Student projects in the Section of Microbiology

Below is a list of suggested bachelor and master projects along with smaller student projects (“fagprojekter”) in the Section of Microbiology. If a project has sparked your interest or you have a suggestion for a similar project please feel free to contact the projects supervisor(s).

The main objective of the research in Section of Microbiology is to evaluate the extent of genetic flow within the natural microbial communities and the responses to environmental perturbations. State-of-the art techniques form the basis of our research and teaching. Below is an outline of our areas of research.
Soil biodiversity and function

Microbiology of thawing permafrost

Project type: Masters, Bachelor

Permafrost covers 20% of the land area in the northern hemisphere and is estimated to contain half of all soil organic matter on our planet. Due to global warming permafrost is thawing over large tracts of the Arctic. Thawing increases the decomposition by microorganisms of the enormous stock of organic material stored in permafrost soil. During the decomposition carbon dioxide, methane and other greenhouse gases are released to the atmosphere, potentially increasing global warming.

Potential topics:

- The links between CO₂ production in permafrost samples and the communities of fungi and bacteria
- Microorganisms in a changing world: How soil bacteria and fungi react to global change experiments in the field
- Nitrogen fixation in the Arctic
- Biogeography of soil bacteria, fungi and microeukaryotes in the Arctic
- Microbiology at landscape scale – extending plot scale measurements to landscape scale

This list is by no means exhaustive. Many other projects involving activity or diversity of microorganisms in the Arctic are possible.

The projects typically combine molecular tools, e.g. quantitative PCR approaches, sequencing of DNA and/or cDNA and enzyme assays, with analyses of soil and other environmental parameters. If relevant for the project, field work in Greenland is a possibility.

The work will be done within Center for Permafrost, CENPERM, a Center of Excellence financed by the Danish National Research Foundation. You can find more information about CENPERM at [www.CENPERM.ku.dk](http://www.CENPERM.ku.dk).

Key words: Microbial communities, permafrost, greenhouse gases, ecology, microbial physiology

Link to project homepage: [www.cenperm.ku.dk](http://www.cenperm.ku.dk)

Supervisor: Anders Priemé: aprieme@bio.ku.dk
Microbial responses to drying and rewetting

Project type: Masters

In many globally important ecosystems, climate change leads to more extreme fluctuations in precipitation and temperatures involving more severe drought and rainfall events.

Fluctuations in precipitation will affect soil moisture availability, which regulates the activity of soil microbes. A massive pulse of carbon dioxide is often observed when water is added to a dry soil, but the status of the different groups of soil microbes under dry conditions and following rewetting is unknown. The question under study is how drying and/or rewetting affect CO$_2$ emissions and microbial activity status.

The project may involve incubation of soil samples in the laboratory, quantitative PCR on key genes involved in microbial responses to thawing or wetting, transcriptomic analyses of soil microbes (with a focus on rRNA transcripts revealing the activity response of different groups of soil microorganisms), and gas flux analyses. You will learn techniques to measure general soil parameters, analyse rRNA, and estimate release of CO$_2$ from soil.

Starting date is flexible and upon agreement.

Keywords: Climate change, transcriptomics, microbial physiology, extreme events, soil microbes

Supervisors: Anders Priemé: aprieme@bio.ku.dk
How low can you go – activity of permafrost microorganisms at very low temperature

Project type: Masters, Bachelor

Arctic microorganisms are subjected to low or very low temperatures. In the deep permafrost soil, bacteria are known to be active at -39 °C. In surface soils, arctic bacteria and fungi survive not only the low temperatures during the long arctic winter but also the physiological stresses related to freezing-thawing cycles and changes in osmolarity.

The project aims to study the responses of bacteria and fungi in arctic soils to temperature changes near or below the freezing point, e.g.:

- life at the edge: the lowest temperature for detectable microbial activity
- the low temperature response of the microbial processes producing or consuming greenhouse gases (carbon dioxide, methane and nitrous oxide)
- the mechanisms used by microorganisms to cope with freezing
- the mechanisms used by microorganisms to initiate or enhance activity upon thawing.

The work may involve transcriptomic analyses of pure cultures of bacteria and fungi as well as of complex microbial communities in permafrost soil. Measurements of greenhouse gas production and sequencing of microbial genomes may also be included in the work.

The projects will be carried out within Center for Permafrost, CENPERM, a Center of Excellence financed by the Danish National Research Foundation. You can find more information about CENPERM at www.CENPERM.ku.dk.

Key words: Microbial physiology, extreme biology, transcriptomics, permafrost, low temperature biology

Link to project homepage: www.cenperm.ku.dk

Supervisor: Anders Priemé: aprieme@bio.ku.dk
Sociomicrobiology

CRISPRs on Plasmids: Fight Fire with Fire

Project type: Masters
Main area: CRISPR; Plasmids; Microbiology; Bioinformatics

Project Description:
CRISPR/Cas systems are prokaryotic adaptive immune systems which target foreign genetic elements such as viruses and plasmids. The application of these systems is revolutionizing gene editing both in research but also in the clinical setting. Given the novelty and great diversity within these systems, still very little is known about their mechanism and evolutionary role in natural environments. Although much attention has been given to their importance in combating phage predation, their impact on the interplay between host and plasmids is generally overlooked. Plasmids play an important role in bacteria’s ability to quickly acquire new abilities (ie. antibiotic resistance), and one could imagine that a trade-off between an effective immune-system and the ability to uptake beneficial plasmid-encoded traits is a defining feature of pathogenic vs. benign bacteria. However, the playing field is even more complex, as some plasmids are known to encode CRISPR/Cas system components, whilst others may potentially encode antiCRISPR proteins.

The goal of this project is to bioinformatically describe CRISPR/Cas systems on plasmids and using selected plasmids with defining CRISPR/Cas related features, experimentally investigate the interplay between promiscuity and plasmid uptake. Another key part will be investigating novel CRISPR/Cas system found on the plasmids experimentally.

You will be working with a team containing both bioinformaticians and experimentalists.

Methods used:
You will have a great deal of influence of the direction of your project, but it can include Bioinformatics, Data Analysis and Systems Biology, Molecular Microbiology, Flow Cytometry

Supervisors:
Jonas Stenløkke (jsmadsen@bio.ku.dk), Joseph Nesme (joseph.nesme@bio.ku.dk), Rafa Pinilla Redondo (rafapinillaredondo@gmail.com)
Master thesis project at Chr. Hansen A/S

Can we use the sequence of particular eps genes to predict polysaccharide functionality?

Project type: Master

Dairy products worldwide generate estimated 500 billion US$ in revenues. Understanding how lactic acid bacteria (LAB) contribute to better dairy products is essential to the industry. LAB have large effects on fermented dairy products, including shelf life, flavor and texture. Production of polysaccharides by LAB has long been associated with the technological, functional and health-promoting benefits of these microorganisms. In particular, the capsular polysaccharides and exopolysaccharides have been implicated in modulation of the texturing properties of fermented products such as cheese and yoghurt. For this reason, screening and selection of polysaccharide-producing LAB has been extensively carried out by academia and industry.

Exopolysaccharides (EPS) and capsular polysaccharides (CPS) are exocellular polysaccharides that at most differ in their degree of attachment to the cell surface: CPS are tightly linked, often covalently, to the cell surface and form a capsule around the cell, whereas EPS are secreted into the extracellular matrix or loosely associated with the cell surface via electrostatic interactions often forming a slime layer. The enzymes involved in the synthesis of these polysaccharides are encoded by eps genes found in clusters.

The aim of the project is to compare the sequence of the epsA gene in more than 200 *S. thermophilus* strains, group the genes based on sequence similarity, and link these groups to phenotypic variations amongst the more than 200 *S. thermophilus* strains. Furthermore, focus will be on improving existing methods for CPS detection, and to develop a reliable high-throughput method for CPS measurement.

We are looking for a motivated student, eager to learn and with scientific curiosity. Furthermore the student should have:

- Know how in molecular biology, general microbiology and/or bioinformatics.
- A drive to develop new methods
- Ability to work independently both in the lab and with analysing data, after receiving appropriate training.

The student will be trained in:

- Work with LAB
- Using Hamilton Microlab Star robot for liquid handling and high throughput screening
- CLC software for genome data analysis

*Supervisors*: Vera Kuzina Poulsen, Gunnar Øregaard and Anders Priemé.

Email: dkvekp@chr-hansen.com
Name a new parasite!

Project type: Masters, Bachelor.

Analysis of genetic diversity in the intestinal parasite *Endolimax* has led to the recognition that the genus comprises not only one (*Endolimax nana*) but at least two ribosomal lineages that differ by approximately 15% or maybe more.

The clinical significance of infection by *Endolimax* is uncertain, and it is possible that different lineages may have different clinical impact.

We have access to *Endolimax* DNA from the newly recognized lineage and primers that specifically amplify the small subunit rRNA gene of *Endolimax*; this gene is typically used for taxonomic inferences, including naming of new species.

We are now ready to perform a study similar to what we have already been doing on a related parasite, namely *Iodamoeba* (PMID: 21940643).

Objectives/Aims

We hope that you would help us characterize this newly recognized ribosomal lineage. The work will involve PCR, TA cloning, clone sequencing (using either Sanger or NGS), sequence alignment, and phylogenetic analysis, with a view to mapping the entire SSU rRNA gene and naming the new lineage.

We aim to publish the results in a journal such as *Molecular Biology and Evolution*, and we have a lot of NGS data displaying intra-strain genetic variability already that can be published in the same paper. We would offer you authorship as either 1st or 2nd author depending on the time and work invested.

Supervisors:  
Rune Stensvold: run@ssi.dk (41729674) from Laboratory of Parasitology, Statens Serum Institut.  
Søren Sørensen: sjs@bio.ku.dk from Section of Microbiology, UCPH, www1.bio.ku.dk/microbiology.
Master thesis project at Chr. Hansen A/S

Investigating the effect of co-culturing Bacillus strains with LAB starter cultures in milk

Project type: Master project

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

In this project, we want to investigate whether a beneficial effect on e.g. acidification and texture could be obtained by co-culturing LAB and bacilli. A faster acidification in fermented milk is an important factor, as it can increase production capacity due to a faster throughput. To explore mode of action of Bacillus strains in milk, gene technology methods will be used on Bacillus wild type strains.

The student in this project will use and learn the following methods:

- Co-culturing LAB strains and Bacillus strains in milk
- Acidification and texture measurements in fermented milk
- Use of 96-well plates for screening and confirmation of results in larger scale systems like 200 ml baby bottles
- Analytical techniques to investigate sugar consumption and flavor production
- Use of Hamilton Liquid Handling robot
- General microbiology
- Genetic techniques to modify Bacillus wild type strains

Since this is a new concept and area for us, we need the student to be proactive and curious.

**Supervisors:** Karin Bjerre, Mette Dines Cantor and Anders Priemé
Contact: dkkbj@chr-hansen.com or dkmdc@chr-hansen.com
Optimization of microbial encapsulation techniques for isolation of keratin degrading microbial species.

Project type: Master project

Europe –especially Denmark- has a serious protein deficit as animal production which is reliant on soy protein imported from South America. Whereas, significant protein food chain loss is worsened by waste and inefficient by-product use. Waste management is a key industrial process, as it can significantly contribute to reducing global environmental footprint of an activity, while optimizing the process by valorization of non-noble materials into high quality products with significant added value. Therefore, it is necessary to develop novel technology to convert biological residual resources into new alternative high value products. Microbial cooperation, resulting from billions of years of evolution in multispecies consortia, is central to all natural processes and optimizes natural energy flow and decomposition. Investigation and manipulation of synergistic microbial interactions require development of high throughput techniques.

Keratin packs into a semi-crystalline state in nature (hair and feather), where keratin monomers are highly cross-linked with disulphide bonds (R-S-S-R). Conversely, biological and biochemically-mediated processes have already been attempted with, for instance, proteases produced by pure cultures of *Bacillus sp.* And several proteases (keratinases) able to degrade keratin and belonging to the subtilizing family of serine proteases have been already characterized. However, these technologies are hampered by the compact nature of keratin in the feather fibers and the extensive disulfide bonding.

Therefore, the one gene, one protein, one product dogma underpins most modern commercial industrial biotechnology. Application of state of the art techniques will take this further by elucidation and application of the roles of multiple microorganisms in consortia gained through millennia of evolution. Bead encapsulation of cells relies on the incorporation of cells into beads of 25-35 micron in diameter, small enough to be sorted in a fluorescence activated cell sorter (Figure 1). Although the beads are small, they are large enough to support growth of few cells up to a micro-colony of 500-5000 cells per bead. In effect, this is equivalent to a well in a microtiter dish allowing for assay of enzymes activities that are not sensitive enough to be scored on single cell level. This gives us the possibility to monitor the relative decrease in fluorescence signal, as the labelled substrate of interest gets degraded by active extracellular enzymes secreted by the encapsulated cells in comparison to control beads.

The aim of this project is to design and apply a high throughput screening approach coupling encapsulation of cells in micro-beads of agarose or alginate coupled to fluorescence-based activity assays to generate and screen a wide panel of synthetic consortia with flow cytometric identification of promising candidates coupled to development of innovative keratin degrading assays.

**Supervisors:** Søren J. Sørensen: sjs@bio.ku.dk
Master thesis project at Chr. Hansen A/S

Molecular tool development for bioprotective strains

Project type: Master project

In Europe alone 29 million tons of dairy products are lost or wasted every year according to a study by the UN Food and Agriculture Organization (FOA) from 2012. Worldwide this amounts to around 20% of all dairy products produced. A considerable portion is due to spoilage through contamination, flavor degradation and the general stability and texture of the product. Chr. Hansen A/S has developed a product range that helps to prolong shelf life in a natural way by protecting the food against yeast and mould growth. In this student project we would like to develop molecular tools that will help us understanding and improving our cultures even more.

We expect from the potential candidate:

• to be curious in understanding complex mechanisms
• to have a solid knowledge in general molecular biology tools (e.g. PCR, cloning) and the will to expand on this
• to be a team player, as many of the techniques will be shared within the department.

What you can expect from us:

We offer an exciting opportunity to be part of an R&D department in an international company. At the end of the project we believe that the student has gained insight into bioprotective mechanisms including different screening methods and has learned about molecular tools in industrial strain backgrounds.

Supervisor: Solvej Siedler: dksosi@chr-hansen.com
Bioinformatics approaches for assembly and characterization of novel circular complete plasmids from publically available *Escherichia coli* genomes

**Project type: Master project**

Nowadays the wide application of next generation sequencing technologies has provided us with huge amounts of primary nucleotide sequences of various bacterial species available in public databases. A number of recent studies focusing on comparative genomic characterization have discovered vital genetic mutations linked with bacterial adaptive evolution\(^1\). However, these studies rarely pay attention to recovery of putative circular plasmids, a class of important mobile genetic elements carrying resistance and virulence genes, from deep genomic sequencing of single species. The aim of this study is to utilize and improve an integrative bioinformatics approach to *in silico* identify complete plasmid sequences based on whole genome shotgun sequencing projects of *Escherichia coli* (1,606 WGS projects deposited in the GenBank database, Nov 2013). Novel plasmid type that occurs in *Escherichia coli* populations will be characterized and compared to the known ones. The spectrum of predicted circular plasmid sequences will provide promising targets for experimental validation and provide useful genetic context for future research into plasmid diversity of *Escherichia coli*.

This project depends on one or two master students with a good working knowledge of genetics and programming (Perl, Python, R etc). Experience in analysis of high throughput sequencing data would be preferred.


**Supervisors:** Søren J. Sørensen: sjs@bio.ku.dk
Master thesis project at Chr. Hansen A/S.
Department of Bacterial Physiology and Strain Improvement.

Determination and characterization of Bacillus spp. genetic traits involved in plant growth promotion.

Project type: Master project

According to the United Nations World Food program, feeding the projected world population of 9 billion people by 2050 will require that agricultural yields increase by 70-100%. Plant growth promoting bacteria (PGPR) are beneficial bacteria that possess the ability to colonize the roots and/or inner plant tissues and promote plant growth by either acting as biofertilizers or via biological control of plant disease.

During this master thesis project the candidate will help developing novel and creative microbial solutions to increase agricultural productivity. The student will work with *Bacillus* natural isolates and will apply state-of-the-art molecular microbiology techniques in order to genetically manipulate them. The candidate will implement a number of screening assays for plant growth promotion traits. In addition, selected strains will be physiologically characterized in depth.

Requirements:
- We search for a highly motivated student, eager to learn and with an innate scientific curiosity.
- Previous knowledge or experience in molecular biology, general microbiology techniques and/or bioinformatics would be an advantage.
- We would appreciate responsible and mature students that can show certain independence in their research after receiving the adequate training.

The candidate will receive training/will acquire knowledge on:
- Genetic transformation of *Bacillus* strains: Electroporation, phage transduction and transformation of naturally competent cells.
- Generation of mutant strains.
- State-of-the-art molecular biology techniques.
- qPCR
- Fluorescent microscopy
- Bacterial physiology

Supervisors: Patricia Dominguez-Cuevas, Rute Neves and Søren Sørensen
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Origin and evolution of the bacterial antibiotic resistome

Project type: Master project

Background
Microbial communities of natural environments are extremely diverse (Torsvik et al., 2002) and can be considered major reservoirs of resistance genes, referred to as the bacterial resistome (D’Costa et al., 2007). There is debate, however, about the current estimates of the resistome. In a study by Sommer et al. (2009), the reservoir of antibiotic resistance genes in the microbial gut microbiota of healthy individuals was described. Interestingly, the resistance-conferring genes encoded proteins that were 100% identical to hypothetical proteins of unverified function in GeneBank (Sommer et al., 2009). Other studies have suggested that resistance determinants originate from ancestral housekeeping genes that encode proteins with antibiotic binding functions, serving other cryptic functions (Morar and Wright, 2010). These studies indicate that chromosomal housekeeping genes – once overexpressed – can be identified as antibiotic resistance genes (Sommer et al., 2009), suggesting a new mechanism by which the resistome develops: recruitment of housekeeping genes on to higher copy number mobile genetic elements (MGEs). However, the impact of this mechanism in shaping the resistome of microbial communities is hitherto unknown.

Objectives/Aims
In this project, we will investigate if “actual” antibiotic resistance genes are in fact derived from housekeeping genes that become over-expressed by high copy number or transcription activity when acquired by plasmids, thus resulting in an antibiotic resistant phenotype.

Experimental approach
To link over-expression of housekeeping genes to the development of resistance phenotypes, we will investigate whether a selective pressure by antibiotics can “push” housekeeping genes from chromosomes onto plasmids.
1. Genomic DNA from antibiotic-sensitive isolates will be purified and cloned into high expression E. coli vectors.
2. These will be transformed into an antibiotic hypersensitive E. coli strain.
3. Cells will be screened for resistance towards various antibiotics.
4. Sequence analysis will reveal if resistant clones contain expression vectors carrying housekeeping genes, and thus if these clones are related to over-expression of housekeeping genes from the sensitive isolates.

References: 

Supervisors: Søren Johannes Sørensen: sjs@bio.ku.dk
Microbiomes

Unravel and map eukaryotic diversity within the human gut

Project type: Masters

Background: Application of next-generation sequencing (NGS) to genomic DNA extracted from sewage offers a unique and cost-effective opportunity to study the genetic diversity of intestinal parasites. In this study, we used amplicon-based NGS to detect and differentiate taxa within several common luminal intestinal parasitic protists (CLIPPs), namely Entamoeba, Endolimax, Iodamoeba, and Blastocystis, in samples from Swedish sewage treatment plants.

Materials/methods: Influent sewage samples were subject to DNA extraction and PCR-based amplification using three primer pairs designed for amplification of eukaryotic DNA and targeting nuclear ribosomal DNA. PCR products were sequenced using ILLUMIINA sequencing, and resulting DNA sequences were annotated to species and subtype level using the in-house BION software. Data on Entamoeba moshkovskii were included, although this parasite is mostly thought of as free-living.

Results: A total of 26 samples from 8 treatment plants in 7 cities in Middle and Southern Sweden were available for analysis. Blastocystis and Entamoeba moshkovskii were detected in all samples, and most samples (n=19) were positive for Entamoeba coli. We moreover detected Entamoeba histolytica, Entamoeba dispar, Entamoeba hartmanni, Endolimax nana, and Iodamoeba bütschlii in 1, 11, 4, 10, and 7 samples, respectively.

Research question: Using online clustering and BLAST tools, we are now interested in mapping the genetic diversity within these parasitic protists.

It will also be possible to use our automated R script to generate data on diversity (bacterial and fungal data are available for all samples too), create PCoA plots, top-ten most abundant genera, etc. for each of the samples. This is mostly thought of as an exercise. However, we have data from human and animal faecal samples that can be looked into in the same way, with parasite, bacteria and fungal data available.

We are hoping to be able to publish the results.

Supervisors:
Rune Stensvold, Department of Bacteria, Parasites & Fungi, Infectious Disease Preparedness, SSI (run@ssi.dk)
Søren Johannes Sørensen, Department of Biology, Section of Microbiology, UCPH (sjs@bioku.dk)
Microbial abundance and diversity in dust samples collected from homes of kids with asthma.

Project type: Master project

Asthma is the most common chronic disease in children and its incidence has doubled in the recent half-century, now afflicting 10% of all schoolchildren. Evidence suggests that environmental exposures in early life, such as an altered diet and a perturbed microbiome, may lead to low-grade inflammation progressing to disease. Different factors have been associated with the risk of developing pneumonia and bronchiolitis in young children such as daycare attendance, duration of breastfeeding, crowding, siblings, environmental tobacco smoke exposure, low socioeconomic status, and male sex, however these factors only explain a minor proportion of the variation. Microbial exposure during early life seems to play an important role for innate immune functioning and maturation of the adaptive immune system and influence susceptibility to disease. It has been previously demonstrated that neonates colonized in the hypopharynx with Streptococcus pneumoniae, Haemophilus influenzae, or Moraxella catarrhalis have an increased risk of childhood asthma and recurrent wheeze in at-risk children (Vissering et al., 2013). Therefore, we hypothesized that bacterial colonization of the neonatal airway could also affect susceptibility to pneumonia and bronchiolitis during early childhood.

In this study, we aim to investigate the possible role of microorganisms (bacteria and fungi) present in the dust collected from kids’ home the Copenhagen Prospective Studies on Asthma in Childhood2000 (COPSAC, http://www.copsac.com/) and the risk of developing asthma.


Supervisors: Søren J. Sørensen: sjs@bio.ku.dk
Martin Steen Mortensen: martin.mortensen@bio.ku.dk
Genetic characterization of bacterial human virulence genes in non-host environmental bacteria

Project type: Master project

Introduction
Bacterial pathogens continue to cause problems for humans by the continuous evolution of known and emerging pathogens. Approximately 160 new bacterial infectious diseases have been discovered between 1940 and 2010. While the mechanisms by which many pathogenic bacteria cause disease are relatively well-described, the evolutionary origin of virulence is not fully understood. Bacterial virulence determinants causing disease in humans may evolve through complex host-pathogen interactions but their origin may also lie in non-human reservoirs amongst the environmental microbiomes. Recent studies have demonstrated that bacterial genes normally associated with clinical bacteria causing disease in humans may be widespread in the outer environment, e.g. in soil, seawater, hot springs, the deep-sea etc. It has been suggested that the virulence genes play an important role in the survival of environmental bacteria. Furthermore, a high degree of sequence conservation between the environmental and clinical sequences suggest that the virulence genes found in clinically relevant bacteria likely have an environmental origin.

The project
Bacteria possessing genes with high sequence similarity to known virulence genes from clinically relevant bacteria have previously been isolated from a beech wood soil. Surprisingly, some of the genes were observed in bacterial phyla that were hitherto not known to contain such genes. Expression assays further showed that the genes were functional and in some cases expressed, giving the isolates a virulent phenotype.

The purpose of the project will be to genetically characterize the virulence genes and their genetic context (the flanking genes) in the atypical host bacteria to understand how they are regulated, to assess their evolutionary origin, and possibly explain how they have been transferred to the unexpected hosts. The work will involve testing of the ability of the bacteria to express the genes (transcription and virulence assays), whole-genome sequencing of the bacteria and bioinformatics (annotation of the sequences, identification/characterization of the operons involved, and phylogenetic analyses of the genes).

More information


Supervisors
Niels Kroer (nk@bio.ku.dk)
Søren J. Sørensen (sjs@bio.ku.dk)
Metagenomic and metatranscriptomic analysis of keratin degrading microbial communities in microcosms.

Project type: Master project

Europe—especially Denmark—has a serious protein deficit as animal production which is reliant on soy protein imported from South America. In addition, significant protein food chain loss is worsened by waste and inefficient by-product use. Waste management is a key industrial process, as it can significantly contribute to reducing global environmental footprint of an activity, while optimizing the process by valorization of non-noble materials into high quality products with significant added value. Therefore, it is necessary to develop novel technology to convert biological residual resources into new alternative high value products. Microbial cooperation, resulting from billions of years of evolution in multispecies consortia, is central to all natural processes and optimizes natural energy flow and decomposition. Recent advancements in understanding, investigation and manipulation of synergistic microbial interactions by high throughput techniques such as metagenomics and metatranscriptomics analysis are useful tools to investigate energy and environmental footprints and nutritional yields to address coming societal challenges. Keratin packs into a semi-crystalline state in nature (hair and feather), where keratin monomers are highly cross-linked with disulphide bonds (R-S-S-R). Conversely, biological and biochemically-mediated processes have already been attempted with, for instance, proteases produced by pure cultures of Bacillus sp. And several proteases (keratinases) able to degrade keratin and belonging to the subtilizing family of serine proteases have been already characterized. However, these technologies are hampered by the compact nature of keratin and the extensive disulfide bonding (Fig. 1).

Therefore, the one gene, one protein, one product dogma underpins most modern commercial industrial biotechnology. Application of state of the art techniques will take this further by elucidation and application of the roles of multiple microorganisms in consortia gained through millennia of evolution.

The aims of this project are to:

- Investigate microbial community involved in keratin degradation using next generation sequencing of rRNA genes and ITS region.
- Use next generation sequencing of metagenome and metatranscriptome to Characterize bacterial and fungal species and genes involved in conversion of keratin in nature and use this as basis for constructing stable microbial consortia.

Supervisors: Søren J. Sørensen: sjs@bio.ku.dk

Figure 1: The complex structure of β-keratin.
Investigating the effect of gastrointestinal and food consumption of vertebrate animals on microbial structure and diversity using MiSeq amplicon sequencing of 16S rRNA gene.

**Project type:** Master project

**Research Hypothesis:** The vertebrate animals gastrointestinal tracts structure and food habits can influence gut microbial communities and it can be used as a characteristic to investigate animals’ evolution.

Perspective: Animals are divided into two major groups vertebrate and invertebrates based on presence of vertebral column, also known as backbone or spine. All animals are heterotrophs, which mean that they feed directly or indirectly on organic matter produced by other living organisms. Large animals are often subdivided based on the food source into carnivores, herbivores and omnivores. The gastrointestinal tracts (GIT) of vertebrates are different in length and compartmentalization, which has enabled vertebrates to occupy diverse habitats and exploit different feeding strategies. The aim of this study is to investigate the correlation between microbial community in animal gut and their classification and if the GIT structure and feeding habit will influence microbial gut communities in animals. In addition we will try to build a tree based on relatedness between different the animals included in the study based on type of microbial communities detected in their gut and how much it is correlated with other classification systems.

In this study, 100 animals from Copenhagen zoo representing different groups of vertebrates will be investigated. Fecal materials will be collected from these animals and DNA will be extracted. The variable 4 region of the 16S rRNA gene will be amplified and will be sequenced using MiSeq platform from Illumina. Sequencing data will be analyzed using the QIIME-pipeline.

**Supervisors:** Søren J. Sørensen: sjs@bio.ku.dk  
Martin Steen Mortensen: martin.mortensen@bio.ku.dk
Multispecies biofilm

In the laboratory bacteria are rarely grown as biofilms, although this is the way many bacterial species live in nature. A biofilm protects the cells from predation, antibiotics and physical stress among other things and the close proximity of the cells help facilitate social interactions including a high rate of horizontal gene transfer. The majority of genes necessary for biofilm formation are found on plasmids, again giving a direct link between biofilms and horizontal gene transfer. Biofilms are a large problem in both medical and industrial environments because biofilm forming bacteria are very hard to remove. Research in bacterial biofilms and especially multispecies biofilms is a large field within microbiology and is of great interest in the medical industry. Besides, multispecies biofilms are suitable models for studying essential aspects of social evolution of bacteria (sociomicrobiology).

Cooperation in multispecies biofilm formation

Project type: Bachelor, Master

Project description:
Bacteria normally live in so-called biofilms - dense multi-species communities of microorganisms embedded in a self-made matrix of extracellular polymeric substances. Biofilms are therefore found wherever bacteria are, which is pretty much everywhere. Studying bacterial biofilm formation is important not only to improve our understanding of basic bacterial biology but also due to clinical issues because bacteria residing in biofilms become more tolerant to disinfectants, antimicrobial agents and phagocytosis than their planktonic counterparts.

Most research on biofilm biology has so far focused on mono-species biofilms although this hardly reflects what we find in nature. We are therefore, at Section of Microbiology, especially interested in how multi-species biofilms are established and function.

In this research topic you will get the opportunity to study how and why different species of bacteria cooperate to form biofilms, if the presence of some species provides a protective effect on other species and hereby shield them from inactivating compounds? Why species incapable of biofilm formation by themselves may be present in multispecies biofilms – which is particular concern with respect to pathogenic bacteria. How the biofilm phenotype affects horizontal gene transfer? And many other pressing questions related to bacterial interactions in multispecies biofilms.

Techniques covered:
Classical bacteriology techniques
Biofilm cultivation
Cloning and other molecular techniques (PCR, qPCR etc.)
Fluorescent microscopy
Flow cytometry
Genomics/Transcriptomics/Proteomics

Supervisor(s):
Mette Burmølle: burmolle@bio.ku.dk
Søren Sørensen: sjs@bio.ku.dk

Project home page: http://www1.bio.ku.dk/microbiology/research/multispecies_biofilm/
The interconnection between biofilm formation and horizontal gene transfer

Project type: Bachelor, Master

Keywords: Biofilm formation, horizontal gene transfer, social evolution, sociomicrobiology, plasmids, molecular microbiology

Over the last few years we have been studying natural plasmids that encode genes that enables biofilm formation. This research has indicated that biofilm formation and plasmid biology are interconnected and act as a positive loop that promotes both through various mechanisms. Grasping this connection helps us to understand how social bacterial traits can evolve and this is very important when looking at how, why, where and when bacteria evolve into e.g. pathogens or commensals of humans.

We have, until now, been looking specifically at plasmids that prime biofilm formation via type 3 fimbriae (typically incX1 plasmids), K88 fimbriae and type 4b shufflon pili (incl-cluster). We will be able to use these plasmids as the starting point of further research projects, which will be convenient as we already have the full nucleotide sequences, cloned alternative and knockout mutants available.

This project has multiple aspects and can be directed according to your interests, whether they lean towards medical microbiology, ecology, or sociomicrobiology. The main techniques you will be using are based in molecular biology

References: Burmølle et al., 2008 Microbiology, Rankin et al., 2010 Heredity

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Differential gene expression in multispecies biofilm

Project type: Master

Keywords: cooperation, biofilm, gene expression, transcriptomics

In most environments, bacteria are part of multispecies biofilms and the biofilm mode-of-growth is predominant among most bacterial species. In biofilms, bacteria are enclosed in a self-produced polymeric matrix and more protected from disinfectants and antimicrobial agents than their planktonic counterparts.

It has been observed that the protective effect of biofilms may be further enhanced in multispecies biofilms due to various interactions. The presence of some species provides a protective effect on other species and may shield them from inactivating compounds. Therefore, species incapable of biofilm formation by themselves may be present in multispecies biofilms, which is of particular interest – and concern – with respect to pathogenic bacteria. These observed changes are likely to be caused by changes in gene expression when bacteria are forming biofilms in mixed communities compared to single species biofilms. The genes that are regulated differentially are however not yet identified.

We have identified a model system composed of four soil isolates interacting synergistically with respect to biofilm formation, sequenced their genomes and transcriptomes under specific conditions. Two student projects are related to this study:

1) Differential gene expression in single and multispecies biofilms:
Based on the transcriptomes of single and multi species biofilms, genes expressed differently under these two conditions will be identified. Primers specifically suitable for quantitative PCR, targeting these genes will be designed. This enables validation/exploration of differential gene expression under different conditions at different stages in biofilm development.

2) Metabolic networks
The inter-specific interactions in biofilms may in some cases be caused by metabolic complementation. Such metabolic interactions/dependencies may be reflected in the genes encoding metabolic functions, and can be identified by constructing bioinformatic “metabolic networks”. Based on the genome sequences of the four species mentioned above, metabolic networks will be generated to possibly explain the observed synergistic effect in biofilm formation.

Students with special interest in biofilms and inter-species interactions, interested in working with the model biofilm on a different (self-defined?) project are also welcome to contact us.


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Bacterial cooperation in multispecies biofilms associated to food production devices

Project type: Bachelor, Master

Keywords: Food contamination, multispecies biofilms

Bacteria cause many serious and sometimes lethal human infections. A significant route of entrance of bacteria into the human body is through food contaminated with pathogenic bacteria. Some food-borne pathogens contaminate the food-processing environment and colonize this together with commensal bacteria, and several species, including pathogens, have been isolated from food production devices.

The contamination of food production devices is likely to be caused by bacterial biofilm formation on these. In a biofilm, bacteria are enclosed in a self-produced polymeric matrix and more protected from disinfectants and antimicrobial agents than their planktonic counterparts. Therefore, bacterial biofilms on equipment may be hard to eradicate.

It has been observed that the protective effect of biofilms may be further enhanced in multispecies biofilms due to various interactions including conjugation. The presence of some species provides a protective effect on other species and may shield them from inactivating compounds. Therefore, species incapable of biofilm formation by themselves may be present in multispecies biofilms, which is of particular interest – and concern – with respect to pathogenic bacteria.

The main aim of this project is to identify the characteristics and prevalence of bacterial cooperation in association to biofilm formation of isolates from food processing environments. Screening of combinations of multiple species from bacterial strain collections will provide information on the prevalence of synergetic bacterial interactions. By use of species-specific detection techniques (qualitative PCR or similar), species dynamics including identification of the species important for the observed synergy will be identified. The ability of the multispecies biofilm to support growth of relevant bacterial pathogens associated to food contamination may also be explored under different growth conditions.


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Social interactions and population structure

Project type: Master project

In the natural environments bacteria live side by side and interact in both synergistic and antagonistic ways. They cooperate and compete in a complex network of interactions, and it is hypothesized that this network is not random, but is correlated with the bacteria present in the specific environment and the niches of the interacting bacteria.

The main focus of the project will be to test if there is extensive inhibition between bacteria isolated from the same microenvironments or if they rather inhibit strains from other environments. The former could be due to for example nutrient competition between individual cells and rampant mixing of populations, while the latter could be due to prior selection and a more stable co-existence. First step will be to isolate bacterial strains from an environment of choice; all the strains have to be isolated from the same microenvironment, and the initial part of the project will probably be an optimization of this.

Subsequently, it can be tested which isolates inhibit each other and the specificity of this inhibition.

The project can provide important knowledge on the social evolution of bacteria, but can also be interesting in a clinical and biotechnological perspective, since the experiments can reveal the potential of natural isolates as an alternative to the problematic broad-spectrum antibiotics.

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Regulation of plasmid mediated biofilm formation

Project type: Master or Bachelor

Many bacterial species are known to attach to surfaces or other cells and establish biofilms which offers them several advantages compared to the planktonic lifestyle. First, the biofilm mode may facilitate synergistic interactions and gene transfer among different species. Second, biofilms may be crucial for bacterial survival in many environments, including humans, where biofilms are involved in various persistent infections. Consequently, the formation of bacterial biofilms can cause both health and environmental related problems making biofilm research an important issue.

Today many plasmids occurring in the natural environment are known to be involved in biofilm formation. We have isolated a conjugative plasmid, pIS15_43, and strains carrying this plasmid have been identified as being very good biofilm formers. From subsequent annotation of the plasmid we know that it belongs to the family of IncX1 plasmids and surprisingly seems closely related to another well-known IncX1 plasmid, pOLA52, that recently was found to be involved in biofilm formation (Norman et al., 2008). Like pOLA52, the pIS15_43 plasmid carries the mrk genes encoding the type 3 fimbriae, known to be involved in biofilm formation in Klebsiella (Burmølle et al., 2008). Another interesting feature of this plasmid is the presence of pieces of the regulatory domains, EAL, HN-S and Hha, upstream the mrk genes. These domains are recognized as being indirectly involved in biofilm formation through regulation of the intracellular level of the second messenger cyclic-di-GMP (Hengge, 2009).

However, a degenerated version of EAL lacking normal enzyme activity is found on plasmid pIS15_43 and many other IncX1 plasmids. Despite being degenerated, this domain may play a central role for plasmid mediated biofilm formation. Recent experiments indicate that the function of degenerated EAL may be the opposite of the function of the intact version of EAL, as knocking out the degenerated EAL domain on plasmid pIS15_43 has resulted in a decrease in biofilm formation. The question is whether the degenerated EAL domain acts as a transcriptional regulator of the mrk gene cassette? In this project molecular microbial approaches including gene cloning, gene knock-outs and complementation studies will be designed to further investigate the regulatory role of the degenerated EAL domain.


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Growth of fungi on sustainable coatings with wood as underlying surface.

Project type: Bachelor or Master

In this project we are interested in investigating how different fungi are able to invade and grow on coatings on wood. Most fungi are not able to degrade coatings on wood since the environment eg. rain hardly can supply the fungus with enough nutrition to sustain growth to a degree that overgrown painted wood surfaces look “dirty”. Wood contains nutrition, which might become available for the microorganism when the water vaporizes from the wood to the surface of the coating.

So the question is: Are the nutrition from the wood leaching up through the coating, meaning that these compounds can be transported up to the surface due to the watershifts, based on eg. rain and morning dew? Is it possible to develop a coating where the nutrition is made unavailable for the microorganism?

The project is part of a project sponsored by Ministry of Science, Innovation and Higher Education - (Højteknologi project).

Supervisors: Bo Jensen: boje@bio.ku.dk
Comparison of degradation of wood in Denmark and Malaysia

Project type: Bachelor or Master

Before new products for the protection of wood can be approved, they undergo a field test, demonstrating that the product is effective under realistic conditions. To perform these tests under Danish conditions can take up to 12-15 years before results are available. For a company that will market a new product, this is unacceptable. In order to accelerate the process, the product is therefore tested under tropical conditions where degradation is not stopped by low temperature or low rainfall in parts of the year. In this way the results are achieved within 3-5 years.

Since it is very different climate zones - and continents that the tests are carried out under, there is a desire to determine the organisms that have degraded the subjects in Denmark and Malaysia, respectively

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In collaboration with Morten Klamer—Teknologisk Institut, Tåstrup
Production of hydrophobins from a fungus.

Project type: Bachelor or Master

During growth the mold *Aureobasidium pullulans* produces some small proteins, hydrophobins, which the fungal hyphae exploits as a kind of reverse diving suit, so hyphae can grow in dry areas, but still have access to water. This is of the interest of industries such as paint industry (in development of wood coatings) and pharmaceutical industry (e. investigating microbiological infections of the respiratory system).

The form and function of these hydrophobins are to be investigated in order to make strategies for the control of mold, using the clues in the hydrophobicity of the biochemical properties.

This project is conducted in cooperation with Dyrup, Technological Institute, Life, DTU as well as some small private companies in a Højteknologisk project.

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