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 for Microbiology. If a project has sparked your interest or you have a suggestion for a similar project please fell free to contact the projects supervisor(s).

More projects can be found on our homepage http://www2.bio.ku.dk/microbiology/ under Teaching.

The main objective of the research in the MME group 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.

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**Section of Microbiology**

- **Biofilm**
- **Soil Microbiology**
- **Single Cell and Horizontal Gene Transfer**
- **Metagenomics and Transcriptomics**
Single Cell and Horizontal Gene Transfer

In natural environments the majority of bacterial cells cannot be cultured in the laboratory. However, it is crucial to describe the dynamics and interactions of bacterial communities in natural environments in order to reveal essential evolutionary bacterial traits. As an example the spread of plasmids carrying antimicrobial resistance or other undesirable functions may have evolved through bacterial interactions in complex environments. Consequently, an emerging discipline in microbiology becomes the study of bacterial cells on a cultivation-independent single cell level.

Projects of the single cell group will include emerging single cell techniques such as flow cytometry (FCM), activity targeting fluorescent strains combined with fluorescent in situ hybridization (FISH) and multiple displacement amplification (MDA), a non-PCR based DNA amplification technique.

Other projects of the single cell group include the study of methods for limiting the occurrence of antimicrobial resistance. Many resistance genes are located on mobile genetic elements such as plasmids. New strategies may therefore include the disturbance of plasmid transfer and stability. For these studies we have developed a cultivation independent transfer-reporter approach based on the Green Fluorescent Protein (GFP) that together with Fluorescence-activated cell sorting (FACS) allows us to analyze the prevalence and dynamics of plasmid transfer and stability within bacterial species in complex environments on a single cell level.
Drug impact on plasmid stability when embedded in a high-tech silicone rubber matrix

Project type: Bachelor

Antibiotics are essential for treating several infectious diseases among humans and animals. However, the extensive use of antibiotics is selecting not only for resistant clones but will also increase the spread of antimicrobial resistance among bacterial populations. Since it is difficult to develop new antibiotics at a sufficient rate, new strategies for limiting the spread of resistance must be developed. Horizontal gene transfer (HGT), including the transfer of plasmid-encoded resistance, is today recognized as being the most important factor for the rapid global spread of antimicrobial resistance. Consequently, it becomes crucial to limit the occurrence of resistance through targeted interventions directed at plasmid transfer and stability. Previously, flow cytometric methods using a reporter system based on the expression of Green Fluorescent Protein (GFP) have been used to screen for drugs with the ability to induce plasmid loss (Bahl et al., 2004). For this purpose, the gfp gene has been chromosomally inserted downstream a LacI repressible promoter. When the host contains a conjugative plasmid carrying the lacI gene, expression of gfp is absent. However, when the plasmid is lost, GFP is derepressed and cells become green. Antimicrobial drugs with the ability to induce plasmid loss have already been identified. In this study we would like to test whether the observed plasmid instability effect can be maintained when these drugs are embedded in a newly developed silicone matrix. In addition, the effect on bacterial growth and ability to form biofilm will be studied. The scenario would resemble for instance a tube or a catheter used for drug delivery in therapeutical and medical use. Briefly, silicone rubber has been modified to produce interpenetration polymer networks of silicone rubber and hydrogel material in super critical carbon dioxide. The impregnated hydrogel material functions as a reservoir and transport facility inside the matrix. This technology ensures that a wide range of drugs can be regularly released from the silicone rubber including hydrophilic drugs. See also www.biomodics.com

The morphology of Interpenetrating polymer network (IPN): Virgin Silicone, Interpenetrating polymer network (IPN) and drug impregnated hydrogel material (www.biomodics.com).


Supervisors: Leise Riber: liiber@bio.ku.dk
             Mette Burmølle: burmolle@bio.ku.dk

Barriers in horizontal gene transfer of a synthetic plasmid pX1.0

Project type: Bachelor or Master

Horizontal gene transfer is thought to be the major contributor to bacterial evolution and the recent spread of multi-resistance to antibiotics.
Earlier this year we published pX1.0, the first entirely synthesized conjugative plasmid chromosome. In Silica construction of pX1.0 was done using Gene Designer (v1.1) software provided by DNA2.0 based on a consensus of gene content derived from available IncX plasmid sequences. The designed construct is 30,199 base pairs in size and currently constitutes the quintessential minimal incX1 plasmid. Synthesis resulted in a fully functional IncX1-type plasmid with the ability to replicate stably at a fixed copy number in *Escherichia coli* and transfer horizontally, through conjugation, into other species of *Enterobacteriaceae*. Thus, pX1.0 is the first synthetic self-promoting DNA molecule to transfer between two bacterial species.

We propose to investigate what limits the host range of e.g. IncX1 plasmids to *Enterobacteriaceae* by replacing IncX1 transfer regions with the similar regions from other plasmids, and by doing the same with plasmid stability modules (Figure 1) and origins of replication. This project involves Long-range PCR, cloning, sequencing and trans-species conjugation experiments.

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Leise Riber (Post Doc) [lriber@bio.ku.dk](mailto:lriber@bio.ku.dk)
Plasmid encoded phage resistance in *Salmonella* - battle of two molecular parasites?

**Project type:** This project is available to 1-2 Bachelor or Master students and is part of a collaboration with Henrik Hasman (Food, DTU)

Salmonellosis has been a big problem world-wide and still is. In 2003 *S. Enteritidis* and *S. Typhimurium* were the major serotypes of *Salmonella Enterica* to cause human infection. The phage-type PT1 of *S. Enteritidis* made up 17.8 % of the total cases caused by this strain, being the second most occurring type. The methods of phage-typing to subdivide the serotypes into phage-types have been widely used for many years as a tool to trace an outbreak or a simple infection back to the indigenous source. Studies have now shown that the introduction of an IncX plasmid can change/convert the phage-type of *S. Enteritidis* by protecting the bacterium from infection of some of the bacteriophages. This might have influence on the phage-typing in the future, since a serotype like *S. Enteritidis* with a self-introduced plasmid can change phage-type and by that it can be hard to map the spread of the original type causing the disease.

Also, this phenomenon is extremely interesting from a purely scientific point of view. The potential of plasmids to encode resistance to bacteriophage attack can prove to be a very advantageous selective trait.

The aim of this study is to find the genetic mechanism responsible for the phage-type conversion introduced by the IncX plasmids and to see if other plasmids have the same ability.

**Supervisor:** Lars H. Hansen (Associate Professor): [hestbjerg@bio.ku.dk](mailto:hestbjerg@bio.ku.dk)
Determination of plasmid and Insertion Sequence (IS) copy number by quantitative PCR

Project type: Bachelor or Master

In this project the student(s) will determine how many copies of a plasmid exists in each bacterial cell by the use of qPCR. This method will also be used to follow a change in copy number when subjecting a resistance plasmid bearing strain to increasing concentrations of antibiotics. Finally, qPCR will be used to determine the number of other mobile genetic elements, called IS elements and transposons, in coliform bacteria isolated from both pristine environments and environments subjected to antibiotic pressures (e.g. hospital wastewater). These elements can be present, both on plasmids and bacterial chromosomes and are responsible for much of the shuffling around of resistance genes.

Supervisor:  Lars H. Hansen (Associate Professor): hestbjerg@bio.ku.dk
Metagenomics and Transcriptomics of Plasmids and Genomes

Today, many traditional microbial research techniques have been turbocharged by next generation sequencing and synthetic biology. In the genomics group we apply different sequencing strategies to answer complex microbiological questions. 16S rDNA amplicon sequencing illuminates the microbial diversity of extremely complex microbial communities where each experiment can yield information on millions of individual 16S rDNA sequences, thereby thoroughly describing natural communities. Whole genome sequencing and assembly can inexpensively derive the behavior of individual plasmids, bacteriophages and bacterial species. In our in-house sequencing facility we dispose of both Illumina HiSeq2000 and ROCHE GS FLX (454) sequencing technologies. We have recently sequenced > 100 million 16S rDNA sequences and assembled more than 50 plasmids, 40 phage genomes, 50 bacterial genomes and several metagenomes repeatedly revealing chromosomes and genes encoding completely new traits.

Modern high-throughput sequencing technologies have also made metagenomics and transcriptomics possible by the large amount of sequences generated. Metagenomics is bulk sequencing from an environment to find out what organisms and genes are abundant in that environment. This is the most effective method for studying the function of the organisms in an environment. Transcriptomics, on the other hand, is bulk sequencing of mRNA and is used to unravel what genes are expressed. Both methods are novel and in rapid development both in terms of sequencing technology and in the field of bioinformatics.

As an extension to the new sequencing technologies, the genomics group has started using synthetic biology, to create biology from bits and bytes. Accordingly we have recently (2011) published the rational design and synthesis of a large conjugative plasmid.
Gut Feeling: What’s going on in the mobile pool of the human microbiome?

Project type: “Fagprojekt” (smaller student project), Bachelor or Master project

Keywords: Bioinformatics, human intestinal tract microbiome, MetaHit

The recent landmark study (Metagenomics of the Human Intestinal Tract, MetaHIT) published in Nature in 2010 has contributed a massive amount of new sequencing data (more than 500 gigabases from 124 individuals). Since the ecology of plasmids is virtually unexplored from a metagenomics standpoint, this vast resource represents a goldmine of new information. From the 124 samples over 600,000 contigs have been assembled and more than 3 million non-redundant protein sequences have been derived from these. This is 150-fold more than the human genome equivalent and corresponds to some 1000 bacterial species, which likely represent a large fraction of species associated with humankind intestinal tract. The aim of this study will be to mine the MetaHIT metagenome for plasmid-signature sequences using a number of different search tools (Blast, Hmmer, Usearch) in order to identify possible plasmids and establish the composition of familiar and novel plasmid types or plasmid-associated traits within the “human gut mobilome”.

A strong interest in bioinformatics, but not necessarily experience, is a minimum requirement for this project.

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The airborne microbiome during infancy in a birth cohort and possible association to later development of asthma, eczema and allergy

Project type: Master

Keywords: Pathogenic bacteria, children, asthma, airborne bacteria, high throughput sequencing

Main Objective: Sequencing of the airborne microbiome during infancy in a birth cohort and analysing any association to later development of asthma, eczema and allergy.

Research Hypothesis: The human microbiome in early life is critical for switching children to a trajectory towards the development of asthma, eczema and allergy.

Perspective: If we find support for the research hypothesis, this will provide a target for future prevention strategies for these diseases and a model for other lifestyle diseases.

Rationale and Research Hypotheses: Asthma, eczema and allergy are the most common chronic diseases in children in the industrialized countries and have doubled or tripled in recent decades suggesting strong influence of the exposome. Such interaction between genome and exposome must occur in the very early life, since most chronic cases present themselves within the first year of life. Evidence from our COPSAC birth cohort and others supports a role of the human microbiome in perinatal life on the origins of asthma, eczema and allergy.

Study design: We will utilize the comprehensive clinical data from our COPSAC birth cohort of 411 at-risk children with prospective clinical assessments, through extensive monitoring of symptoms, objective end-points and environmental exposures. We sampled the bedroom air for in the first year of life using PM2.5 cyclone pumping air through filters in the bedroom for 1 week in the first year of life in the COPSAC2000 infants. For capture of DNA in the filters, we will apply the Qiamp circulating nucleic acids kit to centrifuged and frozen samples in WP1. To detect the presence of bacterial DNA, a direct 16S rDNA PCR approach will be applied as well as a multiple displacement amplification (MDA) prior to the PCR step. 16S rDNA and total DNA metagenomics in combination with full genome sequencing of selected bacteria will be performed on all the samples.

This will be supplemented by 454 sequencing to get more in-depth knowledge of the bacterial phylogeny for specific samples showing putative differences. The 750 bp reads produced here will give a species specific resolution, to identify effect causing or indicator microorganisms. Thirdly, when putative organisms of interest have been identified in samples a thorough sequencing effort is done by shotgun metagenome sequencing via 2x100 base paired end sequencing, also on the Illumina HiSeq 2000, to assemble and reveal crucial information about the organisms phenotype, resistance and virulence traits. A subset of MDA positive samples from WP1 will also be shotgun sequenced using a few lanes on the Illumina Hiseq 2000 to reveal viral sequences. All sequencing data will be stored in a dedicated data storage facility at University of Copenhagen with appropriate backups etc. to insure optimal data storage safety.

Supervisors: Søren J. Sørensen: sjs@bio.ku.dk
External supervisor: Clinical Professor Hans Bisgaard, Gentofte Hospital
High throughput sequencing and metatranscriptomic analysis of microbial diversity and activity

Project type: Master

Hypothesis: There is a growing interest in indigenous flora in raw milk and the cultures involved in the secondary maturation of cheeses, including surface maturation, which offer great opportunities to vary the cheeses taste and appearance. The aim is to gain a greater molecular understanding of new prokaryotic and eukaryotic starter cultures and to identify new potential enzymes of importance for taste and aroma formation.

Starter cultures traditionally have been used for the acidification of cheese milk, but there is a growing interest in indigenous flora in raw milk and the cultures involved in the secondary maturation of cheeses. Therefore, there is a need to investigate the microbial diversity in raw milk with the possibility to follow them through the processes of cheese making and ripening, which would promote better understanding of how cheese characteristics vary with respect to microbial growth and activity. Metatranscriptomic analysis of cheese will give insight into genes expressed, and thus insight into the potential metabolic and biosynthetic pathways, which will help in silico prediction of the potential cellular processes involved in metabolic pathways important for formation of flavors and aromas.

Cheese samples will be collected throughout the process of cheese making and ripening. RNA will be extracted from cheese samples and the mRNA will be enriched using different rRNA removal. The mRNA enriched samples will be reverse transcribed and sequenced on an Illumina Genome Analyzer (Illumina) or Genome Sequencer FLX (Roche) following the manufacturers’ instructions. The resulting millions of sequences will then be traced back to the originating organisms and genes, and the species composition and potential metabolic and biosynthetic pathways in cheese making and ripening will be characterized. This project depends on one or more students with fair knowledge in molecular biology and experience in UNIX and Perl programming is advantageous.

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Vultures - disperser of leprosy and anthrax, or antibiotic megastore?

Project type: This project is available to 1-2 Masters students and is part of a collaboration with Tom Gilbert (SNM, KU) and Tomas Sicheritz-Ponten (CBS, DTU).

New World vultures are one of the most charismatic creatures of the Americas, famous for their love of rotting carrion. While to many disgusting, the fact that their diet is such raises some intriguing questions of general microbiological, and even medical importance. On the one hand, the fact they hunt and scavenge on mammals such as armadillos, that naturally carry bacteria pathogenic to humans such as leprosy, suggests that the vultures themselves may enter the chain as a tool for dissemination of such bacteria. In short, if a vulture eats leprosy containing armadillo, does this survive its digestive system, and emerge at the other end in a viable form? Is so, are vultures driving a growing leprosy epidemic? And what about other pathogenic bacteria such as anthrax? On the other hand, the fact that vultures spend large amounts of time with their heads inside rotting carcasses, and subsequently ingest the meat, without developing sores on their faces or indeed dying of bacterial related food poisoning, suggests that they may have in built mechanisms to resist such effects. Examples might be competitive bacterial communities inside the gut or on the face of the bird, or even evolved forms of antibiotic resistance.

The aim of this MSc project will be to investigate the questions outlined above. Obtaining sterile sampled vulture skin, gut and fecal material from our collaborator Dr Gary Graves at the Smithsonian Institute, the project will combine conventional PCR analyses with second generation high-throughput sequencing approaches to first investigate for the prevalence of pathogens such as leprosy and anthrax in the birds (and their feces!), and secondly begin to characterize the metagenomic composition of their skin and gut, in order to investigate the interplay with the bacterial community on the ingested bacteria.

Supervisor: Lars H. Hansen (Associate Professor) hestbjerg@bio.ku.dk
Sequencing assembly and annotation of the yeast genome *Debaryomyces hansenii* isolated from Danish cheese

**Project type: Master**

*Debaryomyces hansenii* is one of the yeast species most frequently isolated from a large diversity of natural sources including fruit, air, water and soil, but most frequently from processed food products, where it contributes to development of the bacterial community, aroma formation, inhibition of undesirable contaminants and stabilization of color. *D. hansenii* is halophilic yeast with some strains being able to grow at NaCl concentrations up to 24% (w/v). The species *D. hansenii* is a highly heterogeneous based on phenotypic differences and optimal growth condition.

A *D. hansenii* strain was isolated from Danish cheese and was found to have different physiological characteristics than the sequenced reference strain (*D. hansenii* var. *hansenii* (type strain CBS 767)). Therefore, the aim of this project is to sequence the genome of the Danish strain, to assemble and annotate its genome and to compare with the genome of the reference strain. This project depends on one or more students with fair knowledge in molecular biology and experience/interest in UNIX and Perl programming.

**Supervisors:**
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The childcare institutional environment – a heaven of the pathogens

*Biodiversity and proliferation of pathogens in multispecies biofilms*

**Project type:** Bachelor or Master

**Keywords:** Childcare environment, high throughput sequencing, biodiversity, pathogenic bacteria.

In this project we are interested in looking at the species diversity and the proliferation of bacteria (and possibly viruses) in a childcare environment. The project is part of a major initiative to increase welfare and health in Danish children’s institutions. For more information, see: [www.sibprojekt.dk/index.html](http://www.sibprojekt.dk/index.html). The project provides a unique opportunity to get hands-on experience with state-of-the-art high throughput sequencing, where we will be able to generate data of a very high quality. Generally, it is believed that we are only able to identify 1-10% of all bacteria using growth medium. Therefore, the possibility to make sequence-based biodiversity studies is a unique opportunity that most other laboratories do not have the facilities to do. Additionally, we are also interested in isolating the essential bacterial species from the institutional environment. We are relatively free to approach and focus the project in a way that suits you. So, if you would like to work with biodiversity focused on pathogens, we can fashion an exciting and very relevant project. There are many interesting questions that we have the opportunity to ask and answer.

**Supervisors:** Jonas Stenløkke Madsen: jsmadsen@bio.ku.dk
Søren J. Sørensen: sjs@bio.ku.dk
New food enzymes; screening, production and characterization

Project type: Bachelor or Master

In collaboration with Chr. Hansen A/S, Hørsholm, projects are available for the development of enzymes that will improve the production of food enzymes. The projects will examine questions such as: how are new cheese enzymes developed, how is enzyme production regulated and how specific textures or flavours can be obtained in cheese or yogurt. During the project the primary host organisms will be strains such as Aspergillus, E. coli and Bacillus. The tasks and techniques will typically include molecular biology, protein chemistry and practical usage of enzymes in dairy applications. Individual projects will be tailored to the needs of the individual students interests and requirements.

Supervisor:  Bo Jensen (Associate Professor): boje@bio.ku.dk
Soil Microbiology

Bacteria are the most abundant organisms in soil in terms of numbers with approximately $10^7$ cell per gram soil. This makes them a key player in nutrient turnover in all environments, and the study of soil microbial ecology is vital for understanding the effects of climate change on our planet. Furthermore, soil is a large reservoir both for pathogens and for plasmids carrying genes encoding resistances to natural occurring antibiotics. Soil microbiology is one of the oldest areas of research in the section and one where we have extensive expertise.

CLIMAITE-Bacterial and archaeal response to global warming in soil

Project type: Master (30-60 ECTS)

Keywords: Pyrosequencing, Global Warming, Microbial Diversity, Bacteria, Archaea qPCR, Molecular Microbial Ecology, Bioinformatics.

How does the microbial community in soil react to global warming? Do we see a changed community structure? Will some bacterial and archaeal genera use the changed environmental conditions to flourish and dominate the soil biosphere? Do we find a changed ecosystem functioning due to global warming?

These basic research questions are still unanswered. In the project the newest molecular techniques like realtime qPCR and pyrosequencing will be used to explore the bacterial and archaeal communities in soil. Quantifying and sequencing the bacterial and archaeal soil community will help to solve some of the unanswered questions regarding the impact of global warming on the microbial soil communities.

The suggested project is part of a large-scale interdisciplinary research project - CLIMAITE – that aims to determine the effects and impact of global warming on a Danish ecosystem. The project has been running since 2006 and has contributors from six different research institutions, for further info regarding the CLIMAITE project see [www.climaite.dk](http://www.climaite.dk).

The applicant will get hands on experience and working independently with some of the newest molecular techniques in microbiology like qPCR and sequencing. Since the project is in the cross field between ecology, microbiology and molecular biology focus can be on everything from classical ecology to hard-core bioinformatics depending of interested.

Feel free to contact one of the following for further information or an informal talk.

*Supervisors:  Lasse Bergmark: lbergmark@bio.ku.dk  
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Global change, greenhouse gases, microbial activity and diversity

Project type: Bachelor or Master

Keywords: Decomposition, enzyme activity, nitrous oxide

The different climate models predict future changes in temperatures and distribution of precipitation. Microbial activity will respond to such changes and can easily be tracked by measuring products like nitrous oxide and carbon dioxide.

As a result of increasing precipitation large land areas may become water saturated and temporarily covered by water. Consequently anaerobic processes as denitrification and methane production may become dominating in these areas. Soil temperature and moisture have also importance for the production and emission of greenhouse gases. Formation of nitrous oxide (and carbon dioxide) as metabolic products will be examined in soil samples incubated at different temperatures and moisture contents and gases measured by gaschromatography.

Climate change will also influence soil microbial diversity as result of increasing temperatures, changing composition and amounts of nutrients and a number of other soil factors. There are many ways to address the study of Microbial diversity including available microscopic, physiological and molecular methods. Several approaches are possible such as isolation and identification of different groups of microorganisms, determination of the ratio between bacteria and fungi, substrate utilization or determination of ribosome DNA sequences extracted from soil.

Experiments may be established in the lab or as field measurements examining effects of climate events and the gases could be measured by gaschromatography or by a photoacoustic detector.


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Biofilm

Microbial communities are often organized in biofilms, which are recognized as bacteria and other microorganisms attached to a surface and embedded in a self-produced matrix. Biofilms are present on a variety of surfaces – from stones and plants in aquatic environments to water distribution tubes, air conditioning facilities, and also in our own bodies, for instance in the digestion system. Additionally, infectious diseases often involve biofilm formation by pathogenic bacteria.

Bacterial cells present in biofilms are known to differ phenotypically from those living as planktonic cells. The gene and protein expression patterns are changed and the cells exhibit increased tolerance towards antimicrobial agents compared to their planktonic counterparts. In natural environments, biofilms often consist of multiple bacterial species, which may result in competition, but also in a range of advantages for the bacteria, including the possibility of co-metabolism and gene exchange. It has been reported that some species are only able to form biofilm in the presence of other species and that resistance towards antimicrobial agents is enhanced in multispecies biofilms when compared to single species biofilms. In situ studies of multispecies biofilms are therefore essential parts of the description of microbial communities.

In the Molecular Microbial Ecology group, we work with several aspects of biofilm biology and ecology. We are especially interested in interactions within bacteria from different species resulting in synergies and cooperation. We work mainly with bacterial isolates from natural environments (soil, water etc.), but we also have collaboration with The National Serum Institute, Faculty of Health (KU) and others, where more clinical problems are addressed.

Student projects can be defined in many areas of biofilm research. Below, you will find suggested student projects, which are closely related to the ongoing research in the biofilm area. You are also very welcome to suggest your own project related to this topic and we will then help you to further define this project and – if suitable – include external collaborators.
The interconnection between biofilm formation and horizontal gene transfer

Project type: “Fagprojekt” (smaller student project), Bachelor or 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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The impact of bacterial growth phases on the ability of biofilm formation

Project type: Bachelor

Keywords: Growth phases, physiological changes, biofilm

The ability of natural bacterial isolates to form biofilm has in several cases been observed to vary much in relation to the physiological state of the bacterial cell. Detailed knowledge on this interdependency is of major importance when evaluating the ability of biofilm formation by individual species and multispecies communities. Biofilm formation is important for the assessment of the virulence of bacterial species. Therefore, it is vital to investigate the effect of bacterial physiology on biofilm formation in order to develop appropriate and precise protocols for assessing biofilm formation.

This project will be based on a number of natural bacterial isolates known to form different levels of biofilm. Samples from different growth phases will be evaluated with regard to biofilm formation. Also, the impact on biofilm formation of prior short or long-term colony growth will be evaluated.


Supervisor: Mette Burmølle: burmolle@bio.ku.dk
Differential gene expression in multispecies biofilm

Project type: Master

Keywords: Synergism, 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.

The aim of this project is to perform transcriptome analysis of bacterial species present in multispecies biofilms. The gene expression in these mixed communities will be compared to that of monospecies biofilms and thereby lead to an identification of the genes involved in multispecies biofilm synergism. More specifically, mRNA will be isolated from mono- and multispecies biofilms, and cDNA libraries will be constructed and sequenced by high throughput sequencing. Obtained sequences will be quantified and assigned to the specific bacterial genomes.

This project is suited for the student with interests in biofilm and gene expression, and who would also like to get hands-on experience with transcriptome analysis and high throughput sequencing.


Supervisor: Mette Burmølle: burmolle@bio.ku.dk
Bacterial cooperation in multispecies biofilms associated to food production devices

Project type: Bachelor, Master

Keywords: Synergism, cooperation, biofilm, horizontal gene transfer

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 and by use of species-specific detection techniques (t-RFLP or qualitative PCR), species important for the observed synergy will be identified. Furthermore, the role of conjugal gene transfer in relation to biofilm stability and synergism will be evaluated, as conjugation has in several cases been demonstrated to enhance biofilm formation.


Supervisor: Mette Burmølle: burmolle@bio.ku.dk
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.

References:  

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Enzyme and antibody technology in the battle against biofouling

Project type: Master

AEROZYME is an EU financed project with the objective of developing an enzyme-based marine paint that will keep ships’ hulls free from biofouling. Biofouling is attachment and growth of marine organisms such as bacteria, algae and barnacles to ships and underwater structures. Biofouling is a huge economic problem in the marine industry both because it is expensive to remove but also because it can reduce a vessel’s performance thereby increasing fuel consumption. Current antifouling coatings have proved to be highly toxic to marine life.

Being the active paint ingredient it is of vital interest to be able to detect the enzyme activity and distribution on a painted surface.

To protect the enzyme from the solvents used in paint production a technology has been developed where the enzyme is encapsulated in an aerogel. By using a self-polishing paint system, fresh enzyme will constantly be exposed on the painted surface, thereby hindering organism settlement.

The student project is to develop technologies (assays) to measure the paint enzyme concentration and activity. Antibody technology will be used as an approach to detect the enzyme on the paint surface. It is also of interest to understand how the protein is encapsulated in the aerogel particles. A combination of different technologies will be used to illustrate this. The project will therefore comprise of the development of ELISA and other antibody “sandwich” techniques.

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