solani with no antimicrobial effect against the oomycete Pythium aphanidermatum. In constrast, nunapeptin is most potent against *Pythium aphanidermatum* in addition to *Fusarium* sp. To investigate the genetic regulation of both peptides, we have examined the diversity of LuxR-type regulators across the genomic island including upstream and downstream regions flanking the peptide biosynthetic genes. Functional analysis by knockout and complementation studies together with liquid chromatography – high resolution mass spectrometry (LC-HRMS) showed loss and gain of both antifungal activity and peptide synthesis. Current studies are aimed at unravelling further the complex regulation and mode of action of both peptides in order to develop effective microbial biocontrol agents (mBCAs).

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[O12] METABOLIC ENGINEERING FOR BIO-BASED CHEMICALS: TECHNICAL PROGRESS AND COMMERCIAL REALITIES

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Over the last decade several companies – both start-ups and large companies – have explored the commercial opportunities of metabolic engineering and fermentation processes. The interest has grown along with environmental concerns and a desire to replace existing petrochemicals with new, sustainable products. Novozymes is one of the companies that have invested significantly in this area in order to develop new and efficient fermentation processes. Key learnings from both technology and commercial development will be shared.
In this presentation it is shown that (1) rational strain design has come a long way, but it is still very unpredictable, (2) there is still an important role for "traditional microbiological approaches" like mutagenesis/screening and adaptive evolution, (3) there are other considerations like e.g., downstream processing that can make/break a fermentation process and (4) finally, the commercial realities are harsh, and have not been favourable for expansion of the technology platform. Collaboration partners change strategy and volatile oil/sugar prices can change the cost competitiveness of technologies in a short period of time.
Examples from two metabolic engineering projects will be highlighted in the presentation: Malic acid, an acidulant with a potential to be a renewable building block and 3-hydroxypropionic acid, a precursor of the commodity chemical acrylic acid. In both projects the purpose was to design an efficient fermentation process based on glucose as the carbon source.

[O13] MAKING YOGURT SWEET USING NATURAL APPROACHES FOR STRAIN IMPROVEMENT

Ana Rute Neve[1]
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Yoghurt is a popular and widespread fermented milk product, nutritionally rich, and frequently recognized as a health promoting food. Industrial yoghurt is manufactured with commercial starter cultures that contain strains of the lactic acid bacteria *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. During the fermentation carried out by this microbial consortium, the milk sugar lactose is converted mainly to lactic acid, which contributes to the characteristic acidic flavor of yoghurt. To meet consumers´ preferences for taste often additional ingredients, including colors, flavors, sucrose or artificial sweeteners, and texturizing agents are added to the yoghurt. Current market preferences are for yoghurt products with a thick texture and a mild, sweet taste. However, health-conscious consumers are concerned about addition of

food additives and the extra calories from added sucrose to their yoghurt. Thus, the use of improved starter cultures that can ferment milk into a yoghurt with the desired properties (mild, sweet taste) is at demand. Here, Chr. Hansen´s approach to generate such a commercial starter culture for yoghurt will be presented.

[O14] DEVELOPMENT OF AN EFFICIENT GLYCEROL UTILIZING *SACCHAROMYCES CEREVISIAE* STRAIN VIA ADAPTIVE LABORATORY EVOLUTION

Tomas Strucko[1], Katharina Zirngibl[2], Elsayed TT Mohamed[1], Adam Feist[1] and Kiran R Patil[2], Jochen Forster[1]
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With increasing interest in biosustainable technologies, the need for converting available non-saccharide carbon sources most efficiently is emerging. Highly abundant crude glycerol, a major waste residue in biodiesel production, has attracted attention as a cheap carbon source for microbial fermentation processes. The most commonly known microbial cell factory, the yeast *Saccharomyces cerevisiae*, has been extensively applied for the production of a wide range of scientifically and industrially relevant products using saccharides (mainly glucose) as carbon source. However, it was shown that popular wild-type laboratory yeast strains, commonly applied in metabolic engineering studies, did not grow or grew very slowly in glycerol medium.
In this work, an adaptive laboratory evolution approach to obtain S. cerevisiae strains with an improved ability to grow on glycerol was applied. A broad array of evolved strains, which exhibited a significant increase in the specific growth rate and a higher glycerol consumption rate, were isolated. The best performing strains were further analyzed by classical genetics and whole genome re-sequencing in order to understand the molecular basis of glycerol catabolism in yeast. The knowledge acquired in this study may be further applied for rational *S. cerevisiae* strain improvement for using glycerol as a carbon source in industrial biotechnology processes.
This work is a part of the DeYeastLibrary consortium financed by ERA-IB
DeYeastLibrary - Designer yeast strain library optimized for metabolic engineering applications
http://www.era-ib.net/deyeast-library

[O15] DEVELOPING DUAL-ACTION BIOMATERIALS FOR BONE REGENERATIVE APPLICATIONS: ENHANCING CELLULAR GROWTH AND PREVENTING BACTERIAL BIOFILMS

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Titanium (Ti) is a widely used material for surgical implants; total joint replacements (TJRs), screws and plates for fixing bones and dental implants are forged from Ti. Whilst Ti integrates well into host tissue approximately 10% of TJRs will fail in the lifetime of the patient through a process known as aseptic loosening. These failures necessitate revision arthroplasties which are more

complicated and costly than the initial procedure. Finding ways of enhancing early (osseo)integration of TJRs is therefore highly desirable and continues to represent a research priority in current biomaterial design. One way of realising improvements in implant quality is to coat the Ti surface with small biological agents known to support human osteoblast formation and maturation at Ti surfaces. Lysophosphatidic acid (LPA) and certain LPA analogues offer potential solutions as Ti coatings in promoting both human osteoblast maturation and reducing aseptic loosening. My presentation provides evidence for the successful bio-functionalisation of Ti using LPA. This modified Ti surface heightened the maturation of human osteoblasts, as supported by increased expression of alkaline phosphatase. These functionalised surfaces also deterred the attachment and growth of *Staphylococcus aureus*, a bacterium often associated with implant failures through sepsis. Collectively the findings provide evidence for the fabrication of a dual-action Ti surface finish, a highly desirable feature towards the development of next-generation implantable devices.

[O16] VASCULAR CATHETER-ASSOCIATED INFECTIONS CAUSED BY *STAPHYLOCOCCUS AUREUS:* PATHOGENESIS AND PREVENTION

Thomas Emil Andersen[1]
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Being among the bacteria most frequently isolated from colonized catheters, *Staphylococcus aureus* also has one of the highest risks of spreading to the bloodstream leading to sepsis and metastatic infections such as endocarditis, osteomyelitis, meningitis and septic arthritis. The main pathophysiological steps are initial device adherence and biofilm formation, vascular dissemination, reattachment to and colonization of vessel walls at distant locations, extravasation through the endothelium and invasion of tissue. We have worked on elucidating key steps in this disease progression by studying the formation and dissemination of *S. aureus* biofilms, using an in vitro model that simulates the physiological growth conditions near implanted catheter surfaces. Selected results from this study will be presented. Furthermore, the presentation will briefly describe a recently initiated university-industry project that aims to develop catheter materials with thrombosis and biofilm-resistant properties. In this project, a novel impregnation technology enables incorporation of biocompatible hydrogels into existing catheter materials. The hydrogel component in the hybrid material is intended to passively shield from activation of coagulation/inflammation and bacterial adhesion. Drug loading and release properties provided by the embedded hydrogel furthermore allows impregnation with antithrombotic and antimicrobial drugs.

[O17] BIOFILM-MEDIATED ORAL DISEASES

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The oral microbiota is highly diverse and comprises more than 700 different bacterial species, and the composition of the resident oral microbiota is believed to be critically involved in maintenance of oral homeostasis. Periodontitis, affecting around 50% of the adult population in the western world, is the most common chronic inflammatory disease. Periodontitis results in degradation of the tooth supporting tissues, ultimately giving rise to tooth loss. Compositional changes of the local oral biofilm are considered a prerequisite for initiation, progression and maintenance of periodontitis disease activity. Interestingly, oral bacterial DNA from bacteria involved in the pathogenesis of periodontitis has been identified in atherosclerotic plaques, which is why pathogenic alterations of oral biofilms might have implications even in distant body sites. The salivary microbiota, an integral part of the oral microbiota, is considered a conglomerate of bacteria shed from various oral surfaces, and our group has recently reported the composition of the salivary microbiota in patients with periodontitis to differentiate from that of orally healthy individuals. This finding suggests that spill-over of bacterial biofilm from local periodontitis lesions is dispersed in saliva. Thus, saliva-based screening of the oral microbiota might be a valuable tool for identification of periodontitis risk patients at pre-clinical stages, which by early intervention may positively influence both oral- and general health status of the individual.

[O18] DIRECT INTERSPECIES ELECTRON TRANSFER IN METHANOGENIC ENVIRONMENTS

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Electroactive microorganisms have been shown to grow using electrodes either as an electron acceptor (electrogens) or donor (electrotrophs). The property to use solid electron acceptors and donors and therefore release or take up electrons via extracellular mechanisms is poorly understood. Most strains in pure culture are not adaptively evolved to grow on solid electron donors and acceptors, but rather on soluble substrates. Recently we've shown that Geobacter species, which are good current producers (good electrogens), are also able to interact syntrophically with cytochrome-containing methanogens - belonging to Methanosarcinales. In contrast, electrogenic Geobacter could not grow in co-culture with methanogens that do not contain cytochromes, and are strictly H2 or formate utilizers. The interaction between Geobacter and cytochrome-dependent methanogens relies on pili- and extracellular cytochromes of Geobacter – which are crucial for extracellular electron transfer but not soluble electron transfer. Until now direct interspecies electron transfer (DIET) was only described in co-cultures and in digesters treating brewery waste. However, cytochrome-containing methanogens are often times encountered in coexistence with Geobacter in anaerobic, iron rich environments. To investigate if

DIET associations could happen in aquatic sediment, we investigated Baltic Sea sediments rich in Geobacter and Methanosarcina. We significantly enriched these organisms using a conductive support material previously shown to stimulate DIET. During incubations with stable isotopes we verified 13C-transfer between microorganisms and found evidence for syntrophic acetate oxidation based on DIET. This expands our knowledge about DIET environments to marine sediments and challenges current models of methanogenesis in such environments.

[O19] DISCOVERY AND ENVIRONMENTAL SIGNIFICANCE OF PHOTOTROPHIC GEMMATIMONADETES BACTERIA

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Photosynthetic bacteria emerged on Earth more than 3 Gyr ago. Despite a long evolutionary history, the photosynthetic ability has been reported in only a few bacterial phyla. Recently, we isolated a bacteriochlorophyll a-producing strain AP64 belonging to the poorly characterized bacterial phylum Gemmatimonadetes1, named Gemmatimonas phototrophica2, from a freshwater lake in the western Gobi Desert. It contains fully functional type 2 photosynthetic reaction centers but performs a photoheterotrophic lifestyle. Its genome contains a 42.3-kb–long photosynthesis gene cluster (PGC). The organization and phylogeny of its photosynthesis genes suggests an ancient acquisition of PGC via horizontal transfer from purple phototrophic bacteria, raising an interesting point that photosynthetic ability can be transferred between distant bacterial phyla. Yet, we know very little about the distribution and significance of phototrophic Gemmatimonadetes bacteria in the environment. By searching for a biomarker gene, Mg protoporphyrin IX monomethyl ester oxidative cyclase (acsF), in metagenome databases, we identified nine metagenomes that contained sequences possibly originating from phototrophic Gemmatimonadetes bacteria, including three from Danish wastewater treatment plants, two from soil samples, one from a biofilm sample in a sulfur spring, and three from freshwater lake samples. Phylogenetic analysis revealed a high diversity of phototrophic Gemmatimonadetes bacteria in the environment. An almost complete 37.9 kb long photosynthesis gene cluster (PGC) was reconstructed from the Odense wastewater metagenome (OdenseWW). A similar but more fragmented PGC was also retrieved from an Aalborg wastewater metagenome (AalborgWW-2). The gene composition and arrangement of the PGCs assembled from OdenseWW and AalborgWW-2 metagenomes were identical to those in the PGC of G. phototrophica, only differing in a large non-photosynthesis-gene insert present between puhABC and pufBALMC. We conclude that while phototrophic Gemmatimonadetes bacteria seem relatively rare in natural environments, their large diversity and presence in disparate niches suggests a long evolutionary history of photosynthesis in the Gemmatimonadetes phylum.

[O20] ANAMMOX AND THE ”NEW” NITROGEN CYCLE

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Microbes play a key role in controlling the availability of bioavailable nitrogen in natural ecosystems, thereby affecting primary production and the global carbon cycle. Since the early 20th century, three microbial processes – nitrogen fixation, aerobic nitrification, and anaerobic denitrification – were recognized as key steps in the biogeochemical nitrogen cycle. Recent studies have, however, demonstrated novel microbial pathways of nitrogen transformation, and one of these, anaerobic ammonium oxidation coupled to nitrite reduction (anammox) represents a shortcut in the classical cycle and an alternative sink for fixed nitrogen. Anammox is carried out by a specialized group of obligately anaerobic and autotrophic bacteria of the Planctomycetales, known for their exceedingly slow growth and resistance to isolation. Nonetheless, this metabolism is widespread in aquatic systems and ubiquitous in marine sediments and anoxic waters where rates, surprisingly, may even exceed those of classical denitrification. Aiming at a detailed mechanistic understanding of the role of anammox in the marine nitrogen cycle, we are exploring the dynamics of the anammox process, its environmental controls, interactions with other microbial nitrogen transformations, and the ecology of anammox bacteria in marine systems. This presentation provides an overview of our current understanding marine anammox bacteria and their biogeochemical significance.

[O21] GERM THEORY: MEDICAL PIONEERS IN INFECTIOUS DISEASES

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In the 2,500-year history of western medicine, the idea that microorganisms could cause disease in a human being is a comparatively recent one - an accepted notion for only about 125 years. However, the road to the Germ Theory was anything but a logical, straight path. Through a series of biographies, Dr. Robert Gaynes will describe the genesis of the germ theory of disease and present the inside stories of medical pioneers such as Edward Jenner, the discoverer of vaccination, who had to face down scores of naysayers, Louis Pasteur whose chance discovery led him to the idea that virulence of microbes can be altered, Robert Koch who discovered the bacterium that causes tuberculosis, Joseph Lister who found a way to make surgery safe from bacterial infections, and Alexander Fleming who discovered penicillin. Although these discoveries are some of the most important contributions in the history of medicine, surprisingly, nearly all of these contributors struggled to have their work accepted. These inspiring lessons can inform strategies for tackling current crises in infectious diseases as we reexamine everything we thought we knew about developing vaccines to try to produce an HIV vaccine and how we should be using antibiotics. But before we can figure out where we should be going, we should examine how we got here.

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Poster abstracts

Antimicrobial resistance

[P1] ASSESSMENT OF METHODS TO QUANTIFY LIVESTOCK-ASSOCIATED MRSA IN PIG HERDS

Julie Elvekjær Hansen[1], Anna Irene Vedel Sørensen[1], Carmen Espinosa-Gongora[1], Anders Rhod Larsen[2], Jesper Larsen[2], Robert Skov[2], Karl Pedersen[1]

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There is a lack of knowledge about the actual MRSA loads on infected farms. The present study was undertaken to assess methods to quantify LA-MRSA in animals and in the environment in pig herds, to be able to study a potential connection between the level of LA-MRSA and the risk for human exposure, and to estimate the effect of intervention strategies.

Sampling was conducted at a confirmed MRSA infected farm. Nasal samples from sows (n=5), piglets (n=5) and weaning pigs (n=18) were collected with dry swabs, and piglets and weaning pigs were sampled behind one ear with a moistened swab. The swab material was extracted, and MRSA was quantified by direct plating from dilution 10^0 and 10^{-1} onto selective agar. Colonies were counted, and counts expressed as cfu/swab for each sample. Airborne MRSA in the weaning section was quantified by calculation of cfu/m^3 of various air volumes, collected with two different sampling devices, Sampl'air Air sampler and Sartorius MD8 air sampler. Flies were sampled and enriched prior to MRSA determination.

All tested animals (28/28) were found MRSA-positive, with a nasal MRSA load in the range 6.6×10^1-3.9×10^4 cfu/swab and skin load, range 1.1×10^1-2.6×10^5 cfu/swab. Both air sampling devices were able to detect MRSA, but MD8 gave a more stable detection level of MRSA from the different volumes. In sections with animal activity, flies were found to carry MRSA, but found MRSA-negative when no animals were present.

Fast quantification of the animal MRSA load in pig herds is possible by direct plating of either nasal or skin swab samples. The MD8 air sampler provides fast environmental quantification, and it was possible to detect MRSA in flies with animal contact, which demonstrates their potential as environmental MRSA carriers.

[P2] ADAPTIVE LABORATORY EVOLUTION OF *ESCHERICHIA COLI* REVEALS ARDUOUS RESISTANCE DEVELOPMENT TO A COMBINATION OF THREE NOVEL ANTIMICROBIAL COMPOUNDS AND TO THE SHORT AMP P9-4

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Antimicrobial peptides (AMPs) were for long considered as promising new antimicrobials since resistance was not expected. However, adaptive evolution experiments have demonstrated that bacteria may indeed develop resistance also to AMPs. However, we and others hypothesize that the risk of resistance development decreases when two or more compounds are combined as compared to single-drug treatments.

The purpose of this study was to determine if resistance could develop in *Escherichia coli* ATCC 25922 to the peptidomimetic HF-1002 2 and the AMPs novicidin and P9-4. The mentioned compounds were applied alone and in a combination of three in an adaptive evolution approach.

All the lineages exposed to HF-1002 2 and three out of four lineages exposed to novicidin adapted to 32 x MIC, after approximately 350 generations. Conversely, only one out of four lineages exposed to the combination reached adaptation to 32 x MIC. This shows that resistance to novicidin and HF-1002 2, administered alone, developed more easily than it occurred in lineages exposed to the combination of three drugs. This result further supports combinatorial treatment as a way to circumvent resistance development.

Surprisingly, none of the lineages exposed to P9-4 was adapted to 32 x MIC. This indicates that this short-length antimicrobial peptide may be a promising candidate for further optimization for future application in clinical settings.

[P3] SUBLETHAL CONCENTRATIONS OF ANTIBIOTICS CAUSE SHIFT TO ANAEROBIC METABOLISM IN *LISTERIA MONOCYTOGENES* AND INDUCE PHENOTYPES LINKED TO ANTIBIOTIC TOLERANCE

Gitte M. Knudsen[1], Yin Ng[1], Lone Gram[1]
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Introduction: The foodborne pathogen *Listeria monocytogenes* can cause the severe infection listeriosis, which havs up to 20-30% mortality, but if discovered in time, it can be treated with antibiotics. Most antibiotics are bacteriostatic against *L. monocytogenes*. This could be due to the coexistence with antibiotic-producing organisms during its saprophytic lifestyle. To determine if tolerance could be induced or potentially alter virulence, we investigated the transcriptome after exposure to sublethal antibiotic concentrations.

Results: Four antibiotics caused induction of the alcohol dehydrogenase gene *lmo1634* and repression of *alsA* and *lmo1992*, which are involved in acetoin production leading to more ethanol and less acetoin production. This shift in central metabolism indicates a shift from aerobic to

anaerobic metabolism, that could reduce oxidative stress and be a survival strategy in response to antibiotics. We investigated the antibiotic tolerance of a *Δlmo1634* mutant, however; it was comparable with the wild-type in a killing assay. *L. monocytogenes* encodes a second alcohol dehydrogenase *lmo1179,* which potentially could cause a redundant pathway and this is under further investigation. The concentration of acetoin and ethanol are also currently under investigation.

Conclusions: Consistent with other studies, we hypothesize that *L. monocytogenes* when exposed to antibiotics alters its metabolism from aerobic to anaerobic metabolism, and this could prepare the organism to withstand lethal concentrations of antibiotics.

[P4] DETECTION AND CHARACTERIZATION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) AND METHICILLIN-SUSCEPTIBLE *S. AUREUS* (MSSA) FROM DANISH RETAIL MEAT PRODUCTS

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Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) has increasingly been detected from meat products and has created more and more attention from a health food safety perspectives[1]. This study investigates the occurrence and genetic characteristics of MRSA and MSSA from Danish retail meat products.

Methods: Meat samples were purchased from Danish supermarkets, including turkey meat (non-Danish origin; n=23), chicken breast (n=21), chicken legs (n=21) and pork chops (n=20). *S. aureus* was isolated by selective media, identified by MALD-TOF MS, checked for presence of the *mecA* gene by spa-*mecA* multiplex PCR, sequenced for *spa* type and further screened for antibiotic resistance according to the guideline of Clinical and Laboratory Standards Institute (CLSI).

Results: 18 of 85 meat samples were -positive including 11/23 of turkey meat, 4/20 of pork chops, 2/21 of chicken breast and 1/21 of chicken leg. 68% of *spa* types corresponded to CC398 (*spa* t034, t011, t108 and t2582), 11% to CC8 (t008) and one isolate to t1430. MSSA were detected from 48 of 85 meat samples. The predominate *spa* types of MSSA were t034 (CC398) and t3478 (CC5), followed by t091 (CC7), t1333 (CC30), t273 (CC1), t337 and t1273, respectively.

Conclusions: The large majority of tested retail meat samples carried *S. aureus* (78%). Turkey meat has the highest occurrence of MRSA, followed by pork chop and chicken meat products. The most commonly found *spa* type is t034 (CC398) from both MRSA and MSSA isolates.

References:

[1]Normanno, G., et al., Occurrence, characterization and antimicrobial resistance of enterotoxigenic *Staphylococcus aureus* isolated from meat and dairy products. *International Journal of Food Microbiology*, 2007. 115(3): p. 290-296.

[P5] ENCAPSULATION OF ESSENTIAL OILS FOR USE IN FOOD PRESERVATION

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Introduction: Plants produce antimicrobial compounds that could potentially replace synthetic chemicals in food preservation. Essential oils contain aromatic compounds, and some of these work in defense of the plant. Application of essential oils in food is limited by their effect on taste and smell, as addition of oils in effective antimicrobial concentrations will impact the organoleptic properties of the food. We therefore need to modify the oils to increase their antimicrobial effect.

The aim of this project is to encapsulate an essential oil in order to increase the contact between bacteria and oil. Encapsulation of essential oils was obtained by production of simple and coated emulsions that were spray-dried in order to obtain a solid powder.

Methods: Emulsions were characterized in terms of size, zeta-potential and loading capacity. Furthermore, the morphology was examined by scanning electron microscopy and confocal laser scanning microscopy, which showed that coated emulsions tended to aggregate. We measured release profiles by dialysis followed by UV-vis spectroscopy.

The efficacy of emulsions in killing bacteria was investigated by Minimum Bactericidal Concentration Assays against *Escherichia coli* K12 and *Listeria monocytogenes*.

Results and conclusions: The encapsulation was successful in making the oil more effective for both bacteria tested. Coating had either no effect or a negative effect, which was surprising. The reason might be found in aggregation of coated emulsions that was not seen for simple emulsions.

For comparison, the developed system is not as effective as nisin, which is another natural antibacterial, against Gram-positive bacteria. However, nisin is not effective against Gram-negative bacteria, which is where our system proved to be more effective. Therefore, we believe that the developed system complements very well what already exists.

[P6] REVERSIBLE ANTIBIOTIC TOLERANCE INDUCED IN *STAPHYLOCOCCUS AUREUS* BY CONCURRENT DRUG EXPOSURE

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Introduction: Resistance of *Staphylococcus aureus* to beta-lactam antibiotics has led to increasing use of the glycopeptide antibiotic vancomycin as a life-saving treatment for major *S. aureus* infections. Coinfection by an unrelated bacterial species may necessitate concurrent treatment with a second antibiotic that targets the coinfecting pathogen.

Methods: Screening of antibiotic compounds inducing transient cross resistance to other antibiotics was done using MIC susceptibility testing and agar plating. Epigenetic induction of vancomycin tolerance by colistin was subsequently investigated using DNA microarray combined with susceptibility testing of defined mutants.

Results: While investigating factors that affect bacterial antibiotic sensitivity, we discovered that susceptibility of *S. aureus* to vancomycin is reduced by concurrent exposure to colistin, a cationic peptide antimicrobial employed to treat infections by Gram-negative pathogens. We show that colistin-induced vancomycin tolerance persists only as long as the inducer is present and is accompanied by gene expression changes similar to those resulting from mutations that produce stably inherited reduction of vancomycin sensitivity (vancomycin-intermediate *S. aureus* [VISA] strains).

Conclusions: As colistin-induced vancomycin tolerance is reversible, it may not be detected by routine sensitivity testing and may be responsible for treatment failure at vancomycin doses expected to be clinically effective based on such routine testing.

[P7] NOVEL PATH TOWARDS COLISTIN RESISTANCE IN *PSEUDOMONAS AERUGINOSA* DURING CHRONIC INFECTION INVOLVES POLYMORPHISMS IN UNCHARACTERIZED GLYCOSYLTRANSFERASE GENE

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Introduction: Antibiotic resistance development in the gram-negative bacterium *Pseudomonas aeruginosa* is an increasing problem. The effect of colistin, one of the few last resort drugs commonly given to cystic fibrosis (CF) patients, is dependent on the lipopolysaccharide (LPS) structure. We have identified a novel gene cluster, which is involved in colistin susceptibility in chronically infecting *P. aeruginosa* strains. The gene cluster contains two uncharacterized glycosyltransferases and a gene of unknown function. During chronic infection of CF patients one of the glycosyltransferase genes is prone to mutation.

Methods: The glycosyltransferase single nucleotide polymorphism (SNP) was reverted to the reference genotype in a clinical isolate and in parallel introduced into the laboratory reference strain PAO1 to provide a clear background for mutational analysis. We evaluated minimal inhibitory concentration by microbroth dilution, virulence in an amoebae model and LPS structure by visualization in a silver-stained gel.

Results: Reversion of the SNP to reference genotype resulted in increased colistin susceptibility, reduced virulence in an amoebae model and altered LPS structure. The results indicate that this glycosyltransferase polymorphism is needed for the clinical strain to be fully virulent. However, introducing the SNP into PAO1 did not result in altered phenotypes. These results reveal this uncharacterized glycosyltransferase as a novel *in vivo* path to colistin resistance by LPS modification.

Conclusions: Colistin resistance development *in vivo* occurs via multiple paths. Here a novel pathway for the development of colistin resistance was described. It involves mutations in a hitherto uncharacterized glycosyltransferase.

[P8] IS THE RIBOSOME TARGETED BY ADAPTIVE MUTATIONS?

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Introduction: RNA polymerase and ribosomes, composing the macromolecular synthesis machinery (MMSM), carry out the central processes of transcription and translation, but are usually seen as mechanical elements with no regulatory function. Extensive investigations of gene regulation and the high degree of evolutionary conservation of the cellular MMSM tend to support this view. However, under certain selective conditions the machinery itself may be targeted by adaptive mutations, which result in fitness-increasing phenotypic changes. Here we investigate and characterize the role of ribosomal mutations in adaptive evolution.

Methods: Several mutations in ribosomal genes have been identified in the genome analysis of nearly 700 *Pseudomonas aeruginosa* isolates from infected cystic fibrosis patients. Among these mutations we have repeatedly identified insertions, deletions and substitutions in specific ribosomal genes. The bacterial phenotypes of the mutated strains will be investigated.

Results: Preliminary assays show that mutant strains have reduced growth rate and an altered antibiotic resistance pattern. The selection for mutations in ribosomal protein genes is partly explainable by the antibiotic treatment of the patient. However, other mutations cannot be directly associated with antibiotic resistance.

Conclusions: Clarification of the potential pleiotropic consequences of the 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.

References:
Marvig *et al.* Nature Genet. 2015 Jan; 47(1):57-64.

[P9] OUTER MEMBRANE VESICLES AS A POTENTIAL VACCINE CANDIDATE IN COMBINATION WITH THE PUTATIVE ANTIGENS GTXA-N AND FLFA FOR UNIVERSAL VACCINATION AGAINST *GALLIBACTERIUM ANATIS*

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Introduction: *Gallibacterium anatis* is a cause of salpingitis and peritonitis in poultry, resulting in decreased egg production and increased mortality worldwide. High antigenic diversity and a high level of antibiotic resistance make prevention and control of disease difficult. Outer membrane vesicles (OMVs) are produced by virtually all Gram negative bacteria, and several studies have shown their potential as vaccine candidates. Current experiments aim at exploring the potential of OMVs as a cost-efficient alternative to traditional vaccines.

Methods: To produce OMVs in large amounts, we have created a Δ*tolR* mutant of *G. anatis.* A previous large study has identified the recombinant protein GtxA-N and the fimbrial subunit protein FlfA as promising vaccine candidates (Bager et al., 2014). In current experiments the Δ*tolR* OMVs is used in combination with GtxA-N and FlfA as vaccine. Protective potential will be evaluated after a challenge infection with *G. anatis*, and antibody production will be evaluated by ELISA. Immunoreactive components will be identified using 2-dimensional western blot.

Results: The Δ*tolR* mutant of *G. anatis* was engineered and shown to release large quantities of OMVs compared to the wild-type (Bager et al., 2013). In the currently running study chickens were vaccinated twice with OMVs together with recombinant GtxA-N or FlfA. The potential as vaccine is currently being assessed.

Conclusions: Future studies will focus on the use of the OMVs as antigen carriers for efficient delivery of cross-protective antigens. The use of Δ*tolR* OMVs could offer a promising and cheap alternative to traditional vaccines.

References:
Bager *et al.* 2013. Vet. Microbiol., 167:565-72; Bager et al., 2014, Vet Res., 45:80.

[P10] STAPHYLOCOAGULASE, AN EXPLOITABLE INTRA- AND INTERSPECIFIC PUBLIC GOOD

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Staphylococcus aureus and *Pseudomonas aeruginosa* are major causes of community–acquired infections and two of the leading causes of nosocomial infections. The ability of these pathogens to colonize their hosts depends upon the cooperative production of extracellular factors. Previous studies have shown that *S. aureus* has the ability to "hijack" the host's coagulation cascade by producing the prothormbin-activating protein, staphylocoagulase (*coa*). Given that this phenotype is important during infections, yet subjected to exploitation, we postulated that *coa* is a "public good", a molecule that provides benefit on a both intra- and interspecies level. We used an experimental evolution approach by culturing these pathogens together in a clinically relevant *in vitro* wound model. Our experiments were started using three bacterial strains: a wild-type *S. aureus* that produces *coa*, a *coa* mutant, and a wild-type *P. aeruginosa*. The relative fitness of each strain was monitored while propagating the bacterial populations through several rounds of culturing. We observed that: (i) *coa* is vital in developing a bacterium-derived matrix; (ii) there are fitness costs associated with *coa* production; and (iii) it is a trait that is exploitable on a both inter- and intra-species level. In agreement with our prediction, we observed that *P. aeruginosa* and *coa* mutant displayed enhanced antibiotic tolerance when present with wild-type *S. aureus* in comparison to that in a monoculture. Our results provide explanations as to (1) how such cooperative behaviors can affect the population dynamics and (2) why *coa*-negative *S. aureus* are often isolated from clinical infections.

Biofilm

[P11] RESENSITIZATION OF ANOXIC *PSEUDOMONAS AERUGINOSA* BIOFILMS WITH REOXYGENATION USING 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 oxygen (O_2) depletion due to polymorphonuclear leukocyte activity. The mechanisms involved in the activity of antibiotics on biofilm are not completely clear. However, accumulating evidence suggests that killing by bactericidal antibiotics may involve formation of hydroxyl radicals (OH·) during aerobic respiration, and that lack of O_2 increases the tolerance. Accordingly, we speculate that reoxygenation of O_2-depleted biofilm may improve susceptibility to ciprofloxacin by restoring aerobic respiration required for generation of toxic amounts of OH·. We expected to achieve reoxygenation of O_2-depleted biofilm by hyperbaric O_2 treatment (HBOT) since the enhanced O_2 partial pressure increases the driving force for diffusion and the diffusion distance according to Fick's Law. Therefore, PAO1 biofilms were established in LB media with agarose and nitrate in microtiter plates for 3 days. Fresh LB media with nitrate was replaced on top of the biofilm daily. 3-day-old biofilms were treated with ciprofloxacin in two-fold dilutions from 0.0625 to 16 mg/L. The biofilms were further incubated for 2-4 hours ± HBOT (100% O_2, 2.8 bar). Successful reoxygenation of the biofilm during HBOT was documented by the O_2 indicator methylene blue showing O_2 penetration all through the biofilm after 4 hours. Furthermore, we demonstrated enhanced bactericidal activity of ciprofloxacin in *P. aeruginosa* agarose biofilm during HBOT after 4 hours of treatment. In conclusion, we have shown that reoxygenation of anoxic biofilm by HBOT enhances the bactericidal activity of ciprofloxacin presumably by engaging aerobic metabolic pathways associated with increased susceptibility. We suggest that combining ciprofloxacin with HBOT may be considered for treatment of *P. aeruginosa* biofilm infection.

[P12] TREATMENT OF HOSPITAL WASTEWATER WITH A COMBINED ACTIVATED SLUDGE AND BIOFILM PROCESS, HYBAS™

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Introduction: Hospital wastewater contributes with input of pharmaceuticals into municipal wastewater. The combination of suspended activated sludge and suspended biofilm (Hybas™) is suggested as treatment for improved removal of pharmaceuticals from hospital wastewater.

Methods: A pilot plant was constructed with a series of Hybas™ reactors to investigate the potential removal of pharmaceuticals. General parameters were measured (DOC, COD and nitrification), and both batch and continuous flow experiments were conducted for degradation of pharmaceuticals.

Results: Removal of organic matter and nitrification mainly occurred in the first reactor. Pharmaceuticals were efficiently removed, with the highest removal rate in the first reactor. However, when normalized to biomass on carriers, the last reactor showed the highest removal rates. In the batch experiment 16 out of 26 compounds were assessed to degrade more than 20% of the respective pharmaceutical within the Hybas™ system. Several pharmaceuticals known to be recalcitrant in activated sludge treatment were significantly degraded including some iodinated contrast media. The continuous flow experiment showed similar results. However, some pharmaceuticals had negative elimination efficiencies, which can be ascribed to pharmaceuticals excreted as conjugates and are de-conjugated during wastewater treatment.

Conclusions: The tested combination of sludge, Hybas™, and MBBR showed removal of not only COD and nitrogen, but also normally recalcitant pharmaceuticals. This system can therefore be a solution for hospital wastewater treatment.

References:

Casas et al 2015, Science of the total environment 530-531. Casas et al, 2015, Water Research 83.

[P13] STRONG BACTERIAL FLOCS ARE IMPORTANT FOR FILTRATION PROPERTIES OF MEMBRANE BIOREACTORS

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Introduction: Bacterial consortia in the form of flocs play a key role in wastewater treatment. Recently, membrane bioreactors (MBRs) made their entry into the field due to the advantages of high effluent quality and low footprint. However, in the conventional activated sludge process there is a selection for flocculated bacteria, while dispersed bacteria are removed with the effluent. For MBR biomass, single bacteria will be retained by the membrane, so a high content of planktonic cells and soluble extracellular polymeric substances (EPS) may be observed (Christensen et al., 2015). The aim of this project was to investigate whether increased levels of planktonic bacteria deteriorate the membrane performance due to fouling.

Methods: A submerged flat sheet membrane laboratory scale set-up was used to test the filtration properties of different fractions of biomass and model solutions. Key filtration parameters were modelled and bacteria were visualised using fluorescent microscopy.

Results: Filtration of the supernatant with single cells resulted in poor filtration properties in terms of flux decrease over time compared to the flocculated biomass. Increasing the number of planktonic cells resulted in a higher degree of fouling.

Conclusions: We demonstrated that the floc properties are very important for membrane performance in MBRs and that particularly the planktonic cells cause problems. Future studies should establish methods to ensure good flocculation.

References:

Christensen, M.L., Keiding, K., Nielsen, P.H., Jørgensen, M.K., 2015. Dewatering in biological wastewater treatment: A review. Water Res. doi:10.1016/j.watres.2015.04.019.

[P14] H_2S PRODUCTION BY *PSEUDOMONAS AERUGINOSA*

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Introduction: *Pseudomonas aeruginosa* is a Gram-negative pathogen, which is known to cause chronic infections in humans. During infection *P. aeruginosa* is likely exposed to oxidative stress during the encounter with phagocytes and antibiotic treatment. We speculate that *P. aeruginosa* can protect itself against oxidative stress by producing H_2S. It has long been recognized that *P. aeruginosa* is able to produce H_2S from cysteine, but even though *P. aeruginosa* contains genes involved in assimilatory sulphate reduction, so far H_2S production by assimilatory sulphate reduction has not been reported. Therefore, we have investigated if *P. aeruginosa* is able to produce H_2S from sulphate, sulphite and thiosulphate in addition to H_2S production from L-cysteine.

We studied the pH (from 5.5 to 8), time and ABTG medium to generate H_2S from L-cysteine, sulphate, sulphite and thiosulphate by using an amperometric H_2S microsensor. A significant increased H_2S accumulation was detected in samples with pH 6.5-8 in ABTG medium supplemented with L-cysteine and Sulphite.

Methods: H_2S concentrations were recorded with an amperometric H_2S microsensor mounted in a motorized PC-controlled profiling setup.

Results: We saw significantly increased H_2S accumulation in PAO1 cultures supplemented with L-cysteine. Statistical significance was determined by using a two-way ANOVA test. *, ($p<0.05$)

Conclusions: We have shown that our methods enable measurements of accumulation of H_2S in cultures of *P. aeruginosa* in ABTG supplemented with L-cysteine, as expected**.** We intend to investigate the effect of oxidative stress on H_2S production by *P. aeruginosa*, by comparing the effect of supplemental sulphate, sulphite and L-cysteine on the H_2S accumulation during oxidative stress.

[P15] OVEREXPRESSION OF FUNCTIONAL AMYLOID IN *PSEUDOMONAS AERUGINOSA* STRONGLY AFFECTS PHYSIOLOGY AND BIOFILM FORMATION

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Introduction: Recently, we described a novel functional amyloid operon (*fap*A-F) within the *Pseudomonas* genus[1]. Overexpression of the *fap* operon leads to enhanced aggregation. It is, however, not known if a regulatory connection exists between functional amyloids and biofilm formation or virulence. To answer this, the facultative pathogen *P. aeruginosa* PAO1 wild-type (PAO1wt) and a Fap overexpression mutant (PAO1pFap) were compared.

Methods: Samples were characterised by electron and confocal microscopy. Additionally, the proteome was quantitatively analysed by LC-MS/MS.

Results: Microscopy of the cultures showed the deployment of the Fap fibrils on the PAO1pFap cell surface. Furthermore, clear cell aggregation could be observed. Label-free protein quantification revealed significant physiological adaptations as more than 500 proteins significantly changed in abundance between PAO1wt and PAO1pFap (p-value < 0.05, twofold change, ~2800 proteins quantified).

Conclusions: The non-mucoid *P. aeruginosa* PAO1[2] could be converted into an immobile and mucoid phenotype by overexpression of Fap fibrils, although the functional amyloids themselves likely do not have any regulatory function. Many of the proteomic changes are associated with pathology and biofilm formation, which suggests the possible importance of these amyloid structures in chronic infections of pseudomonads.

References:
[1]Dueholm, M. S. *et al.* Functional amyloid in Pseudomonas. *Mol. Microbiol.* **77,** 1009–1020 (2010).
[2]Wozniak, D. J. *et al.* Alginate is not a significant component of the extracellular polysaccharide matrix of PA14 and PAO1 *Pseudomonas aeruginosa* biofilms. *Proc. Natl. Acad. Sci. U. S. A.* **100,** 7907–7912 (2003).

[P16] EVALUATION OF A COMMERCIAL BROAD-RANGE PCR ASSAY FOR IDENTIFICATION OF BACTERIA IN BIOFILM INFECTIONS

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Introduction: Biofilms are known to cause persistent infections in the human body. The gold standard for diagnosing infections is culturing. However, biofilms are, in contrast to planktonic bacteria, difficult to culture[1]. An alternative to culturing is broad-range PCR amplification of conserved genes with variable regions in order to identify the pathogens. The purpose of this study was to evaluate a commercial semi-automated broad-range assay for identification of pathogens in biofilm infections.

Methods: Tissue samples from patients suspected of having a biofilm infection were collected under sterile conditions and cultured at the Department of Clinical Microbiology, Rigshospitalet. Tissue samples were subsequently processed with the Universal Microbe Detection (UMD) SelectNA kit (Molzym, Germany). During this process human DNA was degraded and DNA from the microorganisms was extracted in the SelectNA instrument. A real-time PCR that amplifyies the 16S was performed and positive samples sequenced.

Results: 21/37 samples were positive with the UMD assay, and 10/37 were positive by culture. Comparing the two methods, 57% of the samples gave the same result with both methods, 32% were positive with the UMD assay but culture negative, 3% were UMD negative and culture positive, and in 8% different organisms were identified. Half of the UMD positive and culture negatives were considered to be true positives, and the remaining were either possible pathogens or contaminants.

Conclusions: The UMD kit showed a higher positivity rate than culturing and identified additional true pathogens. The assay shows promise for detection of bacteria in biofilm infections. Currently, more samples are being processed.

References:
[1]Costerton et al. 2011 PMID 21204998

[P17] CARBON SOURCE INFLUENCES POPULATION HETEROGENEITY IN *PSEUDOMONAS PUTIDA* KT2440 BIOFILMS

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Introduction: *Pseudomonas putida* is well known as a potential cell factory for many different biochemicals. Biofilm-based production can be advantageous for possibly toxic products due to increased chemical tolerance and robustness. Biofilm cells frequently differentiate, which challenges the benefits of biofilm-based production, and knowledge about factors driving the heterogeneity is therefore of importance.

Methods: Biofilm flow chamber systems connected to confocal laser scanning microscopy were used to study biofilm structures of *P. putida* KT2440 at different carbon conditions. Subsequent plating of mature biofilm allowed for variant selection followed by pheno- and genotypic analysis.

Results: Structure and cell differentiation in mature *P. putida* KT2440 biofilms were highly dependent on the type of carbon source utilised. Low glucose concentrations (0.3 mM – 10 mM) did not alter biofilm structures nor motility, biofilm capability nor growth rate. However, increasing the glucose concentration (15 - 30 mM) introduced filamentous cell structures, as well as variations in colony morphology.

Filamentous structures were also observed when using citrate (1 mM – 50 mM), and different morphotypes appeared. These morphotypes showed a large variation in swimming motility, biofilm formation and growth rate, and whole genome sequencing revealed alterations in cyclic-di-GMP-related genes involved in biofilm development.

Conclusions: Low concentrations of glucose seem to allow a more homogenous *P. putida* KT2440 biofilm. In contrast, citrate as a carbon source induces cell differentiation and seems associated with an apparent selection for mutations of genes involved in cyclic-di-GMP-mediated global regulation.

[P18] TAGGING OF BACTERIA WITH FLUORESCENT MARKERS FOR STUDIES OF SPATIAL DISTRIBUTION IN A MULTISPECIES BIOFILM

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In natural environments, multispecies biofilm is the predominant bacterial way of living. Biofilms are spatially structured communities containing one or more bacterial species resulting in interactions among the attendants. The high bacterial diversity in soil makes it more suitable for establishment of multispecies biofilm, but the impact of interspecies interactions and spatial organization is less described.

Synergy in biofilm formation was previously demonstrated in a four-species soil consortium composed of *Stenotrophomonas rhizophila, Xanthomonas retroflexus, Microbacterium oxydans* and *Paenibacillus amylolyticus* [1], which could be the result of an organized spatial distribution of the bacteria in the biofilm. To further investigate this, we, in this study, apply different systems to fluorescently tag these four interacting species. *X. retroflexus* and *S. rhizophila* were tagged in with GFP (green fluorescent protein) and mCherry by use of the Tn7-based vector system. Successful construct insertion was confirmed by fluorescence microscopy and flow cytometry analysis. The two Gram-positive bacteria strains, *M. oxydans* and *P. amylolyticus*, will be tagged by use of reporter plasmids carrying fluorescence genes. Biofilms will be inoculated and cultured under shear flow using a BioFlux system. This approach allows investigation of the spatial distribution of the individual species by establishing the biofilm of the tagged versions of the four species.

References:

[1]Ren D, Madsen JS, Sørensen SJ, Burmølle M (2015). High prevalence of biofilm synergy among bacterial soil isolates in cocultures indicates bacterial interspecific cooperation. ISME J, 9. 81-89.

[P19] COMMUNITY SYSTEMS MICROBIOLOGY OF EXOPOLYSACCHARIDES BIOSYNTHESIS AND BIOREFINING FROM USED WATER STREAMS

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Introduction: Alginate-like exopolysaccharides are key structural materials of microbial biofilms. This natural feature is exploited here to examine the mechanisms of biosynthesis of these high-value biopolymers of biotechnological interest, and their biorefining in open mixed microbial cultures from the organic loads carried by wastewater.

Methods: High-throughput molecular methods are used on top of sequencing batch experiments conducted at bench scale with acetate as sole carbon source to investigate whether the key polyphosphate-accumulating phylotype "*Ca.* Accumulibacter phosphatis" involved in the microbial community of granular sludge biofilms engineered for an intensified biological treatment of wastewater can play a significant role in the production of bacterial alginates.

Results: The formation of granular biofilms by "*Ca*. Accumulibacter" was highlighted to connect with exopolysaccharides biosynthesis. The accumulation of alginates from 11 to 39% (±1-5% m/m, dry weight) in the biomass during the transformation of activated sludge flocs into granules directly correlated (r=0.90) with the enrichment of this organism from 0.1 to 60% of the bacterial community. A high-grade enrichment of "*Ca.* Accumulibacter" (>95%) obtained in suspension further displayed an equal substantial content (37%) of alginates.

Conclusions: These results strongly support that "*Ca.* Accumulibacter" might harbour interesting genetic signatures, explored within exopolysaccharide synthetic networks.

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[P20] CHARACTERISATION OF BIOFILM FORMATION IN ACHROMOBACTER SPECIES ISOLATED FROM CYSTIC FIBROSIS PATIENTS

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Introduction: *Achromobacter* is an emerging pathogen in cystic fibrosis (CF). It has the capacity to form biofilm *in vitro*. Biofilm formation increases pathogenesis, yet biofilm formation has not been extensively investigated for this species. The aim of this study is to investigate biofilm formation in clinical isolates of *Achromobacter* to gain knowledge about biofilm composition, dispersal factors, antimicrobial tolerance, and to visualize *Achromobacter* biofilms in sputum from CF patients.

Methods: *Achromobacter* biofilm structure and matrix composition was investigated by staining cells and matrix components and visualized by confocal microscopy. We challenged the biofilm matrix with enzymes and investigated increase in antimicrobial tolerance by comparing minimal inhibitory concentration (MIC) to minimal biofilm eradication concentration (MBEC). Sputum samples from CF patients were embedded in paraffin, sliced and visualized using PNA-FISH.

Results: We characterized *Achromobacter* biofilm formation in two clinical CF isolates. Our findings showed one isolate forming a surface attached biofilm and one forming aggregates encased in polysaccharides. Enzymatic treatment reduced biofilm formation and caused dispersal. Antimicrobial treatment showed increase in MBEC up to 2000 times MIC. *Achromobacter* aggregates in sputum samples in structures resembling biofilm.

Conclusions: Clinical isolates of *Achromobacter* has the capacity to form biofilm *in vitro*. eDNA, polysaccharides and proteins are part of the extracellular matrix. Biofilm formation increases tolerance towards antimicrobials. Visualization of *Achromobacter* in sputum samples suggests that it forms biofilm in sputum within the CF lung.

[P21] THE LIPOPEPTIDE BIOSURFACTANT VISCOSIN ENHANCES DISPERSAL OF *PSEUDOMONAS FLUORESCENS* SBW25 BIOFILMS

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Pseudomonads produce several lipopeptide biosurfactants that have antimicrobial properties, but also facilitate surface motility and influence biofilm formation. Detailed studies addressing the significance of lipopeptides for biofilm formation and architecture are rare. Hence the current study sets out to determine the specific role of the lipopeptide viscosin for *Pseudomonas fluorescens* SBW25 biofilm formation, architecture and dispersal, and to relate *viscA* gene expression with viscosin production and effect. Initially, we compared biofilm formation of SBW25 and the viscosin-deficient mutant strain SBW25Δ*viscA* in static microtiter assays. These experiments demonstrated that viscosin had little influence on the amount of biofilm formed by SBW25 during the early stages of biofilm development. Later, however, SBW25 formed significantly less biofilm than SBW25Δ*viscA*. The indication that viscosin is involved in biofilm dispersal was confirmed by chemical complementation of the mutant biofilm. Further, a fluorescent bioreporter showed that *viscA* expression was induced in biofilms 4 hours prior to dispersal. Subsequent detailed studies of biofilms formed in flow-cells for up to 5 days revealed that SBW25 and SBW25Δ*viscA* developed comparable biofilms dominated by well-defined mushroom-shaped structures. Carbon-starvation was required to obtain biofilm dispersal in this system. Dispersal of SBW25 biofilms was significantly larger than of SBW25Δ*viscA* biofilms after 3 hours, and importantly, carbon-starvation strongly induced *viscA* expression, in particular for cells that were apparently leaving the biofilm. Hence the current study points towards a role for viscosin-facilitated motility in dispersal of SBW25 biofilms.

Bioremediation

[P22] A COMPARATIVE STUDY ON ADHESION AND MINERALIZATION CAPACITY OF BACTERIAL PESTICIDE DEGRADERS INTENDED FOR SAND FILTER INOCULATION

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Introduction: Sand filters inoculated with specific pesticide degrading bacteria may be used to remediate pesticide polluted drinking water. However, the efficiency is hampered by lack of retention and loss of activity of the bacteria. The phenoxy acid herbicide MCPA has been found in Danish groundwater in concentrations exceeding the EU limit for drinking water (0.1µg L-1). We aimed to find a suitable MCPA degrader, demonstrating adherence and degradation potential in sand filters.

Methods: Four MCPA degraders (*Sphingomonas* sp. PM2, *Sphingomonas* sp. ERG5, *Burkholderia* sp. TFD34, and *Cupriavidus* sp. TFD38) were selected and characterized regarding motility, cell surface hydrophobicity, biofilm formation, adhesion behavior, and ability to mineralize MCPA.

Results: PM2 and ERG5 were nonmotile and hydrophobic, while TFD34 and TFD38 were motile and hydrophilic. All four strains showed low biofilm formation on both polystyrene and glass, although significantly higher on glass. PM2 expressed the highest mineralization potential, displaying no lag phase and reaching > 45% MCPA mineralization at all concentrations (1 µg L-1 – 25 mg L-1). PM2 and ERG5 adhered significantly better to sand than TFD34 and TFD38. No clear correlation between motility, hydrophobicity and biofilm formation and the ability to adhere to sand was found. The adhesion and removal of MCPA was tested in saturated sand columns with a constant inlet of 1 mg L-1 MCPA. PM2 completely removed MCPA for a period of 14 days.

Conclusions: Our results demonstrate that an appropriate degrader inoculum may not be found by screening for certain characteristics important to adhesion, but rather by testing the strains adherence and performance in a mini-scale column set up.

[P23] DICHLORPROP MINERALIZATION BY *SPHINGOMONAS HERBICIDOVORANS* MH - DEPENDENCY ON OXYGEN AVAILABILITY

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Introduction: Phenoxy acid herbicides are common contaminant in Danish aquifers with dichlorprop as one of the most persistent types. Bioaugmentation with specific degrader bacteria may help remediation of this pollutant. We studied *Sphingomonas herbicidovorans* MH (SHMH), able to mineralize dichlorprop, regarding how mineralization of the herbicide and the uptake in the bacterial biomass correlates with the availability of O_2.

Methods: SHMH was exposed to ^{14}C-labelled dichlorprop in batch cultures incubated at a range of O_2 concentrations. Produced CO_2 was collected in NaOH traps and the proportion of mineralized dichlorprop estimated from its content of ^{14}C by scintillation counting. Likewise, the ^{14}C content in the spent medium and in the SHMH cells was measured to establish a ^{14}C mass balance.

Results: Mass balances of ^{14}C could be established along the O_2 gradient (1-26%), with total recoveries between 87 and 97% of initial added ^{14}C-dichlorprop. Within a week, mineralization rates of 70-80% were observed at high O_2 concentrations, while at low concentrations (<3% O_2) less the 30% of the added dichlorprop was mineralized and only 2% found in the microbial biomass.

Conclusions: SHMH mineralized dichlorprop effectively at high O_2 concentrations, while at low O_2 concentrations the degradation rates were much less. The present study contributes with relevant knowledge about O_2 conditions needed for effective bioaugmentation *in situ*.

Evolution

[P24] GENOMIC EVOLUTION OF THE MDR SEROTYPE O12 *PSEUDOMONAS AERUGINOSA* CLONE

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Introduction: Since the 1980's the serotype O12 of *Pseudomonas aeruginosa* has emerged as the predominant serotype in clinical settings and in epidemic outbreaks. These serotype O12 isolates exhibit high levels of resistance to various classes of antibiotics.

Methods: In this study, we explore how the *P. aeruginosa* LPS biosynthesis gene clusters evolve in the population by investigating the phylogenetic relationship among 83 *P. aeruginosa* strains and their serotype. In the process we develop a program for *in silico* serotyping of *P. aeruginosa* isolates, the *P. aeruginosa* serotyper (PAst).

Results: While most serotypes were closely linked to the core genome phylogeny we observed horizontal exchange of LPS genes among distinct *P. aeruginosa* strains. Specifically, we identified a 'serotype island' containing the *P. aeruginosa* O12 LPS gene cluster and an antibiotic resistance determinant (*gyr*A^{C248T}) that has been transferred among *P. aeruginosa* strains. Acquisition and recombination of the 'serotype island' resulted in expression of the O12 serotype in the recipient strains.

Conclusions: This observation demonstrate a strong selective advantage for this type of genomic recombination, and suggest that serotype switching in combination with an antibiotic resistance determinant contributed to the dissemination of the O12 serotype in the clinic. This selective advantage coincides with the introduction of fluoroquinolones in the clinic. With the PAst program isolates can be serotyped using WGS data, and dangerous clones like O12 can be identified quickly.

[P25] EPISTATIC MUTATIONS AND UNPREDICTABLE PHENOTYPES IN *PSEUDOMONAS AERUGINOSA*

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Introduction: *Pseudomonas aeruginosa* is an opportunistic pathogen, able to adapt to stressful environments such as the cystic fibrosis (CF) airways. Adaptation of *P. aeruginosa* to the CF environment is associated with phenotypic changes, such as switch in mucoidy, antibiotic resistance and loss of virulence factors. The phenotypic changes arise from mutations in trans-regulatory elements but are nearly impossible to predict from sequence data alone. Often, the combinatorial effects of few mutations in global regulators give rise to unexpected phenotypes. To understand the epistatic effect and how unexpected phenotypes arise from seemingly unrelated mutations, we have studied two mutations in *P. aeruginosa* transcriptional regulators, sigma factor *rpo*D and *alg*T.

Methods: Chromatin immunoprecipitation coupled to deep sequencing (ChIP-seq), surface plasmon resonance, genetic engineering of *P. aeruginos*a.

Results: We have applied surface plasmon resonance to demonstrate that mutations in *alg*T and *rpo*D reduce the affinity for the core RNA polymerase, enabling competing sigma factors to gain access to the transcriptional machinery. ChIP-seq studies have revealed epistatic effects as the combination of mutations causes far greater impact on gene regulatory networks than single mutations, and that this combinatorial effect gives rise to unpredictable phenotypes.

Conclusion: Gene expression is regulated on a small scale by adjusting simple mechanisms such as the affinity between a sigma factor and the RNA core polymerase, but also on a far more complex scale with entire gene regulatory networks being remodelled due to epistatic effect, underlining the importance of epistasis in phenotypic development and bacterial adaptation.

[P26] SUBSTANTIAL MOLECULAR EVOLUTION IN PROLONGED LATENT *MYCOBACTERIUM TUBERCULOSIS* INFECTIONS IN HUMANS

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Introduction: Despite its central role as a reservoir for active tuberculosis disease (TB), latent *Mycobacterium tuberculosis* (*Mtb*) infections and the underlying persistence mechanisms are poorly understood. The *Mtb* genome in latently infected individuals may hold the key to understanding the processes that lead to reactivation and progression to clinical disease.

Methods: We studied genomic relationships among 14 isolates of *Mtb* from historical and recent Danish clinical strain collections, spanning more than three decades, to investigate 6 putative cases of *Mtb* reactivation, inferred from IS6110 profiles. Single-nucleotide polymorphism (SNPs) patterns were analyzed to identify true cases of TB re-activation, as well as the underlying mutational patterns.

Results: Two parallel cases of latent TB reactivation were identified. We found an average mutation rate of 0.2 – 0.3 over 33 years, as well as evidence for distinct processes such as oxidative damage or natural selection having contributed to mutation accumulation.

Conclusions: Our study shows that distinct processes can shape *Mtb* genomes during latent infection. Most importantly, we document substantial molecular evolution of *Mtb* over three decades, with mutation rates similar to observations from cases of active disease. Our study thus emphasizes the importance of identifying and controlling latent cases.

[P27] INVESTIGATION OF CONTINGENT MUTATIONS IN THE RETS-GACAS REGULATORY SYSTEM IN CLINICAL *PSEUDOMONAS AERUGINOSA* ISOLATES FROM CYSTIC FIBROSIS PATIENTS

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Introduction: *Pseudomonas aeruginosa* is a major pathogen infecting the airways of cystic fibrosis (CF) patients. From a collection of 474 full-genome sequenced *P. aeruginosa* isolates from 34 young CF patients[1], we have discovered parallel evolution in the RetS-GacAS regulatory system[2], a key factor in the reciprocal regulation of acute and chronic infection genes. Mutations in this system occur in a sequential manner, such that mutations in *retS* precede mutations in *gacS/gacA*.

Methods: Using genomics, transcriptomics, and metabolomics (Biolog), we have investigated the effects of these mutations in seven clinical isolates from two patients with two distinct clone types of *P. aeruginosa*.

Results: Mutations in the RetS-GacAS regulatory system have a major effect on the behavior of *P. aeruginosa*. Initially, mutations in *retS* cause a shift towards aggregation/biofilm formation, type VI secretion, and phenazine production. Later, mutations in *gacA/gacS* cause a reciprocal shift by increasing expression type III secretion and motility genes, while decreasing expression of the former. Addtionally, Biolog data show clone type specific shifts in carbon source utilization, where one clone type expands and the other decreases the metabolic repertoires.

Conclusions: The sequential nature of the mutations in the RetS-GacAS regulatory system suggests that different behavioral patterns are needed at different times of infection, where the initial mutation in *retS* results in a defensive behavior, enabling establishment of infection. This is then offset by mutations in *gacA/gacS* leading to a more expansive phenotype by dissemination of the infection.

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[P28] STABILITY AND VARIATION IN THE GUT AND FUNGUS COMB MICROBIAL COMMUNITIES IN FUNGUS-GROWING TERMITES

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Introduction: Gut microbes play a crucial role in decomposing lignocellulose to fuel termite societies, predominantly with protists in the lower termites and predominantly prokaryotes in the higher termites providing these services. However, a single basal subfamily of the higher termites, the Macrotermitinae, also domesticated a plant biomass-degrading fungus (*Termitomyces*), but it has remained enigmatic how this additional symbiont has affected the fungus-growing termite microbiotas.

Methods: We investigated the bacterial community compositions in fungus-growing termites by analyzing:
i) termite guts of five genera (nine species) from the Ivory Coast, and comparing these communities to non-fungus-growing termite and cockroach microbiotas;
ii) fungus combs and termite guts from 25 different colonies from four species and four sites in South Africa over two years. We explored the composition of fungus comb microbiotas in details over time, and whether gut bacteria pass through to the fungus combs during comb formation or not;
iii) 134 caste- and age-differing guts from four macrotermitine species from 12 different colonies in South Africa. This was to test if macrotermitine gut microbiota is influenced by host species, geographic locations, colonies or caste/age differences.
High-throughput sequencing of the 16S rRNA gene was used for all microbiota, which allowed profiling with high resolution bacterial assignments.

Results: We found 42 bacteria forming a core community in the fungus-growing termite guts and accounted up to 68% of the entire bacterial reads. This core was more similar to cockroaches than to other termites, with signals of termite phylogenetic ancestry on gut microbiotas. Large proportions of gut bacteria are detected in the combs, suggesting that these bacteria are likely transferred to fungus combs during comb formations by termite faeces. However, comb communities were more variable compared to the guts, probably because combs are more environmentally exposed and receive an influx of bacteria from

the surroundings, suggesting that comb microbiotas are also influenced by time and environmental conditions. Finally, we found that the gut microbiotas are shaped by termite age and caste more than the species or colony, as gut microbiotas from the same caste in a species are more similar to each other than to gut microbiota from the same species but different castes. This suggests that gut microbiotas are more influenced by the host's age/caste-diet serving the required function.

Conclusions: Our findings suggest that the obligate association with *Termitomyces* has functionally shaped the gut bacterial communities of fungus-growing termites to become more similar to their cockroach ancestors than to other termites. Also, macrotermitine gut microbiotas are hugely influenced by termite castes within a species regardless of colony location, suggesting similar digestive roles played by fungus-growing termite castes in a species. Comb microbiota compositions are not only shaped by gut content after the faecal deposition, but also by time and environmental conditions. Finally, the association with *Termitomyces* has forced Macrotermitinae microbiotas towards a finer adaptation to their lifestyle and a complementarity in the functions.

The better understanding of what microbes characterise these microbiotas will allow for future studies that in targeted ways can explore their roles and impact on the ancient fungus-growing termite symbiosis.

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[P29] PHENOTYPIC PLASTICITY IN THE OPPORTUNISTIC PATHOGEN *PSEUDOMONAS AERUGINOSA*

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Introduction: Evolutionary theory predicts that natural selection will favor individuals who maximize their fitness. In a changing environment, a key mechanism to increase the individual fitness is the ability to adjust behavior in response to changes, also known as phenotypic plasticity. However, how these environmental fluctuations affect a population on a phenotypic and genomic level remains poorly understood. Here we aim to investigate phenotypic plasticity of certain pathogenic traits in our model organism *Pseudomonas aeruginosa*, an organism well known for its extreme adaptability.

Methods: In our experimental design, the bacterial populations are challenged with a changing environment over time. The environment shifts between two different conditions favoring either motility or biofilm formation. The experiment was initiated using a parent wild type *P. aeruginosa* and after a given number of alternating rounds of culturing in swimming agar and static liquid broth, individual clones from the population of bacteria were isolated and their phenotypes characterized.

Results: Our results so far indicates (i) that exposure to varying conditions over time selects for a heterogeneous population of bacteria; (ii) the population diversifies into subpopulations of specialists in motility and biofilm formation, and a small subpopulation of generalists, similar to that of the wild type.

Conclusions: Further studies will uncover which genomic changes these observations are based upon.

General pathogenesis

[P30] COMPARING CULTURE AND MOLECULAR METHODS FOR THE IDENTIFICATION OF MICROORGANISMS INVOLVED IN NECROTIZING SOFT TISSUE INFECTIONS

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Introduction: Necrotizing soft tissue infections (NSTIs) is a group of infections of all soft tissues. NSTI involves necrosis of the afflicted tissue and is potentially life threatening. The gold standard for diagnosis is culture; however molecular methods may potentially identify additional species.

Methods: Tissue samples (n=20) obtained after debridement of patients (n=10) with NSTI were analyzed by standard culture, peptide nucleic acid fluorescence *in situ* hybridization and multiple molecular methods, including quantitative PCR, direct 16S rRNA gene Sanger sequencing, 16S rRNA gene clone library, Ibis T5000 biosensor and 454 pyrosequencing.

Results: For 70% of the surgical samples microorganisms were identified by culture. Some samples did not result in growth (presumably due to administration of antibiotics prior to sampling). The molecular methods identified microorganisms in 90% of the samples, and frequently detected additional species compared to culture.

Half of the patients were found to be infected with *Streptococcus pyogenes*, and several atypical findings were also made including infection by a) Acinetobacter *baumannii*, b) *Streptococcus pneumoniae*, and c) fungi, mycoplasma and *Fusobacterium necrophorum*.

Conclusions: The study supports that many pathogens can be involved in NSTIs, and that no specific "NSTI causing" combination of species exists. This means that clinicians should be prepared to diagnose and treat any combination of microbial pathogens.

[P31] THE SMALL COLONY VARIANT OF *LISTERIA MONOCYTOGENES* IS MORE TOLERANT TO ANTIBIOTICS AND GROWS BETTER WITHIN CACO-2 EPITHELIAL CELLS THAN THE WILD TYPE

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Introduction: Small Colony Variants (SCV) of bacteria are a slow growing phenotype with a pinpoint colony morphology and several specific characteristics. In several pathogens they have been linked to recurrent and chronic infections. SCV of *Listeria monocytogenes* can be generated when exposed to sublethal concentration of triclosan, and in this study, we characterized their tolerance to antibiotics and ability to invade and survive in host cells.

Results: Complementation assays showed that SCV E18 phenotype is caused by a mutation in the heme biosynthesis pathway. Although no difference in MIC, the SCV E18 survived significantly better than the wild type N53-1 (one and three $\log_{10}$ higher CFU/ml) when exposed to super-MIC concentrations of most tested antibiotics, indicating a persister-like phenotype of the SCV. While SCV E18 displayed sensitivity towards oxygen, it was significantly more tolerant of 20mM H_2O_2 as compared to the wild type, with 6.3 $\log_{10}$ CFU/ml and 3.7 $\log_{10}$ CFU/ml, respectively. The SCV E18 had lower survival rate in unactivated macrophages, however, it was able to survive and multiply to almost 100-fold higher CFU/ml than the wild type in CaCo-2 epithelial cells.

Conclusions: This study is the first to demonstrate that the persister-like SCV phenotype of *L. monocytogenes* potentially could complicate treatment by causing an increase in tolerance towards most of the clinically relevant antibiotics, while also enabling the bacteria to persist in the protected intracellular environment.

Global epidemiology

[P32] *SALMONELLA WELTEVREDEN* IN TILAPIA FISH AQUACULTURE SYSTEMS IN GUANGDONG PROVINCE, CHINA

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Introduction: Fish-pig integrated farms, which use pig manure as aquaculture pond fertilizers, are common small-scale production systems in Southeast Asia. However, the use of faecal manure represents a risk for contamination with *Salmonella* spp. The study aimed to determine the prevalence, serovar and antimicrobial resistance of *Salmonella* spp. isolated in integrated tilapia (*Oreochromis niloticus*)-fish and non-integrated tilapia aquaculture farms in Guangdong province, China .

Methods: A total of 78 samples (9 pig feed, 20 fish feed, 9 pig faeces, 20 fish mucus and 20 fish intestine) from 10 tilapia-pig integrated farms and 10 non-integrated farms in Guangdong province, China, were analysed.

Results: *Salmonella* spp. were found in fish mucus (20%), fish intestine (40%) and pig faeces (11.1%) from integrated farms, and from fish mucus (40%) and fish intestine (40%) from non-integrated farms. *Salmonella weltevreden* (76.5%) was the most common serovar showing limited antimicrobial resistance, but not found in pig faeces and feed. DNA fingerprinting by the PFGE methods of *S. weltevreden* showed a clonal relationship, which was supported by their similar antimicrobial resistance patterns (sulfamethoxazole, trimethoprim), as well as most isolates harbouring a 147-kb sized plasmid.

Conclusions: i) *S. weltevreden* is a common serovar in seafood and could have ability to survive and even multiply in tropical aquatic environments; ii) commercial feed does not seem to be a main source of *Salmonella* for both aquaculture systems, and *Salmonella* Typhimurium seems not to be a pathogen which is transmitted from pig to fish in tilapia-pig integrated farm.

[P33] MULTILOCUS SEQUENCE TYPING AND ANTIBIOTIC RESISTANCE OF *STAPHYLOCOCCUS AUREUS* ISOLATED FROM THE BRAZILIAN DAIRY INDUSTRY

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Staphylococcus aureus is a common cause of food poisoning due to enterotoxin production. This is particularly an issue in the dairy industry, where *S. aureus* can contaminate the product e.g. from raw milk or the handlers. In Brazil, soft cheese is mainly produced in small dairy plants where good hygiene practices can be limited. The aim of this study was to determine if Brazilian dairy plants were contaminated by *S. aureus*, and if any clones were persistent. Four dairy plants were sampled during 8 months (398 samples in total). *S. aureus* (n=66) was found in all the dairy plants but the contamination rate varied between the processing plants. Multilocus Sequence Typing was used to type and assess potential persisting sequence types (ST). Seven known STs (ST1, ST5, ST30, ST97, ST126, ST188, ST398) were identified. Three new STs were identified and they all belong to clonal complex (CC) 1, which was the dominant CC in the investigated dairy plants. However, there were no indications of re-occurring (persistent) STs in the plants. The potential health risk of the isolates was assessed by antibiotic resistance and hemolytic activity screening. Resistance levels were low, and all of the isolates were presumptive methicillin-sensitive *S. aureus*. All of the isolates expressed hemolytic activity. The frequent isolation of CC1 strains in Brazilian dairy plants indicates, despite antibiotic sensitivity, a potential health risk to the human consumer.

Industrial usage of microorganism

[P34] ANALYSIS OF THE MICROBIAL COMMUNITY STRUCTURE IN MESOPHILIC BIOGAS DIGESTERS

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Introduction: Anaerobic digestion (AD) is an important technology in the future bio-based energy production. AD of different types of bio-wastes forms biogas, and this renewable energy source plays an important role in the national strategy phasing out fossil fuels. Despite the increased focus on biogas production, the knowledge about the microbial community in AD is still fairly limited. This also is true for the identification of the operational parameters that catalyze optimum methane production. The aim of the current study was to examine the microbial community structure and the effect of temperature increase in mesophilic AD.

Methods: 13 mesophilic sludge-based anaerobic digesters were sampled, and the microbial community structure was determined using 16S rRNA amplicon sequencing. The samples were treated with propidium monoazide (PMA) prior to DNA extraction for live/dead detection using real-time PCR. The temperature effect on the microbial activity and methane production was examined in the mesophilic temperature range at 35°C, 39°C, and 42°C.

Results: Multivariate analysis of the sequenced samples revealed microbial community structures similar to other Danish mesophilic AD. The PMA treatment of the samples induced a shift in the microbial community structure and the qPCR data revealed the presence of 25% live cells. The most abundant bacterial and archaeal orders were *Anaerolineales* and *Methanosarcinales* (acetoclastic methanogens), and the effect of temperature increase correlated with that of the operation temperature in the full-scale plants.

Conclusions: This study gives new insight into mesophilic biogas production and provides important information about microbial communities in Danish sludge-based AD.

Macro ecology

[P35] SOURCES OF HIGH CONCENTRATIONS OF *CLADOSPORIUM* AND *ALTERNARIA* SPORES IN THE AIR OF COPENHAGEN

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Introduction: High concentrations of fungal spores in urban air can been associated with acute respiratory allergies among sensitive individuals. The precise sources of such spores, in particular for episodes of the allergenic genera *Cladosporium* and *Alternaria*, are subject to scientific debate. We hypothesize that the cause of high concentrations of *Cladosporium* spores can be attributed to the same physical mechanism that causes high *Alternaria* spore concentrations, i.e. emissions from distinct vegetation types followed by local or long distance transport[1].

Methods: This hypothesis is tested by investigating a 10-year 3-hourly record of *Cladosporium* and *Alternaria* spores in the air of Copenhagen, Denmark, along with a dedicated study of emissions from agricultural fields under harvest.

Results: Data analysis revealed potential coinciding LDT episodes almost every year for *Alternaria* and *Cladosporium*, e.g from the main agricultural areas in Central Europe. The emission study showed that cereal fields produced large amounts of *Alternaria*, but low amounts of *Cladosporium* spores, while grass seed harvest produced large amounts of *Cladosporium* spores. It is likely that such harvesting periods can cause clinically relevant levels of fungal spores in the atmosphere, and our findings suggest that the same physical mechanism in the atmosphere can cause episodes with co-exposure of both *Cladosporium* and *Alternaria* when the source area is cropped agricultural fields.

Conclusions: It is evident that, with these insights, clinically relevant episodes of allergenic spore concentrations in city air could be simulated using atmospheric transport models.

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[P36] RETURN SLUDGE SIDE-STREAM – HOW TO CONTROL GAOS AND ENSURE SUCCESSFUL EBPR IN HOT CLIMATES

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Introduction: The efficiency of the enhanced biological phosphorus removal process (EBPR) is affected by the competition for substrate between polyphosphate accumulating organisms (PAOs) that remove phosphate and unwanted glycogen accumulating organisms (GAOs) that do not. A robust method to control the proliferation of GAOs in EBPR plants and ensure stable and successful phosphorus removal is needed.

Here we evaluate the effect of a change in design from traditional EBPR to EBPR with return sludge sidestream (RSS) hydrolysis using pilot tests and full-scale surveys.

Methods: The microbial communities in 24 Danish full-scale EBPR WWTPs have been surveyed by 16S RNA amplicon sequencing for up to 9 years. Here we show that RSS influences the PAO-GAO community and may act as a control mechanism of GAO proliferation.

A MBR pilot plant in Paris was constructed to allow the conversion from EBPR to EBPR-RSS by a simple redirection of flow paths. The microbial composition was analyzed by 16S RNA amplicon sequencing (Albertsen et al. 2015) and correlated with the design change.

Results and Conclusion:

- Wastewater treatment plants with and without RSS show differences in microbial composition in favour of PAOs limiting the abundance of GAOs.
- The full-scale observations are confirmed by pilot plant trials and GAOs are depleted and any proliferation is prevented.
- At HRTs above 12-15 h, the RSS fraction is the main driving force enriching for PAOs.
- At 30˚C the RSS design allows for successful EBPR and control of GAOs.

References

Albertsen, M. et al., 2015. Back to Basics – The Influence of DNA Extraction and Primer Choice on Phylogenetic Analysis of Activated Sludge Communities. PLoS One 10

[P37] THE IMPACT OF ICE NUCLEATION ACTIVE BACTERIA ON WEATHER AND CLIMATE – A MODEL STUDY

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Introduction: Certain bacteria may have an impact on the formation of precipitation[1], as they can nucleate ice at temperatures as high as –2 °C in laboratory tests, in addition to being abundant in relevant atmospheric compartments, including cloud water, precipitation[2], and air. However, in climatic modeling studies, the actual impact of bacteria on ice nucleation and precipitation formation has been questioned, due to their low atmospheric concentration compared to dust[3].

Methods: We investigated the sensitivity of modeled cloud ice and other meteorological parameters to the fraction of cloud droplets containing bacterial ice nucleation particles (INP), and thereby the potential atmospheric impact of bacteria in nucleating ice in mixed phase clouds. For this purpose, a module that calculates the probability of ice nucleation as a function of the fraction of cloud droplets containing different types of INP was implemented in a numerical weather prediction model.

Results: Utilizing historic weather data from Scandinavia, it was found that changing the fraction of cloud droplets containing bacterial INP causes a perturbation in the forecast, which leads to differences in several meteorological variables, including cloud ice, solar radiation and precipitation. The fraction of bacteria required for obtaining a certain amount of cloud ice was determined.

Conclusions: We found that we can predict an impact of bacteria on weather variables, with the effect being most pronounced during convective events in local areas.

References:
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Micro ecology

[P38] INVESTIGATION OF POULTRY MEAT SPOILAGE BY 16S rRNA AMPLICON SEQUENCING

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Introduction: Microbial spoilage is a key factor for the limitations in shelf life of fresh poultry meat. Traditionally spoilage studies have focused on culture-dependent methods, where difficult to culture organisms might be overlooked. The shelf life of fresh poultry varies between 8-16 days. Important parameters for ensuring long shelf life are production hygiene, initial microbial counts, storage temperature and packaging method.

We aim to describe the microbiome on retail chicken during shelf life, and identify bacteria involved in spoilage by culture-independent 16S rRNA amplicon sequencing.

Methods: Samples of whole chicken from 5 different flocks, retail packaged in 80% O_2/20% CO_2 atmosphere, were collected at a Danish slaughterhouse, and stored at refrigeration temperature. Sampling of total microflora was performed during shelf life (0-16 days), analysed by 16S amplicon sequencing (Illumina MiSeq), bioinformatic analysis, psychrophilic viable counts, and sensory acceptability.

Results: Acceptable shelf life varied between 8-12 days for the sampled flocks. When spoiled, the dominating microflora consisted of *Brochothrix* and surprisingly *Vagococcus*, and also *Carnobacterium* and *Janthinobacterium* spp.

Conclusions: Presently Danish slaughterhouses analyse total viable counts and have qPCR for *Salmonella* and *Campylobacter*. In the future we hope to develop a qPCR method for quality control, targeted towards important spoilage bacteria identified, potentially improving shelf life and reducing food waste. We also wish to identify critical points in the production chain, by comparing data from retail poultry, production samples at time of slaughter and further sampling from other slaughterhouses.

[P39] MIMICKING SEAWATER FOR CULTURING MARINE BACTERIA

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Only about 1% of marine bacteria have been brought into culture using traditional techniques. The purpose of this study was to investigate if mimicking the natural bacterial environment can increase culturability.
We used marine substrates containing defined algal polymers or gellan gum as solidifying agents, and enumerated bacteria from seawater and algal exudates. We tested if culturability could be influenced by addition of quorum sensing signals (AHLs). All plates were incubated at 15°C. Bacterial counts (CFU/g) from algal exudates from brown algae were highest on media containing algal polymers. In general, bacteria isolated from algal exudates preferred more rich media than bacteria isolated from seawater. Overall, culturability ranged from 0.01 to 0.8% as compared to total cell count. Substitution of agar with gellan gum increased the culturability of seawater bacteria approximately 100-fold; from 8.5×10^1 CFU/ml to 5.2×10^3 CFU/ml, whereas addition of AHLs did not improve culturability on any of the media.
The substitution of agar with gellan gum shows great promise for increasing culturability of marine bacteria, and further studies are ongoing. The AHLs used in this study were selected based on a previous study determining the most common AHLs produced by marine strains of the Vibrionaceae family. However, their effect on culturability could not be fully explained, so also here further studies are being carried out.

[P40] REGULATION OF INTERTIDAL MICROPHYTOBENTHOS PHOTOSYNTHESIS OVER A DIEL EMERSION PERIOD

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Introduction: Microphytobenthos (MPB) are phototrophic communities of intertidal and shallow subtidal benthic systems, responsible for a significant fraction of the total primary productivity of estuaries and coastal ecosystems.

Methods: Changes in biomass and photosynthesis of a diatom-dominated MPB intertidal community were studied over a diel emersion period using a combination of O_2 and scalar irradiance microprofiling, chlorophyll fluorescence and pigment analysis.

Results: Under constant low light, MPB biomass, O_2 concentrations and volumetric gross photosynthesis rates in the photic zone (0-0.5 mm) increased during the first half of the emersion period, starting to decrease almost 2 hours before tidal inundation, showing that photosynthesis is mainly controlled by changes in the productive biomass determined by cell vertical migration. A diel pattern in RLC photosynthetic parameters α (photosynthetic efficiencies at limiting irradiances) and ETR_{max} (photosynthetic capacities at saturating irradiances) was also observed.

Under high light exposure, lower α, ETR_{max} and sediment O_2 concentrations were found when cell migration was inhibited with the diatom motility inhibitor latrunculin A (Lat A), showing that migration is also used by MPB to maximize photosynthesis by reducing exposure to potentially photoinhibitory light levels. Higher de-epoxidation state in sediment treated with Lat A indicates that the involvement of thexanthophyll cycle in physiological photoprotection is more relevant when cells were inhibited from migrating.

Conclusions: Cell migration seems to be the key factor regulating MPB community-level photosynthesis over a diel emersion period and upon changes in light exposure.

[P41] DYNAMICS IN MICROBIAL COMPOSITION AND FUNCTIONALITY OVER A SEASON IN TWO CONTRASTING ESTUARINE SYSTEMS

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Introduction: In aquatic microbial ecology it remains unclear how bacterial community composition and dynamics are coupled to functionality, and whether this putative coupling varies over the season. In this study we address the questions if bacterial community composition can be linked to community function, and how this coupling is affected by environmental conditions during a season. Surface samples were obtained monthly from two estuaries of contrasting nutrient richness and hydrography.

Methods:
Illumina MiSeq
Extracellular enzyme assay
Biolog EcoPlate™
Bacterial respiration
Bacterial production[^{3}H] thymidine
Flow cytometry
C/FDOM
DOC+Nutrients

Results: Bacterial community composition and dynamics was determined using 16S amplicons, showing marked differences between whole (rDNA) and active communities (rRNA). The community activity was estimated through respiration and production, and the growth efficiency was calculated, showing a variance over the year, between 1-38% and 3-39% for the Great Belt and Roskilde Fjord, respectively. Bacterial activity showed pronounced changes with the season,

with the highest rates occurring in connection with phytoplankton blooms, in spring and autumn, at both stations.

Conclusions: In general, the microbial community of Roskilde Fjord appeared to be the most active over the year, which likely is associated with the nutrient load. Further analyses will address the coupling between bacterial community structure and dynamics in bacterial carbon processing.

[P42] OFF-FLAVOUR IN RECIRCULATED AQUACULTURE SYSTEMS

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Introduction: The earthy off-flavor, geosmin, is a secondary metabolite produced by a small group of bacteria containing the geosmin synthetase gene (*geoA*). Geosmin constitutes a problem for aquaculture as it accumulates in fish and reduces the quality.

Methods: Sequencing and analyzing the distribution of *geoA* has allowed us to identify the bacteria harboring the gene, to estimate their abundance, to evaluate their presence relative to specific environmental and operational parameters in aquaculture systems and to investigate relevant parameters that might induce the gene expressions.

Results: This result show that the *geoA* gene has undergone several horizontal gene transfer events and are found in at least six phylogenetic groups covering 0.1% of the community with only a minor culturable fraction.

Conclusions: Distribution of *geoA* within RAS show that geosmin-producing bacteria are more abundant in the water treatment compartments and show high correlation dependencies with the organic loading of the system. Combinations with amplicon sequencing of the 16S rRNA gene in more than 35 RAS from 5 European countries show clear distributional patterns of the microbial communities and correlations with *geoA* presence. Correlational analysis to various process parameters, RAS designs and operational practices has been initiated in order to identify important influencing parameters governing the distribution of geosmin producers. Such correlations are critical for the future optimization and management of full-scale RAS, and can be used as a diagnostic tool in developing strategies to limit the growth of geosmin-producing bacteria.

[P43] CHITIN DEGRADATION IN MARINE BACTERIA

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Introduction: Chitin is the most abundant polymer in the marine environment and the second most abundant in nature. Chitin does not accumulate on the ocean floor, because of microbial

breakdown. Chitin degrading bacteria could have potential in the utilization of chitin as a renewable carbon and nitrogen source in the fermentation industry.

Methods: Here, whole genome sequenced marine bacteria were screened for chitin degradation using phenotypic and *in silico* analyses.

Results: The *in silico* analyses revealed the presence of three to nine chitinases in each strain, however the number of chitinases did not correlate to chitin degrading abilities on chitin agar. Two glycosyl hydrolase (GH) groups of chitinases were identified: GH18 and GH19. Interestingly, all strains had genes coding for GH19 chitinases, which for a long time were believed to be present only in higher plants. Differences in genes related to chitin degradation were found between *Vibrionaceae* and *Pseudoalteromonaceae* families. The sensor kinase, ChiS, which regulates around 50 genes, was found in all *Vibrionaceae* but not in any of the strains from the *Pseudoaltermonaceae* family indicating that the latter has a different chitin regulatory system.

Conclusions: This study has provided insight into the ecology of chitin degradation in marine bacteria. It also served as a basis for choosing a more efficient chitin degrading production strain e.g. for the use of chitin waste for large-scale fermentations.

[P44] MIDAS FIELD GUIDE – AN ONLINE SOURCE OF INFORMATION ABOUT THE MICROBES OF ACTIVATED SLUDGE

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Introduction: Understanding microbial communities in engineered systems is essential for process optimization. Microbial composition can be described using 16S rRNA amplicon sequencing, and putative function of microorganisms can be proposed by classifying sequences to a genus for which the function is known. Environmental sequences are classified using public databases (e.g. Silva). However, genus names for many organisms present wastewater treatment systems are missing therein.

Results: To improve classification for process important organisms, we have developed MiDAS taxonomy, for which the SILVA taxonomy has been manually curated with annotations for all the abundant and important genera in full-scale activated sludge.

Using MiDAS taxonomy, we have collected key organisms in activated sludge wastewater treatment systems, linked their identity with available information on their function and distribution and included this information in MiDAS field guide (www.midasfieldguide.org).

Conclusions: MiDAS taxonomy gives a solid foundation for the study of microbial ecology of the wastewater treatment processes. The online MiDAS field guide links the identity of genera that are important for the wastewater treatment process to details about their morphology, diversity,

physiology and distribution. This will facilitate a better understanding of the ecology of this important ecosystem.

References

McIlroy et al. MiDAS: the field guide to the microbes of activated sludge. Database. 2015; Vol. 2015.

Microbial interactions

[P45] RNASEQ AS A METHOD TO STUDY MICROBIAL INTERACTIONS ARISING IN THE CYSTIC FIBROSIS AIRWAYS

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Introduction: In previous studies from our laboratory, a *Pseudomonas aeruginosa* lineage, named DK2, has been identified and characterized as highly successful, transmissible and persistent over four decades in cystic fibrosis (CF) patients. This lineage underwent substantial phenotypic and genetic changes over time and therefore provides a unique opportunity to explore the impact of those adaptational pathways on its ability to interact with other pathogenic bacteria such as *Staphylococcus aureus*, a pathogen frequently co-infecting the CF airways.

Methods: We have used a novel method to study interspecies interactions between a CF isolate (2003) from the DK2 lineage and a wild-type *S. aureus* JE2. We grew both strains in mono or co-culture on LB agar, harvested RNA from the colonies after a 24-hour period. Subsequently we performed RNA-seq for the different samples. The data were then compared in a pairwise mode to isolate the transcriptomic profiles for each species. The most differentially expressed genes from both species were validated using real-time quantitative PCR.

Results: Interestingly, the greatest expression change was observed in *S. aureus*, where large clusters of genes associated with virulence were differentially expressed, compared with the monoculture condition, while the *P. aeruginosa* DK2 response was much more discrete with isolated genes differentially regulated rather than whole operons or clusters.

Conclusions: According to our data, *S. aureus* would display reduced virulence in the presence of an adapted *P. aeruginosa* DK2 clone, possibly as a consequence of the multiple hostile forces DK2 encountered over time during its long-term adaptation to the CF airways.

[P46] *S. AUREUS* SECRETES A *P. AERUGINOSA*-INHIBITING SUBSTANCE

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Introduction: Chronic infections are often polymicrobial and *Pseudomonas aeruginosa* and *Staphylococcus aureus* are among the most common bacteria causing these infections. It is known that *S. aureus* often precedes *P. aeruginosa* in chronic infections but that *S. aureus* and *P. aeruginosa* later co-exist[1, 2, 3]. Many have shown that *P. aeruginosa* inhibits *S. aureus in vitro*[4]. However, *S. aureus* is not overgrown by *P. aeruginosa in vivo*, and we therefore hypothesized that *S. aureus* is able to protect itself from *P. aeruginosa*. Thus, we wanted to study if and how *S. aureus* is protected from *P. aeruginosa*.

Methods: As *S. aureus* often is present in infections before *P. aeruginosa*, the effect of *S. aureus* supernatant on *P. aeruginosa* was studied. Supernatants from *S. aureus* (8325) ON cultures with and without glucose were obtained. *P. aeruginosa* (PAO1) was grown with *S. aureus* supernatant and growth and survival of *P. aeruginosa* was determined. Streak assays on plates with and without glucose were carried out to determine how *S. aureus* influences *P. aeruginosa* when not grown planktonically.

Results: We found that *S. aureus* secreted a *P. aeruginosa*-inhibiting substance when grown in the presence of low concentrations of glucose. Planktonic *P. aeruginosa* was completely growth-inhibited by low concentrations of *S. aureus* supernatant and was rapidly killed by higher concentrations. The *P. aeruginosa*-inhibiting effect of *S. aureus* supernatant was pH-dependent; killing and growth inhibition of *P. aeruginosa* was seen when pH≤6.4. *S. aureus* inhibited *P. aeruginosa* on plates with glucose when *S. aureus* was streaked one day prior to *P. aeruginosa*. When *S. aureus* and *P. aeruginosa* were streaked the same time, *P. aeruginosa* inhibited *S. aureus*.

Conclusions: With this study we show that *S. aureus* can secrete a *P. aeruginosa*-inhibiting substance. Many have shown that *P. aeruginosa* can inhibit *S. aureus*, however these results could indicate why *P. aeruginosa* is not able to out-compete *S. aureus* in chronic polymicrobial infections.

References:

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[2]Fazli et al. 2009 PMID 19812273
[3]Rudkjøbing et al. 2012 PMID 22211589
[4]Fugère et al. 2014 PMID 24466207

[P47] NETWORK-BASED SELECTION OF MICROBIOME CONSORTIA AND PATHWAYS

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Introduction: Network-based modelling is an emerging strategy for analysis of microbiome data that can model the interactions in microbiological systems. Integration of expression data and network analysis in breast cancer has improved prediction of tumor metastasis. Also, network-based approaches can automatically select groups of features, i.e. pathways of genes that can predict the disease phenotype, hinting at the underlying biological mechanism in a richer way than simply selecting single genes. In the current work the strategy from Huang et al. is adapted to microbiome data.

Methods: The association of specific network nodes, expression with the disease phenotype is computed as mutual information. "Walking around" in the path of network links can determine groups with mean expression that are highly associated to the disease phenotype.
To test the algorithm, we use two public datasets from the field of rheumatoid arthritis. Scher et al. is a 16s dataset, while Zhang et al. is shotgun sequenced. These two datasets, on two different cohorts, are used to evaluate the generalizability of the algorithm.

Results: Re-implementation of the original software can integrate data from common network algorithms. Benchmark results are still in progress

Conclusions: Final conclusions forthcoming.

References:
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[P48] A NOVEL ISOLATION PROCEDURE TO ISOLATE BACTERIA FROM *PENICILLIUM BILAII* HYPHAE

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Deficiency of phosphorus (P) limits plant production in many soils but plant P nutrition may be assisted by microbial associations. Fungi are important P solubilizers and may function as biofertilizers. Mycorrhiza fungal hyphae are colonized by bacteria with positive impact on fungal growth and performance. However, it is unknown if comparable relationships exist between other fungi and their hyphae associated bacteria. The fungus *Penicillium bilaii* can increase plant-available phosphorus thereby improving plant growth, and the objective of the current study was to characterize bacteria associating with *P. bilaii* hyphae in a close-to-natural soil system. We established a microcosm system to establish fungal-bacterial interactions in the soil. Hyphal growth of *P. bilaii* was established on glass cover slides placed in mesh bags and transferred to soil. After incubation, the presence of hyphae-associated bacteria was confirmed by SYBR Green staining and fluorescence microscopy. Quantification of culturable bacteria from washed, colonized cover slips versus un-colonized controls showed a more than 10^2 fold difference supporting the notion that bacteria isolated from colonized cover slips were indeed hyphae-associated. A strain collection of hyphae associated isolates was established re-isolates eliminated by PCR finger-printing. Representative isolates were subjected to 16S rRNA gene sequencing revealing that the hypha-associated bacteria primarily belonged to the genera *Bacillus* and *Pseudomonas*. Future studies will test if growth and activity of *P. bilaii* can be improved by the hyphae associated bacteria in order to improve plant growth.

[P49] DESICCATION INCREASES THE ENDOGENOUS PRODUCTION OF REACTIVE OXYGEN SPECIES AND INDUCES AN OXIDATIVE STRESS RESPONSE IN *PSEUDOMONAS PUTIDA* MT-2

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Plant growth promoting bacteria may experience desiccation in soil and rhizosphere. Desiccation increases the amount of endogenous reactive oxygen species (ROS) in *Pseudomonas putida* mt-2 in pure culture. However, the impact of desiccation on the eco-physiology and performance of beneficial bacteria in complex soil and rhizosphere environments is understudied. In the current study we constructed a panel of fluorescent bioreporters for oxidative stress to enable future single cell studies in complex samples. Transcriptional fusions between *gfp* and promoters for oxidative stress responsive genes were constructed in the plasmid pSEVA237m and transferred to *P. putida.* In parallel, cells with increased intracellular ROS were identified by staining with the oxidant-sensing fluorescent probe H_2DCF-DA. Fluorescence from the bioreporters as well as from cells stained by H_2DCF-DA was detected by flow cytometry. Exposure to desiccation simulated by addition of polyethylene glycol (PEG-8000) to the media increased the proportion of cells with endogenous ROS production. Furthermore, desiccation led to a clear induction of the *katA* and *ahpC* reporters, and even of the *osmC* reporter cells. Production of exopolysaccharides may play a protective role against oxidative stress in *P. putida*. In alginate and cellulose mutants harboring *katA*, *ahpC* or *osmC* reporter plasmids the populations of GFP-positive cells were larger than in the corresponding wild type reporter cells after exposure to PEG-8000. A comparable difference was observed for the proportion of cells with endogenous ROS production detected by means of H_2DCF-DA. Hence, cells residing in biofilms in the rhizosphere could well be better protected against desiccation-induced oxidative stress than planktonic cells.

Prebiotics / Probiotics

[P50] CONTINUOUS EXPOSURE OF *VIBRIO ANGUILLARUM* TO TROPODITHIETIC ACID: GENETIC CHANGES AND INFLUENCE ON VIRULENCE

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Introduction: The fish pathogen *Vibrio anguillarum* is a major problem in aquaculture causing Vibriosis. Bacteria of the *Roseobacter* clade can antagonize pathogenic vibrios in cultures in live feed such as microalgae, rotifers and *Artemia*, as well as in fish larvae. Therefore, roseobacters could be promising as probiotics in fish rearing. Production of the antibacterial compound tropodithietic acid (TDA) by roseobacters is key in the antagonism of vibrios. However, the effects of continuous exposure to TDA on *V. anguillarum* remain unknown. The purpose of this study was to investigate how prolonged TDA exposure affects *V. anguillarum* focusing on the development of resistance towards TDA and changes in virulence.

Methods: Seven lineages of *V. anguillarum* were exposed to increasing TDA concentrations over 300-400 generations and were subsequently genome sequenced. Virulence of the lineages is currently being tested in fish cell infection trials.

Results: Following exposure, four linages reached 1.75 x wild-type MIC and three reached 1.5 x wild-type MIC. Genome sequencing revealed no major changes in the genomes of the lineages. The only virulence-related gene affected was *fli*M, encoding a flagella motor switch protein. However, mutations in this gene were observed in non-exposed controls as well.

Conclusions: In conclusion, TDA resistance does not appear to develop, and the virulence genes of *V. anguillarum* are unaffected by TDA exposure, supporting the application of TDA-producing roseobacters as probiotics in aquaculture.

[P51] ENZYMATIC PRODUCTION OF HUMAN MILK OLIGOSACCHARIDES

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Introduction: Some hydrolytic enzymes are able to catalyze transglycosylation reactions by transferring sugar residues from a donor to an acceptor molecule. This process could be used to synthesize bioactive oligosaccharides such as human milk oligosaccharides (HMO's) which are notoriously difficult and expensive to produce by chemical synthesis. The aim is to identify hydrolytic enzymes, such as fucosidases, N-acetyl-hexosaminidases and β-galactosidases, which can be used for the synthesis of HMO's.

Methods: Formation of transglycosylation products was detected with TLC and HPLC.

Results: A culture-dependent approach involving screening of isolates from a range of hot springs in East Greenland has been done, and one thermophilic isolate, affiliated to *Paenibacillus dendritiformis,* was shown to produce a transglycosylating β-galactosidase and a transglycosylating fucosidase. These enzymes have been expressed recombinantly, and the enzyme-catalyzed transglycosylation reactions were carried out using lactose, galactose and N-acetyl-glucosamine as potential acceptors.

A culture-independent approach made use of a metagenome to express four putative fucosidases in *Escherichia coli*. The activity was confirmed for three of the four enzymes, and investigations of their transglycosylation potential have been initiated.

Conclusions: Investigations will be carried out in order to identify novel enzymes suitable for enzymatic synthesis of HMO's. Enzyme mediated synthesis of HMO's is a promising way to produce large-scale quantities of highly valuable HMO's for use in infant formulas and nutritional products. However, several challenges need to be addressed, such as process optimization and the need for cheap and available substrates.

[P52] *PHAEOBACTER INHIBENS* AS PROBIOTIC BACTERIA IN NON-AXENIC ARTEMIA AND ALGAE CULTURES

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Bacterial diseases are a major constraint in aquaculture, especially in larviculture. Antibiotics that can control pathogens should be avoided due to risk of antibiotic resistance. We have shown in axenic systems of live larval feed that marine *Roseobacter* clade bacteria can antagonize fish pathogens and improve survival of fish larvae. Both pathogens and probionts are likely affected by the natural microbiota, and the purpose of this study was to determine if the probionts would be effective in non-axenic systems.

The growth and interaction of pathogen (*Vibrio anguillarum*) and probionts (*Phaeobacter inhibens*) were studied in an *Artemia* and a *Dunaliella tertiolecta* challenge setup, and a controlled microbiota of four bacteria isolated from aquaculture was added. *P. inhibens* grew well in *Artemia* and *D. tertiolecta* cultures, also with a background microbiota. *V. anguillarum* was decreased markedly (up to four log units) by *P. inhibens* irrespective in presence of background microbiota.

In aquaculture, the live feed is a well-known potential entry and propagation point for the fish pathogens and, hence adding the probiont at this stage would be a logical stage of introduction. This study demonstrates that probiotic bacteria can be introduced at the stage of live feed and have a pathogen reducing effect in both an *Artemia* and a *D. tertiolecta* challenge setup. This can potentially limit the subsequent use of antibiotics for control of pathogenic bacteria.

Productions organisms

[P53] DIFFERENTIAL EXPRESSION OF SMALL RNAS UNDER CHEMICAL STRESS AND FED-BATCH FERMENTATION IN *ESCHERICHIA COLI*

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Introduction: Bacterial small RNAs (sRNAs) are often expressed in response to changing environmental conditions and function to modulate gene expression. Although chemical stress is routinely encountered in microbial processing applications, the cellular response and the involvement of sRNAs in this process is poorly understood. We have used RNA sequencing to map the *Escherichia coli* sRNome during chemical stress and high cell density fermentations with the aim of identifying sRNAs involved in the stress response and those with potential roles in stress tolerance.

Methods: RNA sequencing libraries were prepared from RNA isolated from *E. coli* MG1655 cells subjected to chemical stress with twelve compounds. The strain was also grown under high cell density fermentation conditions, where cells were harvested in four growth phases.

Results: We have discovered over 250 novel intergenic transcripts, adding to the roughly 200 previously reported sRNAs in *E. coli*. There are 84 and 139 differentially expressed sRNAs under fermentation and chemical stress conditions, respectively. In the latter case, approximately 30 exhibit significant expression changes in multiple conditions, suggesting their involvement in a more general chemical stress response.

Conclusions: This study has revealed a wealth of hitherto undescribed sRNAs and an atlas of expression under 17 growth conditions. A significant fraction of the sRNAs exhibit specific expression patterns during fermentation, and a group of them are differentially expressed in the presence of multiple chemicals, suggesting they may play regulatory roles during these stress conditions. These are candidates for improving stress tolerance and our understanding of the regulatory network during fermentation.

[P54] ABSTRACT WITHDRAWN

[P55] EXPLORING MARINE ENVIRONMENTS TO UNRAVEL TOLERANCE MECHANISMS TO RELEVANT COMPOUNDS

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Production of biofuels and chemicals using microorganisms has been a research driver in the last decades. The approach started with the engineering of metabolic pathways for production of compounds of interest, but it was soon realized that tolerance to the compounds being produced was one of the major bottlenecks of this approach. Since then, tolerance engineering of microbial cell factories along with metabolic pathway engineering has been one of the main research focuses.

Microorganisms with natural tolerance to relevant compounds, such as ρ-coumaric, glutaric and isobutyric acids were isolated from Iranian sediment samples. This was achieved by replica plating the strains on plates containing high concentrations of the mentioned compounds.

Thirty-two samples were analyzed and 96 strains isolated. Isolates with high tolerance were grown in presence of high concentrations of the compounds of interest, HPLC analyses were performed in order to distinguish between compound-degrading and tolerant bacteria. This led to the identification of seven tolerant and non-degrading isolates, the most interesting ones belonging to the genera *Bacillus* and *Pseudomonas*. These will be studied using genomic and transcriptomic approaches to identify the tolerance mechanisms used.

Exploring new ecological niches, as contaminated marine environments allows the identification of naturally tolerant bacteria to the compounds of interest and most likely to the discovery of new mechanisms of tolerance.

[P56] AN ANTIBIOTIC SELECTION SYSTEM FOR PROTEIN OVERPRODUCING BACTERIA

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Introduction: Protein overproduction is a major bottleneck for analyses of membrane proteins and for the construction of cell factories. Screening for optimized protein production can be very time consuming. In this study we show that the coupling of antibiotic resistance to poorly produced membrane proteins of *Escherichia coli* can be used as a fast and simple selection system for protein overproduction.

Methods: We designed an expression plasmid encoding the gene of interest and an additional, inducible antibiotic resistance marker. Both genes were linked by a hairpin structure that

translationally couples the genes[2]. Consequently, high expressing gene variants also allow for higher production of the coupled antibiotic resistance marker. Therefore, high expressing gene variants in a library can be determined either by plating the expression library on selection plates or by growing the library in a liquid culture, while both contain high concentrations of the inducible antibiotic.

Results: We designed libraries for membrane proteins of *E. coli,* based on a recently published technique[1] that promises enhanced protein production by optimizing the nucleotides between the Shine Dalgarno sequence and the start codon, an integral part of the translation initiation region. We successfully tested the expression of these libraries with the antibiotic selection system on plates and in liquid cultures.

Conclusions: We successfully implemented the antibiotic selection system and confirmed enhanced protein production when applying the above-mentioned optimization technique.

References:
[1]Mirzadeh *et al.* (2015), ACS Synth. Biol.; 2. Mendez-Perez *et al.* (2012), Metab. Eng.

[P57] ACCURATE DNA ASSEMBLY AND DIRECT GENOME INTEGRATION WITH OPTIMIZED URACIL EXCISION CLONING TO FACILITATE ENGINEERING OF *ESCHERICHIA COLI* AS A CELL FACTORY

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Plants produce a vast diversity of valuable compounds with medical properties, but these are often difficult to purify from the natural source or produce by organic synthesis. An alternative is to transfer the biosynthetic pathways to an efficient production host like the bacterium *Escherichia coli*. Cloning and heterologous gene expression are major bottlenecks in the metabolic engineering field. We are working on standardizing DNA vector design processes to promote automation and collaborations in early phase metabolic engineering projects. Here, we focus on optimizing the already established uracil-excision-based cloning and combining it with a genome-engineering approach to allow direct integration of whole metabolic pathways into the genome of *E. coli*, to facilitate the advanced engineering of cell factories.

[P58] SMALL RNA REGULATORY NETWORKS IN *PSEUDOMONAS PUTIDA*

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Pseudomonas putida is a ubiquitous Gram-negative soil bacterium with a versatile metabolism and ability to degrade various toxic compounds. It has a high tolerance to different future biobased building blocks and various other stringent conditions. It is used in industry to produce some important chemicals and has a potential to be used as an efficient cell factory for various products. *P. putida* KT2240 is a genome-sequenced strain and a well characterized pseudomonad. Our major aim is to identify small RNA molecules (sRNAs) and their regulatory networks. A previous study has identified 37 sRNAs in this strain, while in other pseudomonads many more sRNAs have been found so far.
P. putida KT2440 has been grown in different conditions which are likely to be encountered in industrial fermentations with the aim of using sRNAs for generation of improved cell factories. For that, cells have been grown in LB and harvested in different growth phases, as well as osmotic, membrane and oxidative stress conditions. RNA sequencing data has been analysed with the open source software system Rockhopper, and it has revealed over 180 putative sRNAs. Most of them (86%) seem to be novel and uncharacterized. The majority of the identified sRNAs are trans-encoded and several 3'UTR derived RNA transcripts have been found, as previously described by Chao et al.
Differential sRNA expression is observed across various growth and stress conditions, suggesting that individual sRNAs are functional and exert their regulatory effects under specific conditions. The sRNA response profiles in different conditions will be presented, and sRNAs important for bacterial adaptation under the studied conditions will be identified.

Risk assessment

[P59] EVALUATION OF A CROSS CONTAMINATION MODEL DESCRIBING TRANSFER OF *SALMONELLA* SPP. AND *LISTERIA MONOCYTOGENES* DURING GRINDING OF PORK AND BEEF

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Introduction: The cross contamination model (Møller et al. 2012) was evaluated to investigate its capability of describing transfer of *Salmonella* spp. and *Listeria monocytogenes* during grinding of pork and beef of varying sizes (50 – 324 g) and numbers of pieces to be ground (10 – 100), in two grinder systems.

Methods: Data from 19 trials were collected. Three different evaluation approaches were applied: *i*) an Acceptable Simulation Zone (ASZ) method compared observed with simulated transfer from the proposed model, *ii*) each trial was fitted and its respective parameter estimates were integrated in a Quantitative Microbiological Risk Assessment (QMRA) model (Møller et al. 2015), and *iii*) the Total Transfer Potential (TTP) was calculated for each of the 20 fitted parameter estimates.

Results: The ASZ showed that the Møller et al. (2012) model could only describe seven of the 19 trials to an acceptable extent. However, all transfer curves could be fitted to the model structure proposed by Møller et al. (2015). A positive correlation was found between QMRA risk estimates and TTP for the individual trials.

Conclusions: Results indicated that transfer estimates were not applicable for unlike processing. QMRA risk estimates and TTP both revealed that risk attribution from grinding was mainly influenced by sharpness of grinder knife > specific grinder > grinding temperature whereas the specific pathogen was of minor importance.

References:
[1]Møller, C.O. de A. et al. (2012): JAM 112:90-98;
[2]Møller, C.O. de A. et al. (2015): IJFM 196:109-125

[P60] QUANTIFYING THE GROWTH VARIABILITY OF PATHOGENIC AND SPOILAGE SPORE-FORMING MICROORGANISMS OF INTEREST FOR CHILLED FOODS USING META-ANALYSIS TECHNIQUES

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To ensure the safety and quality of chilled products with a long shelf-life, it is important to control pathogenic and spoilage spore-forming microorganisms that may grow at refrigeration temperatures, among which *Bacillus cereus* and spoilage *Bacillus* spp. are of particular concern for the industry. Meta-analysis techniques can be a strong tool for evaluating the growth potential of these microorganisms under chilled conditions and for identifying control strategies.

The aim of this study was to perform a meta-analysis of growth rates of spoilage and pathogenic psychrotrophic *Bacillus* spp. found in a publicly available predictive modelling database and to use this information for predicting their growth in chilled products.

ComBase was screened for information on growth of *B. cereus* and spoilage *Bacillus* spp. in dairy matrices and laboratory media at temperatures relevant for refrigerated storage including product abuse by the consumer (0-15^{o}C). The simple square root model of Ratkowsky[1] was fitted to the collected datasets, as previously described[2]. This allowed for the estimation of the mean growth rate as a function of temperature.

The quality of extracted data varied depending on the species of microorganism with best dataset obtained for *B. cereus*. The availability of data for spoilage spore-formers was relatively limited. Quantifying the variability of growth rates as a function of temperature can allow for fail-safe shelf life estimations for chilled products. The meta-analysis also helped to identify areas where limited information is available for controlling psychrotrophic spore-formers.

References:
[1]Journal of Bacteriology, 1982. **149**(1): p. 1-5.
[2]Trends in Food Science and Technology, 2012. **25**(1): p. 34-39.

[P61] ABSTRACT WITHDRAWN

[P62] MODELLING AND PREDICTING GROWTH OF PSYCHROTOLERANT PSEUDOMONADS IN MILK AND COTTAGE CHEESE

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Introduction: Predictive food microbiology models have the potential to evaluate the effect of temperature on microbial growth during distribution as well as be used to determine how product characteristics can be modified to reduce growth to an acceptable level.

Methods: Growth kinetics of psychrotolerant pseudomonads were determined using Bioscreen C experiments, challenge tests, storage trial and literature data. The effect of storage temperature and product characteristics on growth rates (μ_{max}) were described using a simplified cardinal parameter model and the gamma concept. The developed broth/Bioscreen C model included the effect of temperature, pH, NaCl/aw, lactic, sorbic acid and their interaction (Le Marc *et al*., 2002). Then, the reference growth rate parameter (μ_{ref}) was fitted to a total of 35 μ_{max}-values from cottage cheese with cultured cream dressing.

Results: The new models were successfully validated, based on bias and accuracy factor, for 59 growth curves of psychrotolerant pseudomonads in dairy products. The acceptable simulation zone method showed the new model for cottage cheese to successfully predict growth of psychrotolerant pseudomonads at both constant and dynamic temperature storage conditions.

Conclusions: The present study developed and validated mathematical models to predict growth of psychrotolerant pseudomonads in chilled milk and cottage cheese with cultured cream dressing. The cottage cheese model can be used to evaluate the effect of product reformulations on growth.

References:
Le Marc *et al*., 2002. Modeling the growth kinetics of *Listeria* as a function of temperature, pH and organic acid concentration. International Journal of Food Microbiology, 73, 219-237.

[P63] ABSTRACT WITHDRAWN

Soil microbiology

[P64] RESPONSES IN ACTIVE MICROBIAL COMMUNITIES AND EXPRESSION OF IMPORTANT FUNCTIONAL GENES IN FOREST AND AGRICULTURAL FIELD SOIL AFTER WOOD ASH ADDITION REVEALED BY METATRANSCRIPTOMICS

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Introduction: Wood ash is increasingly being produced as a byproduct in different renewable energy processes and is mainly regarded as a waste product due to its content of toxic compounds such as heavy metals. Wood ash does, however contain nutrients and possess soil liming capabilities and wood ash is therefore utilized as a soil amendment. Increased use of wood ash as a soil amendment would lead to the return of more nutrients to the ecosystem where the ash originated from, making the whole process more renewable. Wood ash can, however, alter microbial communities and functions in soil systems and can thereby impact on essential microbe-driven processes involved in e.g. C and N turnover. This could lead to changes in the overall soil quality, but knowledge in this scientific field remains sparse.

Methods: We have set up a microcosm experiment using both agricultural field soil and spruce forest soil incubated at 10 °C for 100 days with different concentration of wood ash added. Soil has been collected during incubation for DNA and RNA extraction and measurements of important soil parameters. Using metatranscriptomics, very detailed insight into changes in the active microbial community (rRNA) from all three divisions of life and the expression of genes (mRNA) will be achieved.

Results: Results from the metatranscriptome will, together with results about changes in different important soil parameters, lead to novel and detailed knowledge on how active microbial communities in two soil systems responds to the addition of wood ash and give knowledge on changes in the expression of functionally important genes in the soils.

Conclusions: This poster will present preliminary results and give an overview of future work to be done.

Total sequencing

[P65] POSSIBILITIES AND OBSTACLES IN RECOVERY OF GENOMES FROM ELUSIVE MICROBES IN COMPLEX METAGENOMES

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Introduction: Metagenomics can be used to access genomes from uncultured bacteria in complex environments, but have limitations when the complexity is high and microdiversity is present. In this study, we explored the potentials and obstacles faced when assembling genomes from complex metagenomes using activated sludge as a model system.

Methods: A brute force strategy was used to enrich bacteria from different functional groups and reduce microdiversity. Activated sludge from a municipal wastewater treatment plant was incubated in batch cultures under six different growth conditions over a period of 7 days. Samples from 12 time points were selected for metagenomics and sequenced on the Illumina platform. Metagenomes with reduced complexity, containing interesting species were used for *de novo* metagenome assembly. The metagenome scaffolds were binned to individual genomes using the multi-metagenome approach.

Results: The short-term enrichments decreased the overall complexity and changed the relative abundances of the different community members, which overall improved the binning. This made it possible to extract quality genome bins from a range of different taxonomic groups. However, natural microdiversity persisted and resulted in highly fragmented genome bins.

Conclusions: The use of short-term enrichments can be used to improve metagenomic binning, but microdiversity prevents the recovery of complete genomes.

[P66] "MICROWINE" - MICROBIAL METAGENOMICS AND THE MODERN WINE INDUSTRY

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Introduction: A diverse, complex, and poorly characterised community of microorganisms lies at the heart of the wine – an industry worth over €220 billion globally. These microorganisms play key roles at all stages of the viniculture and vinification processes, from helping the plants access nutrients from the soil, driving the plants' health through protection against pathogens, to the fermentation process that transforms the must into wine with its complex array of aromas and flavours. Given this importance, an improved understanding of the microbial community and its interplay will have significant effects on the wine industry.

Methods: In recent years, 'Next Generation' DNA sequencing has revolutionised many areas of biology, including microbiology, in particular through conferring the ability to characterise microbes on the deep community scale. To exploit the power of such approaches for the benefit

of the wine industry, we have initiated MICROWINE, a 15 ESR Marie Curie Actions European Training Network. The network is constructed as a close collaboration between industry and academic partners, around the theme of the role of the microbial community in the wine production process.

Samples will be collected from vineries in Europe and beyond to study the role of microbes in the areas from plant protection and nutrition, through wine fermentation process, to final product. The aim is, through multidisciplinary collaborations, to combine microbial metagenomic sequencing with e.g. geochemical metadata and by applying powerful computation analyses to unravel the environmental and microbial forces governing the ancient art of winemaking.

[P67] 16S rRNA GENE SEQUENCING AS A TOOL TO STUDY MICROBIAL POPULATIONS IN FOODS AND PROCESS ENVIRONMENTS – LIMITATIONS AND OPPORTUNITIES

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Introduction: Methodological constraints during culturing and biochemical testing have left the true microbiological diversity of foods and process environments unexplored. Culture-independent molecular methods, such as 16S rRNA gene sequencing, may provide deeper insight into microbial communities and their role in food safety. During method optimization, we have identified several factors which distort the characterization of microbial populations, including DNA extraction methods, DNA polymerases, and most importantly the analyzed fragment of the 16S rRNA gene.

Methods: This study investigated microbial communities in meat and the meat process environment with special focus on the Enterobacteriaceae family as a subpopulation comprising enteropathogens including *Salmonella*. Samples were analyzed by a nested PCR approach combined with MiSeq® Illumina®16S DNA sequencing and standardized culture methods as cross reference.

Results: Taxonomic assignments and abundances of sequences in the total community and in the Enterobacteriaceae subpopulation were affected by the 16S rRNA gene variable region, DNA extraction methods, and polymerases chosen. However, community compositions were very reproducible when the same methods were used.

Conclusions: Altogether, we have shown that conclusions from population studies based on 16S rRNA gene sequencing need to be made with caution. Overcoming the constraints, we believe that population studies can give new research possibilities for e.g. interaction studies, identification and growth of indicator organisms, or source attribution.

[P68] INVESTIGATION OF THE PRIMARY TRANSCRIPTOME OF THE PRODUCTION ORGANISM *PSEUDOMONAS PUTIDA*

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Introduction: *Pseudomonas putida* is a nonpathogenic, Gram-negative bacterium and an excellent model organism for biotechnological applications. Due to its metabolic versatility, *P. putida* can grow in different environments including in extreme conditions. It has several genes to degrade xenobiotic compounds, a capability that renders the bacterium useful in bioremediation. Finally, *P. putida* shows a high potential as a cell factory for the production of several compounds[1].

Methods: Here, a differential RNA-sequencing approach (dRNA-seq)[2] is used to gain new insights into the organization of the *P. putida* KT2440 transcriptome, in the presence of citrate or glucose as sole carbon source.

Results: A total of 7937 putative transcription start sites (TSSs) have been identified. 5' RACE experiments have been performed to confirm putative TSSs, and 5' UTR regions have been investigated for conservative motifs in promoter sequence. Finally, RNA regulatory elements have been identified including putative sRNAs and riboswitches. Selected candidate riboswitch sequences have been tested for the study of the ligand-dependent regulatory mechanisms.

Conclusions: By determination of the 5'-ends of transcripts, our study has allowed for the investigation of several biological features of *P. putida*.

References:
[1]I. Poblete-Castro, J. Becker, K. Dohnt, V. M. dos Santos, and C. Wittmann, *Appl. Microbiol. Biotechnol.*, 2012, 93, 2279–90.
[2]C. M. Sharma and J. Vogel, *Curr. Opin. Microbiol.*, 2014, 19C, 97–105.

[P69] GENOME RECOVERY USING METAGENOMICS ENABLE SPECIES-CENTRIC METATRANSCRIPTOMIC INVESTIGATIONS

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Introduction: Examining the complete gene expression of unculturable bacteria in complex communities has been the dream of microbial ecologists for years. However, a major obstacle is the recovery of genomes from the communities. While metagenomics has been proposed to solve the problem, it is extremely rare to be able to recover high quality genomes from complex samples. This is not due to lack of sequencing depth, but to the presence of multiple closely related species, which prevents decent genome assemblies.

Methods: In this work we show that a successful approach is to make an enrichment of the target organisms in laboratory scale reactors. We demonstrate that it is now easy and affordable to extract genomes of all the dominant organisms from reactors due to reduced microdiversity and further use these to examine their individual gene expression profiles by metatranscriptomics.

Results: To demonstrate this, we revisited the bacteria involved in enhanced biological phosphorus removal (EBPR) from wastewater treatment plants using laboratory scale batch reactors. After obtaining complete genomes we successfully examined the gene expression of the dominant species.

Virology

[P70] ISOLATION CHARACTERIZATION AND SEQUENCE ANALYSIS OF *ESCHERICHIA COLI* PHAGES ISOLATED FROM ANIMAL FAECAL SAMPLES

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Introduction: Since Frederick Twort's discovery of bacteriophages in 1915, much effort has been put into understanding phages and their diversity. This is especially true for phages of classic laboratory strains of *E. coli.* Nonetheless, even with a hundred years of studies, the diversity of *E. coli* phages is not fully explored. Here we present the isolation and characterization of 20 *E. coli* phages. All the phages were isolated on *E. coli* MG1655 from a various faecal samples, which includes samples from the polar bear, tasmanian devil, red panda, bactrian camel and the giant anteater. The isolated phages were purified and analysed in regard to their phylogeny, host range, morphology and genetic features.

Methods: Twenty-five were chosen to be sequenced using a novel, rapid method of sequencing which requires a very low amount of DNA which can be obtained from a single plaque.

Results: The sequencing result showed that some of the phages belong to very rarely isolated phage groups and therefore bring new insights to the diversity of *E. coli* phages.

Conclusions:

1. 25 phages were isolated during the experiment.
2. Using the single plaque sequencing, it was possible to determine the complete, contiguous genome sequence of 22 of the phages (88%).
3. 6 of the phages had only one close relative in the GenBank database.
4. 15 of the phages was able to infect one or more of the 6 pathogenic strains used in the experiment.
5. 4 of the sequenced phages were shown to belong to newly discovered taxa containing only one phage each.
6. All these 4 phages contain most of a queuosine biosyntesis operon.

References:

Kot, W. et al., 2014. DPS - A rapid method for genome sequencing of DNA-containing bacteriophages directly from a single plaque. Journal of Virological Methods, *196*, pp.152– 156

Whole genome sequencing

[P71] GENOME SEQUENCES FOR TWO *PSEUDOMONAS JESSENII* FROM COPPER-CONTAMINATED SOIL IN DENMARK

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Introduction: *Pseudomonas jessenii* was found to be a potent functional rhizobacterium against soilborne plant pathogens. *P. jessenii* C2 and *P. jessenii* H16 were isolated from pristine soil and from sieved high Cu soil from the Hygum site, Denmark, respectively.

Methods: Whole-genome shotgun sequencing of *P. jessenii* C2 and H16 was performed using the Illumina HeSeq platform. Genome assemblies were constructed *de novo* using CLCBio Genomic Workbench 7.0. The genes in the assembled genome were predicted with the RAST server database.

Results: *P. jessenii* C2 contained 6,420,113 bp DNA with a G+C content of 59.83%. *P. jessenii* H16 contained 6,807,788 bp, with a GC content of 59.02%. *P. jessenii* H16 contained an additional putative metal fitness/pathogenicity island when compared with *P. jessenii* C2. It encompasses about 50,000 bp. This potential pathogenicity/fitness island harbored several copper resistance determinants including the *cus* determinant that encodes CusABCRS. In addition, genes encoding the P-type ATPase CopA, the multicopper oxidase CueO and CopBDG could be identified. This island also revealed genes encoding NcrA and NcrB. Moreover, genes encoding the mercury resistance determinant *mer*TRCAB are present on this island.

Conclusions: Comparative genomic analysis of those two *P. jessenii* strains suggested acquisition of a fitness island encoding numerous genes involved in conferring resistance to multiple metals as the main mechanism in genome evolution in this environment.

[P72] COMPARATIVE GENOME ANALYSIS OF *CLOSTRIDIUM PERFRINGENS* ISOLATES FROM HEALTHY AND NECROTIC ENTERITIS INFECTED POULTRY AND DISEASED PIGS

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Introduction: *Clostridium perfringens* causes gastrointestinal diseases in both humans and domestic animals. Type A strains are the main cause of necrotic enteritis (NE) in chickens, which is a significant economic issue in the international poultry industry. The NetB and Cpb2 toxins seem to be important for the development of NE in chickens and piglets, respectively, while the role of these toxins is less well elucidated in diseased turkeys.

Methods: We carried out comparative genomic analysis of 40 *C. perfringens* genomes from healthy and NE-suffering chickens and turkeys, and diseased pigs using whole-genome data.

Results: Analyses of virulence gene content including VirR boxes showed that *net*B was primarily found among NE isolates from chickens, while *cpb*2 dominated the isolates from diseased pigs. The pathogenicity loci NELoc-1, -2 and -3 were primarily observed in NE isolates from poultry and most commonly in chickens, whereas only NELoc-2 was common among isolates from diseased turkeys. Furthermore, conjugative plasmid transfer genes were identified in the majority of all isolates, and VirR boxes were found upstream of genes that are essential in the NE pathogenesis.

Conclusions:

- *net*B, NELoc-1 and -3 seem to play an important role in the NE pathogenesis in chickens, whereas *cpb*2 is important in diseased pigs.
- The VirSR two-component system is involved in regulating NE-associated virulence genes.
- Conjugative plasmid genes are widely spread among *C*. *perfringens*.
- WGS is a powerful tool to investigate virulence properties and population genetic structure of *C. perfringens*.

[P73] PREDICTION OF PLASMID CONTIGS IN SPHINGOMONAD GENOMES

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Sphingomonadaceae is an important bacterial family, since they are often associated with degradation of polluting xenobiotics. The genes encoding the degradative enzymes have been investigated for several strains, but many isolates still need to have their genetic features studied. Usually, sphingomonads have large conjugative plasmids which harbor the catabolic genes encoding the enzymes that provide the trait for which a given sphingomonad was isolated. These plasmids need deeper investigation, since they are key elements in the evolution of sphingomonads. An essential step in this research is the identification of plasmid sequences in draft genomes. This can be challenging, since conjugative plasmids are often larger than 100 kbp and contain many repetitive sequences. This often leads to plasmids being broken into several contigs in genome assemblies.

We developed a pipeline that predicts large conjugative plasmids in draft genomes of sphingomonad genomes using various search and filtering steps. The results are displayed in networks and graphs showing similarity to reference plasmids.

While the final pipeline is not fully tested, the initial results indicate that the pipeline can predict conjugative plasmids in sphingomonad draft genomes.

This pipeline allows for automated prediction of contigs that are likely to represent conjugative plasmids in sphingomonads which represents an essential part of the studies of this environmentally important group of bacteria.

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