NTNU
Kunnskap for en bedre verden

Master's theses proposals

Department of Biotechnology

This document contains proposed projects relevant for students at MSc Biotechnology (2- and 5-year study), MSc Aquatic Food Production, the 2-and 5 year MSc Chemical Engineering and Biotechnology (siv.ing).

The different proposals might have different credits. This relates to the proposed theses being planned for different study programmes: the MSc Biotechnology (60 credits), MSc Aquatic Food Production (30 credits) or MSc Chemical Engineering and Biotechnology (30 credits). Some thesis proposals may also be relevant as specialization project (15 credits)

Please contact supervisor directly if you have any questions regarding theses' proposals.

Academic year 2015/16

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Department of Biotechnology

Masteroppgave (60 [ECTS])

Komparativ studie av mesofil og psykrofil uracil-DNA glycosylase 2 (UNG-2) Engelsk titel: Comparative studies of mesophilic and psychrophilic uracil-DNA glycosylase 2 (UNG-2).

Faglærer: Finn L. Aachmann (K4.140; finn.l.aachmann@ntnu.no; 73 59 33 17)
Veiledere: Edith Buchinger og Geir Slupphaug

Bakgrunn:
Uracil finnes ikke naturlig som base i DNA. Den oppstår imidlertid som et resultat av enten feilinkorporering av dUMP i stedet for dTTP under DNA replikasjon eller deaminering av cytosine. Sistnevnte skade gir U:G basepar i DNA, som fører til mutasjon etter neste DNA-replikasjonsrunde. I cellene finnes 4 ulike DNA-glykosylaser som kan fjerne uracil. Dette gjøres ved at bindingen mellom deoksyribosen og uracil kløyves, noe som igjen fører til en flertrinns enzymatisk prosess som gjenoppretter normal cytosin. Den kvantitativt dominerende uracil-DNA glykosylasen i humane cellekjerner er UNG2. Enzymet består av et 25 kDa C-terminalt katalytisk domene, og et N-terminalt 9 kDa regulatorisk domene. Krystallstrukturen av det katalytiske domenet bundet til uracil-holdig DNA ble kartlagt i 1996 av Geir Slupphaug et al., (1). Det N-terminale domenet av UNG2 er hovedsakelig ustrukturert. Dette domenet har flere funksjoner, og modulerer både enzymatisk aktivitet og intracellulær transport av det intakte UNG2-proteinet. I tillegg inneholder Det N-terminale domenet bindingsseter både for PCNA og RPA, som begge er proteiner involvert i DNA-replikasjon. Dette sammenfaller med rollen UNG2 har i utkutting av feilinkorporert dUMP ved replikasjonsgaffelen. Til slutt er det nylig funnet (2) at det N-terminale domenet finnes i ulikt fosforylerte formen *in vivo*. 3 av disse fosforyleringene ser ut til å modulere bindingsstyrken til RPA, katalyseeffektiviteten, samt selve den intracellulære levetiden til proteinet. Det er imidlertid ukjent om dette skjer ved at RPA-bindingen og/eller fosforyleringene medfører strukturendringer i det N-terminale domenet. Nylig ble det også rapportert et nytt protein, Ugene (3)som binder UNG2 N-terminal, og som er oppregulert i ulike kreftceller. Hvordan denne bindingen skjer og om det medfører strukturendringer i det N-terminale domenet, er imidlertid ukjent. I motsatt til human UNG2 er torsk UNG2 adoptert til et koldt miljø og har sannsynlig forskjellige bindingsstyrker til PCNA og RPA enn human UNG2.

Formål:
Uttrykke N-terminalt domene av human og torsk UNG-2 via rekombinant proteinekspresjon og analysere de rensede proteinene med forskjellige biofysiske metoder.

Delmål:
- Uttrykke og rense proteinet både i umærket og isotopmerket form.*
- Vekselvirkningsstudier via NMR titrering og circular dichroism (CD)+ på forskjellige temperaturer
- Kalorimetrisk måling av vekselvirkning med andre proteiner via isotermisk titrerings calorimetri (ITC)+

* Dette er delmål som skal gjennomføres
\+ Her er det opp til studenten å velge etter interesse.

Metoder og teknikker i projektet.
Proteinekspresjon (både umerket og merket med isotoper – ikke radioaktive). Rensing og konsentrering av protein.
Ulike metoder for å studere protein: circular dichroism, kjerne-magnetisk resonans og isotermisk titrerings-calorimetri.

1. **Slupphaug, *et al.*** 1996.. Nature *384*:87-92.
2. **Hagen, *et al.*** 2008. Embo J *27*:51-61.
3. **Guo, *et al.*** 2008. Cancer Res *68*:6118-6126.

Masteroppgave (60 [ECTS])
Karakterisering av nye proteiner for anvendelse i bioraffineri

Engelsk tittel: Characterization of novel proteins for use in biorefineries
Faglærer: Finn L. Aachmann (Tel. 73 59 33 17; E-post aachmann@nt.ntnu.no)
Veileder: Gaston Courtade (Tel. 73 59 06 62; E-post gaston.courtade@ntnu.no)

Bakgrunn: Et lovende fremtidsperspektiv innen "bioøkonomi" er bioraffineri hvor biomasse fra land- og/eller havressurser omdannes til monosakkarider. Disse monosakkaridene kan brukes som byggesteiner i nye polymerer, til produksjon av bioetanol og andre kjemiske forbindelser. Et kritisk trinn i bioraffineringsprosessen er den enzymatiske nedbrytning av biomasse (kitin og cellulose) som er uløselig i vann. Dette er en stor flaskehals for å oppnå en effektiv prosess. I 2010 oppdaget vår samarbeidspartner på NMBU i Ås et enzym som setter fart på degraderingsprosessen gjennom oksidative brudd av glykosidiske bindinger i kitin[1]. Denne enzymatiske funksjonen er et fellestrekk for en ny familie enzymer innen polysakkarid-nedbrytende enzymer som heter lytisk polysakkarid monooxygenaser (LPMO). Den første NMR-strukturen av en LPMO, som virker på kitin, ble strukturoppklart ved vårt institutt i 2011[2] og dens funksjon er også kartlagt i samarbeid med NMBU. Vi har nå nye LPMO som virker på cellulose, og vi er interessert i å oppklare enzymets 3D struktur ved bruk av NMR-spektroskopi, for å forstå mer om enzymets funksjon og virkemåte på substratet.

Formål:
Uttrykke lytisk polysakkarid monooxygenase via rekombinant proteinekspresjon, strukturoppklare og analysere det rensede proteinet med forskjellige biofysiske metoder.

Delmål:
- Uttrykke og rense proteinet både i umerket og isotopmerket form.*
- Opptak av multidimensionell NMR-spektra og tilordning av proteinryggraden og sidekjede.*
- Strukturbestemmelse av proteinet.*
- Vekselvirkningsstudier via NMR-titrering og evt. circular dichroism (CD)+
- Kalorimetrisk måling av vekselvirkning mellom LPMO og andre substrater/proteiner via isotermisk titreringskalorimetri (ITC)+
- Annet+

* Dette er delmål som skal gjennomføres.
+ Her er det opp til studenten å velge etter interesse.

Metoder og teknikker i prosjektet.
Proteinekspresjon (både umerket og merket med isotoper – ikke radioaktive). Rensing og konsentrering av protein. Ulike metoder for å studere protein: circular dichroism (CD), kjerne-magnetisk resonans (NMR), væskekromatografi (FPLC), isotermisk titreringskalorimetri (ITC). Det er mulighet for kortere opphold på NMBU eller på Aalborg universitet hvis kandidaten ønsker dette.

[1] Vaaje-Kolstad, G. V., *et al*. (2010). "An Oxidative Enzyme Boosting the Enzymatic Conversion of Recalcitrant Polysaccharides." Science **330**: 219-222.
[2] Aachmann, F. L., *et al*. (2012). "NMR structure of a lytic polysaccharide monooxygenase provides insight into copper binding, protein dynamics, and substrate interactions." Proc Natl Acad Sci U S A 109(46): 18779-18784.

Advisor / Faglærer:
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Please feel free to stop by my office. If you are interested in these general topics, but have a different problem in mind – let's talk!

Most of these projects can be adjusted to fit a 60sp or 15+30sp profile.

1. Biological network analysis

Network analysis has been central to uncovering important principles of interactome organization. Using tools and approaches from network analysis to combine different types of biological data can generate new insights on cellular processes. Gene expression correlation networks consist of genes that showing strong similarities or dissimilarities in their expression patterns, making it possible to identify important gene clusters associated with a given phenotype or biological function .

a. Gene expression and protein interaction networks in *Arabidopsis thaliana* during stress conditions
Gen uttrykks- og protein interaksjonsnettverk i *Arabidopsis thaliana* under stress betingelser
In this project, the candidate will combine *Arabidopsis* mRNA expression data with its known protein interaction network. This project is in collaboration with Prof. Bones' group (IBI), which has generated transcription data on multiple conditions in *Arabidopsis*. The goal is to identify genes and gene clusters that change behavior during stress conditions, which may lead to the uncovering of functional pathways.

b. Comparative study of gene expression networks in *Synechocystis sp. PCC6803*
Sammenligning av gen-uttrykksnettverk i *Synechocystis sp. PCC6803*
In this project, the candidate will use a recently developed network approach (Voigt, Nowick & Almaas) to study evolution and conservation of gene expression correlations. The candidate will use the comprehensive database cyanoExpress, which contains gene expression data from 177 distinct genetic and environmental perturbation experiments for the cyanobacterium Synechocystis sp. PCC6803. Synechocystis is an important model organism for developing our understanding of photosynthesis, and the research focus will be on using network analysis methodology to study photosynthetic function. Co-mentor: Prof. Martin Hohmann-Marriott.

c. Comparative study of gene expression networks in *Homo sapiens* brains
Sammenligning av gen-uttryksnettverk i *Homo sapiens* hjerne
The ability to understand consequences of genetic variation among humans is a significant challenge in biology. The goal of this project is to use a recently developed network approach (Voigt, Nowick & Almaas) to study the variation of gene co-expression patterns in two regions of human brain: The cerebellum and frontal cortex. The candidate will use the Gene Expression Omnibus dataset GSE36192, which contains gene expression data measured in 911 tissue samples from 396 subjects.

2. Metabolic Engineering

Flux Balance Analysis (FBA) is the central approach in modeling genome-scale metabolism, with its capability for predicting metabolic phenotypes and identifying possible approaches for engineering cellular behavior. A genome-scale model may be generated within 48 hours[3], and the COBRA toolbox[4] in MatLab can be used for analysis of the model.

a. Computational modeling of evolution of genome-scale metabolism
Modellering av evolusjon i genom-skala metabolisme

The goal of this project is to develop new methods based on the FBA framework for emulating processes guiding the loss and acquisition of genes in microbes that live in changing environments. Computational simulation results will be compared with actual organisms. Interest is towards understanding of evolution of antimicrobial resistance.

b. Computational modeling of random metabolic networks
Modellering av tilfeldige metabolske nettverk

The goal of this project is to develop a new method to generate random metabolic networks. The properties of random metabolic network (that are capable of supporting biomass generation) are of great interest when studying robustness properties of metabolic networks, and they can shed light on the viability of potential antibiotic targets. The current project will develop a novel method that can generate a random metabolic network of any size, starting from an initial network. Thus, the method can also be used to study genome expansions, contractions, and the properties of possible minimal genomes resulting from an organism.

[3] http://seed-viewer.theseed.org/models
[4] http://opencobra.sourceforge.net/

Master thesis in Biotechnology, 60 sp

Microbial stabilization: a tool for combating pathogens in aquaculture systems?

Goal

To evaluate whether "microbial stabilization" is feasible a strategy to prevent blooming of pathogens in aquaculture systems.

Background

Fishes are sharing their living environment with high loads of bacteria. In nature, the fishes experience relatively stable microbial environments. In aquaculture systems, the reared animals are exposed to high and unstable loads of bacteria compared to the natural environments. For a number of reared marine species, we have previously demonstrated that stable microbial environments improve growth and survival. Stabilization of microbial communities in the rearing water can be obtained by so-called K-selection, i.e. keeping microbial loads close to the carrying capacity (the maximal microbial population size that the system can support). Recirculating aquaculture systems (RAS) are well suited for exerting microbial K-selection, because the water going in to the rearing tanks has a carrying capacity similar to that of the water inside the rearing tank. K-selected bacteria are typically specialists, characterized by low maximum growth rate, but with the ability to compete when the available resources are limited. In a system where the carrying capacity is dramatically increased in the rearing tank, e.g. by addition of fish feed in a flow-through system, rapid-growing, opportunistic bacteria will bloom. This would be an example of an r-selected system. R-selected bacteria have a high maximum growth rate, but are poor competitors when resources are limited. According to ecological theory, opportunistic rapid-growing bacteria would more easily bloom in an r-selected system with excess of resources. Pathogenic bacteria are typically r-strategist, and according to ecological theory, they would be outcompeted in a K-selected system.
If stabilization of the water microbial community is found to prevent blooming of opportunistic bacteria, this could be a promising and sustainable strategy for preventing pathogenic invasion in aquaculture systems.

Work description

This master thesis will be part of the EU ERANET project MicStaTech, and work closely together with the project researchers. We will use lab-scale continuous bioreactors for creating r- and K-selected microbial communities in both fresh- and marine water. Potential fish pathogens will be introduced to the systems under both K- and r-selected conditions, and the fate of these strains will be monitored by methods like qPCR and flow-cytometry combined with specific probing. For examining the dynamics of the microbial communities in the systems, methods based on analysis of sequence variation in the 16S rRNA gene will be used. Potential techniques are denaturing gradient gel electrophoresis (DGGE) and Illumina amplicon sequencing.

Supervisors: Ingrid Bakke, (Ingrid.bakke@ntnu.no) and Olav Vadstein (olav.vadstein@ntnu.no), Department of Biotechnology, Kari Attramadal (kari.attramadal@ntnu.no), Department of Biology

Master thesis in Biotechnology, 60 (15) sp

Network analysis as a tool for revealing microbial interactions in an anaerobic biogas reactor

Goal

- Develop and analyse microbial community network.

Background

Anaerobic digestion is a process where organic matter is degraded to methane and CO_2 by anaerobic microorganisms. It is widely used for generating renewable energy in the form of biogas from organic waste. The process depends on the activity of a large number of microbes, and thus the microbial communities in anaerobic bioreactors are complex and represent a number of diverse functional guilds, including both bacteria and archaea. The major microbial activities involved can be classified as: 1) bacterial hydrolysis (degradation of organic polymers to soluble organic compounds); 2) acidogenesis (conversion of easily degradable organic matter to volatile fatty acids, VFA); 3) acetogenesis (conversion of VFA to organic acids like acetate); and finally 4) methanogenesis, where methanogenic archaea convert the products from acetogenesis to methane and carbon dioxide.

Tight microbial interactions are required, for example the acetogenic bacteria and methanogenic archaea are interdependent on each other's activities (syntrophic interactions).

In an ongoing research project in collaboration with Telemark University college, we are studying the microbial community dynamics in anaerobic lab-scale bioreactors running on pig manure. From an experiment where we investigated the effect of temperature and ammonia concentration on the biogas production process, we are now generating a large dataset by using Illumina sequencing of PCR products representing a region of the 16S rRNA gene from 71 samples taken from 4 bioreactors run with varying temperatures and ammonia concentrations.

Work description

The student will use this unique dataset to study the structure and dynamics of the microbial communities by use of complex network approaches. During the last decade, network approaches have been applied to a wide variety of biological challenges with great success. In this project the student will use simple programming in python and an existing R-package for network analysis (WGCNA) to generate networks that reflect the composition and dynamics of the microbial consortium. The analysis of the networks will be used to extract knowledge about how environmental conditions and microbial roles interplay to generate functioning communities.

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Co-supervisors: Ingrid Bakke (ingrid.bakke@ntnu.no) and Anna Synnøve Nordgård (anna.s.r.nordgard@ntnu.no), Department of Biotechnology

2 Master thesis in Biotechnology, 60 sp

Colonization of salmon fry in two aquaculture systems representing distinct microbial environments

Goal

The overall aim is to assess the influence of the bacterial communities in the rearing water on the colonization of newly hatched salmon fry. More specifically, the objectives will be to examine

- Whether distinct water microbiota will result in distinct microbiota on eggs, and on skin, gills, and in the intestinal system of fry at different developmental stages
- Whether the egg microbiota influences on the fry microbiota
- The ontogeny of the fry microbiota; i.e. the temporal development of microbial communities associated with the fry
- Isolate bacterial strains from the gut at different developmental stages
- Morphometric and development of the gastrointestinal tract

Background

In all vertebrates the gut microbiota is essential to health, for example through increased energy harvest from the diet and by protection against pathogenic infections. Moreover, the colonization of the intestines after birth/hatching is required for normal development of the immune and digestive systems. Fish embryos develops in bacteria-free environments inside the egg, but as soon as the fish larvae is hatched, it is colonized (skin, gills, and intestinal system). Fishes are sharing their living environment with high loads of bacteria. In aquaculture systems, the reared animals are exposed to high and unstable loads of bacteria compared to the natural environments. The consequences of these unnatural microbial environments are poorly understood, but in previous research projects, we have demonstrated that microbial stability in the rearing water is improving the growth and survival for marine fish larvae. Although Atlantic salmon is by far the most important species in Norwegian aquaculture, the interactions between bacteria in the water and salmon fry has not been investigated. In this project, we will create two rearing systems with different water microbiota. This can be obtained by applying distinct water treatment (e.g. a flow-through system and a recirculating system). The influence of the water microbiota on the colonization process for salmon eggs and fry will be investigated. It is also known that the development of the gastrointestinal tract is influenced by the intestinal microbial community. Consequently, the functional and morphological development of the gastrointestinal tract will be studied.

Work description

We plan that the two master students should work together to perform the rearing of salmon fry at NTNU sealab. One candidate will perform sampling of water, egg, and fry throughout the experiment. The other candidate will perform sampling of fish intestines for characterization of intestinal development. Methodology includes histology and, possibly molecular biology. Flow cytometry will be used for quantifying number of bacteria in the water samples. To investigate microbial communities, the bacterial 16S rRNA gene will be used as a marker for microbial diversity. A region of the gene will be amplified from samples by using so-called universal bacterial PCR primers. The resulting PCR products will be analysed by denaturing gradient gel electrophoresis (DGGE) and possibly by Illumina MiSeq amplicon sequencing. Statistical analyses of the sequencing/DGGE data will include multivariate statistics. Finally, this project will include cultivation and isolation of pure strains from the gut of salmon fry at different developmental stages, for generating a small strain library representing the salmon fry microbiota.

Master 1 microbial ecology
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Co-supervisors: Rolf Erik Olsen (rolf.e.olsen@ntnu.no) and Kari Attramadal (kari.attramadal@ntnu.no), Department of Biology

Master 2 physiology
Supervisor: Rolf Erik Olsen (rolf.e.olsen@ntnu.no) and Kari Attramadal (kari.attramadal@ntnu.no), Department of Biology
Co-supervisors: Ingrid Bakke, (Ingrid.bakke@ntnu.no) and Olav Vadstein (olav.vadstein@ntnu.no), Department of Biotechnology

Master thesis in Biotechnology, 60 sp

Intragenomic variation among *Vibrio* 16S rRNA copies: Significance for lifestyle

Goal

- To map intragenomic microheterogeneity of 16S rRNA in two *Vibrio* strains
- To investigate the effect of life style on the abundance of the different 16S rRNA gene variants at DNA and RNA level, for both strains
- To examine the potential for horizontal gene transfer between the two strains

Background

The number of copies of the rRNA genes varies from 1 to 15 in bacterial genomes. The gene copy number has been found to be correlated to growth strategy. For example, bacteria with high maximum growth rate have been found to have more rRNA gene copies. Usually, the rRNA copies within a genome are relatively similar in DNA sequence. However, in a few species with large number of rRNA copies (*Clostridium paradoxum, Photobacterium profondum, Thermoanaerobacter tengcongensis*, and several *Vibrio* species), extreme genetic variation has been found in short regions of the 16S rRNA gene, even within genomes. This microheterogeneity has been associated with the ability of these bacteria to have different lifestyles. Further, horizontal gene transfer (HGT) is believed to play a role in the generation of this microheterogeneity. This is surprising, because the rRNA genes have been believed to not be subjected to HGT.

In our research group we have been working with two *Vibrio* strains: one is a member of the intestinal microbiota of cod larvae, and the other is a well-known fish pathogen (*V. anguillarum*). From DGGE (denaturing gradient gel electrophoresis) analysis of 16S rRNA fragments, we have observed extreme variation in the 16S rRNA gene from the *Vibrio* originating from cod larval microbiota. Interestingly, the abundance of the different gene variants appears to differ when this V*ibrio* strain is living in water, in the cod larvae, and in a standard cultivation medium.

Work description

In this project, we will examine this phenomenon further, first by mapping the intragenomic variability of the 16S rRNA gene in these two *Vibrio* strains, and further examine the effects of growth condition on the abundance of the gene variants, both at the DNA and the RNA level. Finally we will test whether HRT of 16S rRNA variants between the two *Vibrio* strains can be obtained. The project will involve cultivation of the *Vibrio* strains by distinct strategies (different liquid and agar media, and continuous cultures). Other relevant methods are PCR, qPCR, DGGE (denaturing gradient gel electrophoresis), and DNA sequencing.

Supervisor: Ingrid Bakke (Ingrid.bakke@ntnu.no), Department of Biotechnology
Co-supervisors: Ragnhild I. Vestrum and Olav Vadstein (olav.vadstein@ntnu.no), Department of Biotechnology

Hovedveileder: Main supervisor:	Professor Olav Vadstein, Department of Biotechnology (Olav.vadstein@ntnu.no) Assistant professor Ingrid Bakke, Department of Biotechnology (Ingrid.Bakke@ntnu.no)
Biveileder(e): Co supervisor	Postdoc Kari Attramadal, Department of Biology (kari.attramadal@ntnu.no)
Arbeidstittel på oppgaven (max 20 word): Preliminary titel:	**Fundamental dynamics of the microbial community in land based aquaculture systems**
Kort beskrivelse av oppgaven (max 300 word): Short description of the project:	This master thesis will be part of the EU ERANET project MicStaTech, and work closely together with the project researchers. Water treatment and design of aquaculture systems are typically focused on optimising physicochemical water quality and efforts to maintain bacteria numbers low (i.e. removing organic matter and disinfection). However, high bacteria numbers are not necessarily problematic for the cultured organisms as long as the system is biostable and all other requirements are met. What is novel in this project is that instead of targeting low bacteria numbers, we aim at optimising the systems for biostability and a beneficial composition of bacteria in the rearing water. This focus has implications for the technological solutions and optimal systems design. Elucidating the underlying mechanisms for the influence of water treatment aimed to increase system biostability and the effects on the microbial dynamics is valuable for a broad collection of systems, like recirculating aquaculture systems, aquaponic and hydroponic systems. The objective of this master thesis is to identify factors that are decisive for creating an efficient maturation unit that gives a biostable rearing water resistant to invasion and opportunistic bacteria blooms. This will be examined through experiments for freshwater and seawater at different relevant temperatures examining effects of different retention times and biofilm area per volume on the development of microbial communities. The development of the microbial composition in both biofilm and in the water will be studied with relevant and up-to-date methods for characterization of microbial communities and water quality (e.g. DNA extraction, PCR, DGGE, DNA sequencing, flow cytometry).
Oppgaven passer for (angi studieretning(er)): Suitable for (main profiles):	Marine Coastal Developement, Biology, Biotechnology

Masterprosjekter (60 sp) and TBT4500 projects (15sp)

Working Title

Molecular investigation of the beer brewing process

Beer brewing is not only one of the oldest biotechnological processes; it is also one of the largest. In recent years there has been established many microbreweries in Norway but also the number of home brewers has increased significantly. Beer brewing is a complex biochemical and microbial process with many variables that affect the taste and quality of the finished product: type and composition of malted barley and other grains/ sugar-containing ingredients, development of bitterness and aroma by hop addition during various steps of brewing, water quality and adjustments, temperature profile during mashing, pre-condition and type of yeast, pitch number and fermentation temperature, maturation and storage. At IBT we are developing competence and conducting experiments on the microbial aspect of the beer brewing process with particular focus on challenges of the microbrewery industry.

The objective of the Master's project is to characterize at the molecular level the effects of selection, pre-conditioning and pitch number of yeast and compare with sensory outcome on the finished products. Several brewing in the 30-40 l scale will be performed, and analyses of the vort during the yeast fermentation, storage and finishing will be performed with mass spectrometric instrumentation (LC-MS, GC-MS incl head space injection). Of particular focus will be to characterize the flavor profiles as a function of the variables.

Supervisor

Professor Per Bruheim, (Per.Bruheim@ntnu.no), tlf. 73593321/ 41498495

External collaborator

Thomas Sjue, Austmann bryggeri, Trolla, Trondheim

Masterprosjekter (60 sp)

Working Title

Mass Spectrometric Metabolic Profiling of Cancer:

- Study of Carbon- and Energy Metabolism in Human Cancer Cells

While abnormal metabolism of cancer has been known for over eighty years ("The Warburg Effect" – increased glucose consumption and lactate production) still much remain to be elucidated why and how cancer acquires this change in metabolism. Several recent discoveries have elucidated the role many common oncogenes and tumor suppressors play in reprogramming of metabolic pathways during oncogenesis, and lately it has been revealed that rewiring of metabolism in cancer is not only a consequence of hyper-activation of signaling pathways that instruct cells to grow, but that altered metabolism in itself can play a tumorigenic role. Thus, cancer metabolism is receiving renewed and increasing focus as therapeutic target.

Mass Spectrometry (MS) is the most important technology to investigate metabolite pool (Metabolomics) and metabolic fluxes (Fluxomics) – two central analytical techniques to the study of the Carbon- and Energy metabolism of any biological system.

The main objective of the MSc project(s) is to perform stress response studies on human cancer cell lines using already established MS Metabolic Profiling methodology as well as develop new MS methods on the recently installed MS instrumentation at the NT MS Core Facility. The experimentation will comprise a number of well-known cancer cell lines combined with a number of cytostatics (e.g. DNA damaging agents) and other inhibitors of protein and cellular activity, and monitoring the cellular stress response at the metabolite pool and metabolic flux levels (^{13}C experimentation). The expected achievement of the project is to generate more knowledge about cancer metabolism with potential identification of new cancer therapy targets.

Several MSc students can choose this project. After a period of training in cell cultivation, sample preparation and mass spectrometry will individual MSc projects be developed together with each student. The project can have a biological focus, MS analytical focus, or data analytical/ modeling focus dependent upon the interest of the student.

Supervisors

Professor Per Bruheim, (Per.Bruheim@ntnu.no), tlf. 73593321/ 41498495
Senior engineer Kåre Kristiansen

External collaborator

Professor Marit Otterlei, Department of Cancer Research and Molecular Medicine, Medical Faculty, NTNU

MASTER PROJECTS - SPRING 2015

Supervisor: Prof. Bjørn E. Christensen
Co-supervisors: Ph.D. student Marianne Ø. Dalheim, Prof. Bård H. Hoff (IKJ), Bjørn T. Stokke (IFY). Other colleagues at IBT and internationally are likely to be involved.

1. Oligosaccharide bioconjugates

We work to develop a new type of conjugates between biomedically important oligosaccharides (X) and a reactive linker molecule (ADH: adipic acid dihydrazide). ADH is conjugated to the reducing end of the oligosaccharides through reductive amination, forming a series of conjugates at the sugar chain termini: X_n-ADH (n = 3-10). In this work we work to replace a toxic reducing agent ($NaBH_3CN$) with a non-toxic one (picoline borane). Another goal is to test approaches for coupling at the non-reducing end.

The purpose is (in the next step) to link these oligomer-ADH conjugates to polymer carriers or nanoparticles, forming oligosaccharide clusters that improve binding to receptors (multivalent effect).

The oligosaccharides are predominantly:

- Alginates (M-oligomers, G-oligomers, MG-oligomers)
- Chitin oligomers with M-terminus (M = 2,5-anhydro-D-mannose)
- β-1,3-glucans
- Cello-oligosaccharides
- Hyaluronan oligomers
- (+ various model oligomers: dextran, pullulan..)

Methods:

- Partial degradation to obtain oligomers
- Purification by chromatography
- Colorimetric methods for monitoring DP and reaction
- NMR and MS for structural analysis

Polysaccharide origami: carbohydrate based nanotechnology

The industry and researchers aim to develop nanostructured biomaterials for a wide range of applications. The recent development of DNA origami (Google this) shows that biopolymers can form a variety of predetermined nanostructures of considerable sophistication. On longer term there will be a need to extend this area to include carbohydrates based on natural, renewable resources. We wish to establish a library of di- and multifunctional oligosaccharide conjugates, and screen their nanostructuring properties. In the initial phase we aim to prepare simple conjugates based on those mentioned above, but will in this case make divalent conjugates: X_n-ADH-X_n.

Methods for preparation and structural characterization as above
Physical methods: DLS, AFM ++

Topic for MSc, 60 studiepoeng (1 til 2 studenter)

2. Penetrating the mucus barrier

Masters project associated with the Innovative Medicines Initiative COMPACT project

Veileder: Forsker Catherine Taylor Nordgård, NOBIPOL/Institutt for bioteknologi
Faglærer: Prof. Kurt Ingar Draget, NOBIPOL/Institutt for Bioteknologi

The mucus barrier is composed of secreted mucus together with the cell membrane bound mucins and together these elements substantially protect and control access to the cell surface. This protection is an important part of normal physiological function, however, under certain circumstances, such as in drug delivery, it may be desirable to control and modify the properties of the mucus barrier.

Our work on mucus modification using oligomers, particularly guluronate oligomers from alginate, to modify mucus functional properties has been ongoing for a number of years. Initially we investigated the ability of such oligomers to improve sputum rheology in cystic fibrosis lung disease (phase 2 clinical trials are currently underway based on this research), and more recently research has focussed on improved mucosal drug delivery by the use of such oligomers.

As part of the COMPACT consortium we are now involved in testing guluronate oligomers as enhancers of mucopenetration, and as anti-aggregation agents in combination with a variety of drug delivery systems developed by other consortium members. (More information about COMPACT can be found at www.compact-research.org)

The masters project(s) will be linked this research and the precise topic will be determined prior to the start of the project and will be influenced by current research status and the interests of the student(s).

Nordgård, C. T., Bjørkøy, A., Draget, K. I. Guluronate oligosaccharides as enhancers of nanoparticle drug delivery in the oral cavity. *Bioactive Carbohydrates and Dietary Fibre***. vol. 5 (1). (2015**)

Nordgård, C. T., Nonstad, U., Olderøy, M. Ø., Espevik, T., Draget, K. I.. Alterations in mucus barrier function and matrix structure induced by guluronate oligomers. *Biomacromolecules***. vol. 15 (6). (2014)**

Nordgård, C. T. and Draget, K. I. Oligosaccharides as modulators of rheology in complex mucous systems. *Biomacromolecules* **12, 3084–3090. 2011**

Draget, K. I. Oligomers: Just background noise or as functional elements in structured biopolymer systems? *Food Hydrocolloids* **25, 1963-1965. 2011**

Draget, K. I. and Taylor, C. Chemical, physical and biological properties of alginates and their biomedical implications. *Food Hydrocolloids* **25, 251–256. 2011**

Masteroppgave 2015:

Development of methodology to determine frauds in salmon products

60 sp eller 30 sp

Faglærer: Alexander Dikiy, NTNU, Institutt for bioteknologi (alex.dikiy@biotech.ntnu.no, +47-73597863; K3-410.
Veileder: Shumilina Elena, NTNU, Institutt for bioteknologi (elena.shumilina@biotech.ntnu.no, +47-73591687; KIII-312)

The aim of the work **is to develop a method and protocol to determine salmon frauds utilising Nuclear Magnetic Resonance (NMR).**

Fish is an important nutritive source for human consumption. The world fish food supply has grown dramatically in the last years, with an average growth rate of 3.2 percent per year in the period 1961–2009 (FAO, 2012). Atlantic salmon (Salmo salar) is a popular food product in Norway and worldwide due to its delicate taste and health benefits obtained from its metabolites (peptides, carbohydrates, vitamins, lipids).

However, as it happens with different fish, salmon products can be counterfeit to sell a lower quality product for a higher price. Several methods exist how to determine frauds in fish meat, such as genetic verifications, isotope analysis, etc. However, most of them have several limitations.

Within this project a method will be developed that will allow the detection of fraud products utilising NMR spectroscopy. Firstly, a model of salmon metabolites will be made by analysing both fresh and frozen samples of the fish. Subsequently, various frauds will be purposefully created to assess the validity of the developed methodology. The final stage of the project will be to create a protocol that might be used by authorities to detect counterfeits.

Time requirement: The time required to accomplish the project is 2 semesters. However, it can be shortened for a 30 credit points master thesis.

Education requirement: We welcome both realsfag-students in biotechnology, biology, chemistry and relevant siv.ing. students. No previous experience with the mentioned techniques is required.

Publication of the data: The project results will be published in international scientific journal(s). We welcome students that have obtained relevant results to participate in the publication process as co-authors.

Career opportunity: The accomplishment of the proposed studies and the acquired experience should allow to the trained student to find an employment both at research and industrial organizations.

Masteroppgave 2015:

Determination of quality metabolites in different brands of salmon fillets

60 sp eller 30 sp

Faglærer: Alexander Dikiy, NTNU, Institutt for bioteknologi (alex.dikiy@biotech.ntnu.no, +47-73597863; K3-410.
Veileder: Shumilina Elena, NTNU, Institutt for bioteknologi (elena.shumilina@biotech.ntnu.no, +47-73591687; KIII-312)

The aim of the work is to determine the quality and molecular changes due to storage of different brands of salmon fillets utilising Nuclear Magnetic Resonance (NMR).

Fish is an important nutritive source for human consumption. The world fish food supply has grown dramatically in the last years, with an average growth rate of 3.2 percent per year in the period 1961–2009 (FAO, 2012). Atlantic salmon (*Salmo salar*) is a popular food product in Norway and worldwide due to its delicate taste and health benefits obtained from its metabolites (peptides, carbohydrates, vitamins, lipids).

Different companies sell salmon of varying qualities. For the consumer it is important to know whether the price correlates with the quality of the product and how and for how long the fish should be stored, in order to keep all the metabolites that account for its health benefits.

Within this project, the student will utilise a method developed in our laboratory to test A) the quality (amount of vitamins, amino acids, fatty acids, etc.) of salmon purchased from different producers utilising NMR spectroscopy; B) characterise how these products change their metabolic profile over time and C) characterise how their metabolic profile changes depending on storage temperatures and time.

Time requirement: The time required to accomplish the project is 2 semesters. However, it can be shortened for a 30 credit points master thesis.

Education requirement: We welcome both realsfag-students in biotechnology, biology, chemistry and relevant siv.ing. students. No previous experience with the mentioned techniques is required.

Publication of the data: The project results will be published in international scientific journal(s). We welcome students that have obtained relevant results to participate in the publication process as co-authors.

Career opportunity: The accomplishment of the proposed studies and the acquired experience should allow to the trained student to find an employment both at research and industrial organizations.

Masteroppgave 2015:

Determination of quality metabolites in different brands of cod fillets

60 sp eller 30 sp

Faglærer: Alexander Dikiy, NTNU, Institutt for bioteknologi (alex.dikiy@biotech.ntnu.no, +47-73597863; K3-410.
Veileder: Shumilina Elena, NTNU, Institutt for bioteknologi (elena.shumilina@biotech.ntnu.no, +47-73591687; KIII-312)

The aim of the work is to determine the quality and molecular changes due to storage of different brands of cod fillets utilising Nuclear Magnetic Resonance (NMR).

Fish is an important nutritive source for human consumption. The world fish food supply has grown dramatically in the last years, with an average growth rate of 3.2 percent per year in the period 1961–2009 (FAO, 2012). Atlantic cod (*Gadus morhua*) is a popular food product in Norway and worldwide.

Different companies sell cod of varying qualities. For the consumer it is important to know whether the price correlates with the quality of the product and how and for how long the fish should be stored, in order to keep all the metabolites that account for its health benefits.

Within this project, the student will utilise a method developed in our laboratory to test A) the quality (amount of vitamins, amino acids, fatty acids, etc.) of cod purchased from different producers utilising NMR spectroscopy; B) characterise how these products change their metabolic profile over time and C) characterise how their metabolic profile changes depending on storage temperatures and time.

Time requirement: The time required to accomplish the project is 2 semesters. However, it can be shortened for a 30 credit points master thesis.

Education requirement: We welcome both realsfag-students in biotechnology, biology, chemistry and relevant siv.ing. students. No previous experience with the mentioned techniques is required.

Publication of the data: The project results will be published in international scientific journal(s). We welcome students that have obtained relevant results to participate in the publication process as co-authors.

Career opportunity: The accomplishment of the proposed studies and the acquired experience should allow to the trained student to find an employment both at research and industrial organizations.

Masteroppgave 2015:

Novel biomarkers of cattle lameness

60 sp

Faglærer: Alexander Dikiy, NTNU, Institutt for bioteknologi (alex.dikiy@biotech.ntnu.no, +47-73597863; K3-410.
Veileder: Shumilina Elena, NTNU, Institutt for bioteknologi (elena.shumilina@biotech.ntnu.no, +47-73591687; KIII-312)

The aim of this project is to find novel non-invasive biomarkers of cattle lameness using Nuclear Magnetic Resonance (NMR).

Lameness is a clinical manifestation of several disorders of cattle feet and legs and one of the three most expensive health conditions for farm management. It is a chronic disorder that impacts cattle performance and welfare and can be found in up to 60% of cows in a heard. It was estimated that lame cows have severe pain for at least three months and their gait and posture change accordingly. In addition, lameness has been also found to affect fertility of the animals, thus resulting in additional losses on the productivity of farms. Currently, there are no reliable, quantifiable and easily measurable biomarkers for this disease.

The determination of early and reliable lameness biomarkers would greatly help cattle welfare and farms' economy and is one of the European priorities in dairy care. Within this project, cattle with different degrees of lameness will be studied together with healthy controls. Saliva, milk and hair will be collected and analysed utilising NMR spectroscopy metabolic profiling. Subsequently, statistical tools will be utilised to find biomarker candidates. It will be also studied how other diseases and seasonal variation might affect the found biomarkers.

Time requirement: The time required to accomplish the project is 2 semesters.

Education requirement: We welcome both realsfag-students in biotechnology, biology, chemistry and relevant siv.ing. students. No previous experience with the mentioned techniques is required.

Publication of the data: The project results will be published in international scientific journal(s). We welcome students that have obtained relevant results to participate in the publication process as co-authors.

Career opportunity: The accomplishment of the proposed studies and the acquired experience should allow to the trained student to find an employment both at research and industrial organizations.

Masteroppgave 2015:

Detection of novel biomarkers to predict cattle pregnancy utilising milk metabolic profiling

60 sp

Faglærer: Alexander Dikiy, NTNU, Institutt for bioteknologi (alex.dikiy@biotech.ntnu.no, +47-73597863; K3-410.
Veileder: Shumilina Elena, NTNU, Institutt for bioteknologi (elena.shumilina@biotech.ntnu.no, +47-73591687; KIII-312)

The aim of this project is to find novel biomarkers in order to timely detect pregnancy in cattle utilising Nuclear Magnetic Resonance (NMR).

Early and reliable pregnancy diagnosis is a prerequisite for economically successful dairy farming. An ideal test should be reliable, non-invasive and determine pregnancies from the third week on ward. There are several tests on the market, however, none of them fulfils all the above mentioned criteria.

Within this project, novel biomarkers for pregnancy detection will be searched in milk of pregnant cows. NMR spectroscopy will be utilised to perform metabolic profiling of the bio-fluids of cows at different stages of pregnancies and controls. Subsequently, statistical tools will be utilised to find biomarker candidates. It will be also studied how diseases and seasonal variation might affect the found biomarkers.

Time requirement: The time required to accomplish the project is 2 semesters.

Education requirement: We welcome both realsfag-students in biotechnology, biology, chemistry and relevant siv.ing. students. No previous experience with the mentioned techniques is required.

Publication of the data: The project results will be published in international scientific journal(s). We welcome students that have obtained relevant results to participate in the publication process as co-authors.

Career opportunity: The accomplishment of the proposed studies and the acquired experience should allow to the trained student to find an employment both at research and industrial organizations.

Fatty acid and triacylglycerol synthesis in bacteria and thraustochytrids /
Fettsyre- og triglyseridsyntese i bakterier og thraustochytrider

Faglærer: Trond Ellingsen, SINTEF. E-mail: trond.e.ellingsen@sintef.no

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Background

Fish oil is currently the main source of long-chain ω3-fatty acids (EPA og DHA), for both human consumption and fish feed. Due to limiting wild fish stocks, a further growth of the aquaculture industry will require, new, alternative sources for ω3-fatty acids. Microbially produced fatty acids are already commercially available for the human "high-cost" market, while the production costs have to be reduced for application as a feed ingredient.

DHA is produced by "thraustochytrids" (marine, heterotrophic microorganisms, related to algae). These organism are able to accumulate high levels of lipids (more than 50 % of the dry weight), with a high fraction of DHA. Also some bacteria are able to accumulate high lipid levels, but these do not produce EPA or DHA.

SIINTEF and NTNU have on-going projects where thraustochytrids and the bacterium *Rhodococcus opacus* are studied for production of ω3-fatty acids for use in fish feed. This work involves introduction of the DHA-synthesis pathway in *R. opacus*.

Contents

The aim of the work will be to increase the understanding of which factors that affect the lipid accumulation in *Rhodococcus* and thraustochytrids, and how an increased productivity of DHA can be obtained.

The work can involve:

Studies of the lipid and DHA-production at different growth conditions

Incorporation of external added fatty acids in the triacylglycerol molecules

Identification of bottle-necks in the metabolic pathways by:

- Use of enzyme inhibitors, comparison of mutants etc.
- Analyses of gene expression at different growth conditions and/or in mutants with different DHA-productivities

The work load can be adapted to project + diploma (2015-16, 1 student) and a 2-year Master (1 student).

The selection of strain (thraustochytrids or *Rhodococcus*) will be decided based on the progress of the on-going projects.

Develop Rhodococcus opacus as a platform organism for lipid production
(15 stp, 30 stp, or 60 stp)

Supervisor: Helga Ertesvåg (tlf 73598678 helga.ertesvag@biotech.ntnu.no)

Rhodococcus opacus is an actinomycete that is able to store large amounts of lipids in lipid bodies within its cytoplasm. At NTNU it has been chosen as one contender in a project aimed at producing the essential omega-3 fatty acids DHA and EPA for aquaculture feed. Aquaculture is the most likely way of providing animal protein for the increasing human population, and new sources for omega-3 are necessary in order to increase the production from aquaculture.

In connection with the ongoing MIRA project it is possible to define molecular biology projects tailored to the wishes of the student. Topics could be: optimized vectors and methods. Cloning and characterization of specific proteins like acetyl transferases, elucidation of gene function, making specific gene knock-outs etc. Literature studies or bioinformatic studies could also be included as part of the project.

Since this is a very open project, it will be available for up to three students. Each will be given their separate project. These projects will be specified in a dialog between the student and the supervisor, and will also depend on how far the MIRA project has developed when the individual students are starting up their thesis work.

Polysaccharide production in Azotobacter vinelandii cysts
(15 stp)

Supervisor: Helga Ertesvåg (tlf 73598678 helga.ertesvag@biotech.ntnu.no)

Azotobacter vinelandii is a gram-negative bacterium that will enter a resting stage called cyst upon encountering adverse growth conditions. I have performed a transcriptome study and identified two gene clusters probably involved in producing polysaccharides that are expressed during encystment. No one knows which polysaccharides the clusters are involved in producing.

As a first attempt at unraveling what these genes are doing, I would like to inactivate one gene in each of these gene clusters and analyse the effect on encystment. Avin05390 and Avin30120 are the best candidates for genes to target. For a project work making the constructs in *Escherichia coli* and transferring the plasmids to *A. vinelandii* will be a realistic aim, it will then be possible to continue the work in a 30 stp project.

Goal: Construct the vectors for gene knock out of Avin05390 and Avin30120 and transfer them to *A. vinelandii.*

Thesis work

1. Construct the recombination vector
2. Transfer it to *A. vinelandii.*
3. If time, select mutants.
4. Use bioinformatic tools to present a hypothesis as to which polysaccharides the clusters are producing.

Regulation of alginate biosynthesis in *Azotobacter vinelandii*

Supervisor: Helga Ertesvåg (tlf 73598678 helga.ertesvag@biotech.ntnu.no)

Department of Biotechnology has for decades been interested in bacterial alginate production. Alginate is an industrially interesting, linear exopolymer. Its regulation has mostly been studied in *Pseudomonas aeruginosa* (see [1] and references therein). *P. aeruginosa* is a pathogen and produces alginate under specific conditions. The nitrogen fixating soil bacterium *Azotobacter vinelandii* produce alginate constitutively, thus there clearly is a difference in regulation between those two species. One difference is KinB, this protein is a negative regulator of alginate synthesis in *P. aeruginosa*, while there is no corresponding gene in *A. vinelandii [2*]. In *P. aeruginosa* KinB is downregulating the protease gene *algW*. We have an *algW* mutant of *A. vinelandii*, this strain do not produce alginate. *algD* is the first gene in the alginate biosynthetic gene cluster and will be used to measure alginate gene expression. *algC* is also needed for alginate production, and may be regulated differently.

We also have *A. vinelandii* knock out mutations in *amrZ* and *algB*, both of which are necessary for alginate production in *P. aeruginosa*. However, AlgB does not seem to be necessary in *A. vinelandii*. A vector expressing *algB* has been constructed.

Goal: To compare the role of putative alginate regulators in *A. vinelandii* to the role in *P. aeruginosa.*

Thesis work

1. Review literature to find what is known about alginate regulatory genes and their function in the two genera.
2. AlgW: Construct a vector expressing *AlgW* in *A. vinelandii* and show that this complement the *algW*-mutant. Methods: PCR, cloning, sequencing, transformation and conjugation.
3. Construct plasmids for measuring *algD* expression and *algC* expression using a reporter gene.
4. Compare alginate production and *algD* gene expression in strains lacking *algW* or *algB* with the wild type strain and with strains overproducing these proteins. Alginate is assayed enzymatically, while gene expression can be assayed using a reporter gene.
5. The literature review should have resulted in identification of other regulatory genes that could be inactivated and tested.

References:

1. Damron, F. and G. JB., *Proteolytic regulation of alginate overproduction in Pseudomonas aeruginosa.* Mol Microbiol., 2012. **84**(4): p. 595-607.
2. Setubal, J.C., et al., *The genome sequence of Azotobacter vinelandii, an obligate aerobe specialized to support diverse anaerobic metabolic processes.* J. Bacteriol., 2009. **191**(14): p. 4534-45.

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Medveileder: Stipendiat Iselin Rynning: iselin.rynning@stami.no, tlf. 23 19 51 37
Faglærer: Professor Aage Haugen: age.haugen@stami.no, tlf. 23 19 52 70.

Carcinogenicity of diesel exhaust particles: EMT and epigenetics

Keywords. Diesel exhaust particles (DEP), DNA methylation, microRNA, epithelial-to-mesenchymal transition (EMT), live cell imaging

Background. Air pollution contributes significantly to the development of chronic pathologies. Diesel exhaust particles (DEP) comprise an important part of particulate air pollution and are associated with adverse health effects, including COPD, asthma, allergy and cardiovascular disease. Recently the International Agency for Research on Cancer (IARC) has classified diesel exhaust as a human carcinogen, but the underlying mechanisms are unclear.

Epigenetics is important for the regulation of gene expression, working in concert with transcription factors to turn genes on or off. Epigenetic changes are strongly associated with environmental exposures and are involved in many diseases, including cancer. Little, however, is known about epigenetic mechanisms in carcinogenicity of DEP.

Epithelial-to-mesenchymal transition (EMT) is a process by which epithelial cells lose their cell polarity and cell-cell adhesion, and gain migratory and invasive properties to become mesenchymal like cells. EMT is essential for numerous developmental processes and has been shown to occur in cancer development and progression.

Project description. The thesis will be part of an ongoing project where we study early carcinogenic effects of DEP at the cellular level, focusing on phenotypical changes and related epigenetic mechanisms. The model system will be human bronchial epithelial cells (HBEC). We have previously established HBEC cell lines with cancer like properties after long tern exposure to DEP (26 weeks). In this project we will study how exposure of cell cultures to DEP induces changes in migration/invasion capacity and how that relates to altered gene expression, DNA methylation, miRNA expression, and early carcinogenesis markers (EMT).

Methods. The student will be trained in cell culture and molecular biology techniques. Mechanistic studies will be performed on human lung cells in culture. Epigenetic changes will be analyzed by pyrosequencing of bisulfite treated DNA (methylation) and RT-qPCR (miRNA expression). Results will be compared to DEP induced cell biology markers (EMT) and changes in gene expression (RT-qPCR).

Location. The master project will be performed at the National Institute of Occupational Health (Statens arbeidsmiljøinstitutt, www.stami.no) in Oslo. All necessary laboratory facilities for completion of the master thesis are available. It will be preferable if the student has finished all master courses before starting on the thesis.

Title

Nanowire-mediated electron transport in Bacteria

Advisors

Assoc Prof Martin Hohmann-Marriott
Assoc Prof Hanne Winther-Larsen, School of Pharmacy, University of Oslo
Dr. Rahmi Lale

Goal

Identify pathways that allow bacteria to donate electrons to external electron acceptors, such as iron oxide, manganese oxide or electrodes.

Introduction

There is strong evidence that bacterial pili have a function in donating electrons to iron oxides. However, this electron disposal has so far only been interpreted as to enable respiration of soil bacteria in anaerobic conditions. In contrast, we have collected data that indicates that pili are crucial for iron acquisition in bacteria. We hypothesise that pili are meditating electron donation to iron oxides, thereby converting insoluble ferric iron (Fe3+) into soluble ferrous iron (Fe2+), which can readily been taken up by bacteria. In addition to addressing the role of pili in iron acquisition, our proposal may also provide crucial understanding required to limit iron uptake by infectious bacteria and construction truly renewable photovoltaic devices.

Techniques

The student will use bioinformatics and access databases. To genetically manipulate bacteria the student will perform molecular biological approaches (e.g. PCR-amplification, plasmid construction and transformation.) Sterile culturing techniques will be used to grow bacterial cells and to select transformants. Analytical techniques (imaging and statistical techniques for the determination of growth parameters, HPLC & mass spectrometry for pigment analysis as well as potentimetric techniques) may be used to characterize the performance of the generated strains.

Literature

Reguera G., McCarthy K.D., Mehta T., Nicoll J.S., Tuominen M.T., Lovley D.R. (2005) Extracellular electron transfer via microbial nanowires. Nature, 435: 1098-1101.

Gorby Y.A., Yanina S., McLean J.S., Rosso K.M., Moyles D., Dohnalkova A., Beveridge T.J., et al. (2006) Electrically conductive bacterial nanowires produced by Shewanella oneidensis strain MR-1 and other microorganisms. Proc Natl Acad Sci USA 103: 11358-11363.

Lamb JJ, Hill RE, Eaton-Rye JJ, Hohmann-Marriott MF (2014) Functional Role of PilA in iron acquisition in the cyanobacterium *Synechocystis* sp. PCC 6803. PLoS ONE 9(8):e105761

Title
Modeling of *Nannochloropsis* metabolism in three compartments

Advisors

Prof Eivind Almaas
Assoc Prof Martin Hohmann-Marriott

Introduction

Nannochloropsis is an alga that possesses ideal features for a model organism: Multiple *Nannochloropsis* genomes are being sequenced. Targeted genetic manipulation of the nuclear genome is possible. *Nannochloropsis* grows quickly, using light or external carbon sources [1,2]. *Nannochloropsis* is also a potential supplier of biofuel, as it naturally accumulates oil. We will supervise a project that aims to model of cellular metabolism. If you have additional ideas, please contact us.

Goal

Nannochloropsis possesses three organelles that carry genetic information: nucleus, chloroplast and mitochondrion. How these three compartments interact on a metabolic level will be modeled [3,4] using the sequenced *Nannochloropsis* genomes.

Techniques

The genome-scale metabolic model will be generated by using the online resource: Model Seed (http://www.theseed.org/models/) and hand curation. We will use the modeling framework of Flux Balance Analysis and its implementation in MatLab (Cobra Toolbox) to evaluate metabolic phenotypes.

Literature

[1] Kilian O, Benemann CS, Niyogi KK, Vick B. (2011) High-efficiency homologous recombination in the oil-producing alga *Nannochloropsis sp.*, Proc Natl Acad Sci U S A. 108: 21265-9.

[2] Radakovits R, Jinkerson RE, Fuerstenberg SI, Tae H, Settlage RE, Boore JL, Posewitz MC (2012) Draft genome sequence and genetic transformation of the oleaginous alga *Nannochloropis gaditana*. Nat Commun 3-686: 1-10.

[3] Boyle NR, Morgan JA (2009) Flux balance analysis of primary metabolism in *Chlamydomonas reinhardtii*. BMC Syst Biol, Jan 7;3:4.

[4] Gomes de Oliveira Dal'molin C, Quek LE, Palfreyman RW, Nielsen LK (2011) AlgaGEM - a genome-scale metabolic reconstruction of algae based on the *Chlamydomonas reinhardtii* genome. BMC Genomics. 22;12:4:S5

Title

Structure of cyanobacterial methionine sulfoxide reductase

Advisors

Assoc Prof Martin Hohmann-Marriott
Prof Alexander Dykyy

Introduction

Methionine sulfoxide reductase are crucial for repairing proteins damaged by oxidative stress. Photosynthetic organisms produce reactive oxygen during photosynthetic electron transport. The genome of the photosynthetic cyanobacterium *Syenchocystis* contains three methionine sulfide reductases, suggesting the importance of Methionine sulfoxide reductases for this organism. However, the function of methionine sulfoxide reductases in cyanobacteria is still poorly defined and structural information on sulfoxide reductases in these organisms is still missing.We have developed two strategies that will lead students to establish the physiological role and structure of methionine sulfoxide reductases in a cyanobacterium.

Projects

Structure of cyanobacterial methionine sulfoxide reductase. In this project the student will use molecular techniques to clone a methionine sulfoxide reductase. The protein will then be over-expressed in *E. coli* and purified using biochemical methods. The atomic structure of the methionine sulfoxide reductase will be determined using NMR-spectroscopy and other complementary techniques.

Equipment*:* The Department of Biotechnology is well equipped with all instrumentation required for the project accomplishment. In particular, a new 600 MHz NMR spectrometer equipped with cryoprobe is available at our NMR center.

Previous experience*:* No previous experience with NMR spectroscopy or molecular biology techniques is required. This is an opportunity to learn state-of-the art NMR spectroscopy and complementary techniques.

Publication of the data*:* The proposed study represents absolutely novel research and, therefore, the project results will be published in international scientific journal(s).

Career opportunity: The accomplishment of the proposed studies and the acquired experience should allow to the trained students to find an employment both at research and industrial (pharmaceutical and biotechnological) organizations.

Literature

Ezraty B, Aussel L, Barras F (2005) Methionine sulfoxide reductases in prokaryotes. Biochim Biophys Acta 1703: 221-229.

Aachmann FL, Sal LS, Kim H-Y, Marino SM, Gladyshev VN, Dikiy A (2010) Insights into function, catalytic mechanism, and fold evolution of selenoprotein methionine sulfoxide reductase B1 through structural analysis J Biol Chem 285: 33315-33323.

Tarrago L, Laugier E, Rey P (2009) Protein-repairing methionine sulfoxide reductases in photosynthetic organisms: Gene organization, reduction mechanisms, and physiological roles. Molecular Plant 2: 202-217.

Title
Biobricks - Developing a genetic toolbox

Advisors

Assoc Prof Martin Hohmann-Marriott
Dr Rahmi Lale

Introduction

Genetic manipulation is an important tool to engineer organisms for biotechnological applications. This manipulation is simplified by adhering to design principles that have been formulated into the BioBrick standard [1].

Goal of this thesis project

The goal of this project is to implement functional BioBricks in model micro organisms (*E. coli*, *Synechococcus*, *Nannochloropsis*, *Chlamydomonas*, *Sacchromyces).*

Techniques

The student will genetically manipulate bacteria to construct BioBricks. These BioBricks could be for example: Antibiotic resistance genes, reporter systems (GFP [2], luciferase, fluorescing RNA [3]), protein-engineering systems [4], etc. This work will involve molecular biology (e.g. PCR-amplification, plasmid construction and transformation). Sterile culturing techniques will be used to grow the selected model organism (*E. coli*, *Synechococcus*, *Nannochloropsis*, *Chlamydomonas*, *Sacchromyces)* in order to select transformants. Suitable analytical techniques will be used to verify the function of constructed BioBricks.

Literature

[1] Ho-Shing O, Lau KH, Vernon W, Eckdahl TT, Campbell AM (2012) Assembly of standardized DNA parts using BioBrick ends in *E. coli*. Methods Mol Biol. 852: 61-76.

[2] R. Y. Tsien (2008) Constructing and exploiting the fluorescent protein paint box. Nobel Lecture

[3] Paige J.S., Wu K.Y., Jaffrey S.R. (2011) RNA mimics of green fluorescent protein Science, 333: 642-646.

[4] Volkmann G, Mootz HD (2013) Recent progress in intein research: from mechanism to directed evolution and applications. Cell Mol Life Sci 70:1185-206.

Title
Nano-scale imaging technique application and development

Advisors

Assoc Prof Martin Hohmann-Marriott
Dr Rahmi Lale

Introduction

Nano-scale imaging is revolutionized by interfacing established electron microscopy techniques with molecular biology and computational approaches. Synergies arising from these techniques including three-dimensional imaging of entire organisms and the visualization of tagged protein complexes.

Goal of this thesis projects

The goal of this thesis is to visualize photosynthetic organisms and the location of protein complexes.

Techniques

The student will genetically manipulate a photosynthetic organism in order to label protein complexes. Techniques that will be utilized include PCR-amplification, plasmid construction and transformation. Sterile culturing techniques will be used to grow photosynthetic model organisms and to select transformants.

Microscopy approaches will be used to visualize cell structure and protein locations within a cell. Computational approaches will be used to assemble images into three-dimensional models of imaged cells.

Literature

Hohmann-Marriott MF, Sousa AA, Azari AA, Glushakova S, Zhang G, Zimmerberg J, Leapman RD. (2009) Nanoscale 3D cellular imaging by axial scanning transmission electron tomography. Nat Methods. 6: 729-31..

Diestra E, Fontana J, Guichard P, Marco S, Risco C. (2009). Visualization of proteins in intact cells with a clonable tag for electron microscopy. J Struct Biol. 165: 157-68.

Hohmann-Marriott MF, Roberson RW. (2009) Exploring photosynthesis by electron tomography.
Photosynth Res. 102: 177-88.

Risco C, Sanmartín-Conesa E, Tzeng WP, Frey TK, Seybold V, de Groot RJ. (2012) Specific, sensitive, high-resolution detection of protein molecules in eukaryotic cells using metal-tagging transmission electron microscopy. Structure. 20: 759-66.

Title

Construction of 'open source' laboratory equipment and programs

Advisors

Assoc Prof Martin Hohmann-Marriott
Dr Rahmi Lale

Introduction

Technologies that can be used to analyze biological samples in a high-throughput fashion are often not available or only available as costly custom-built solutions. In addition a large number of current laboratory equipment does not benefit from interfacing with modern information technologies to allow on-line monitoring of procedures and acquired data. Recently 'open source' implementations of laboratory equipment have been developed that demonstrate the potential of student-designed instrumentation to accelerate synthetic biology approaches.

Goal of thesis projects

The goal of the Master's Thesis projects is to design and implement 'open source' laboratory equipment that automates and monitors synthetic biology approaches. Concrete examples are instrumentation to assess protein stability or microbacterial growth, as well as robotic liquid handling systems.

Techniques

Depending on the student's interests and abilities a variety of project can be defined. Arduino and Raspberry Pi micro-controllers, as well as cell phones and tablets may be used as control and communication devices. The physical instrument can be constructed using standard parts (e. g. lego) or be printed using 3D printers. Depending on the developed device, evaluation of the device performance may also involve synthetic biology approaches.

Literature

Heidi Ledford (2010) Life Hackers. Nature 467: 650-652

Smith ZJ, Chu K, Wachsmann-Hogiu S (2012) Nanometer-scale sizing accuracy of particle suspensions on an unmodified cell phone using elastic light scattering. PLoS One. 7: e46030.

Lamb JJ, Eaton-Rye JJ, Hohmann-Marriott MF. (2012) An LED-based fluorometer for chlorophyll quantification in the laboratory and in the field. Photosynth Res. 114 :59-68.

Lamb JJ, Eaton-Rye JJ, Hohmann-Marriott MF. A Cost-effective solution for the reliable determination of cell numbers of microorganisms in liquid culture. Curr Microbiol. In press.

Title
Hydrogen production in cyanobacteria

Advisors

Assoc Prof Martin Hohmann-Marriott

Introduction

Hydrogen production by photosynthetic organisms is a promising pathway for the converting solar energy into chemical energy. Some green algae possess hydrogenases that are energetically poised to produce hydrogen. However, the electrons that are used to make hydrogen are extracted from water. Oxygen that is produced during water-splitting and destroys the hydrogenase, making hydrogen production unsustainable in alga. Cyanobacteria possess a photosynthetic machinery that can interface with algal hydrogenases. Our laboratory develops methodologies in cyanobacteria that will allow the separation of oxygen and hydrogen production.

Goal of this thesis project

The goal of this thesis is to engineer a cyanobacterium so it produces efficiently algal hydrogenases and hydrogen efficiently.

Techniques

The student will genetically manipulate photosynthetic and non-photosynthetic bacteria. This will involve molecular biological approaches (e.g. PCR-amplification, plasmid construction and transformation). Sterile culturing techniques will be used to grow bacterial and to select transformants.

Literature

Posewitz MC, King PW, Smolinski SL, Zhang L, Seibert M, Ghirardi ML (2004) Discovery of two novel radical S-adenosylmethionine proteins required for the assembly of an active [Fe] hydrogenase. J Biol Chem. 279: 25711-20.

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Czech I, Stripp S, Sanganas O, Leidel N, Happe T, Haumann M (2011) The [FeFe]-hydrogenase maturation protein HydF contains a H-cluster like [4Fe4S]-2Fe site. FEBS Lett. 585: 225-30.

Posewitz MC, King PW, Smolinski SL, Smith RD, Ginley AR, Ghirardi ML, Seibert M. (2005))Identification of genes required for hydrogenase activity in *Chlamydomonas reinhardtii*. Biochem Soc Trans. 33: 102-4.

Title
Creating 'open source' laboratory equipment and programs

Advisors

Assoc Prof Martin Hohmann-Marriott
Dr Rahmi Lale

Introduction

High-throughput techniques are revolutionizing the biological sciences. However technologies that can be used to analyze biological samples in detail are often not available or only available as costly custom-built solutions. In addition a large number of laboratory equipment does not benefit from interfacing with modern information technologies, which allows on-line monitoring of procedures and acquired data. Recently 'open source' implementations of laboratory equipment have become available. These open source instruments range from gel boxes to PCR machines. Ideas for specific instrumentation or programming projects by students are encouraged.

Goal of thesis projects

The goal is to built 'open source' versions of established and novel laboratory equipment and computer programs that automate procedures and data acquisition. Interfacing of these instruments with hand held devices may be an additional focus.

Techniques

Depending on the students interests and abilities a variety of project can be defined. Arduino and Raspberry Pi micro-controllers, as well as cell phones and tablets may be used as control and communication devices. The physical instrument can be constructed with standard parts (i.e. lego) or printed by 3D printers. Depending on the developed device, evaluation of device performance may also involve molecular biology and sterile culturing techniques.

Literature

Heidi Ledford (2010) Life Hackers. Nature 467: 650-652

Smith ZJ, Chu K, Wachsmann-Hogiu S (2012) Nanometer-scale sizing accuracy of particle suspensions on an unmodified cell phone using elastic light scattering. PLoS One. 7: e46030.

Lamb JJ, Eaton-Rye JJ, Hohmann-Marriott MF. (2012) An LED-based fluorometer for chlorophyll quantification in the laboratory and in the field. Photosynth Res. 114 :59-68.

Lamb JJ, Eaton-Rye JJ, Hohmann-Marriott MF. A Cost-effective solution for the reliable determination of cell numbers of microorganisms in liquid culture. Current Microbiology 67: 123-12.

Title
Bacterial arrays for horizontal gene transfer studies

Advisors

Dr Rahmi Lale
Assoc Prof Martin Hohmann-Marriott
Assoc Prof Marit Sletmoen

Introduction

Horizontal gene transfer (1), aka conjugation (HGT) is a process where transmission of DNA occurs between a donor and a recipient organism. HGT plays a significant role in bacterial evolution including the spread of antibiotic resistance. The details of conjugation are yet to be understood (2), and a technology allowing continuous tracking of high numbers of single bacterial cells would be advantageous. We propose to use bacterial microarrays to address the influence of the following variables on the conjugation process:

. the distance between donor and recipient cells,
. the size and location of the genes that are to be transferred (on a chromosome or on a plasmid).

Bacterial microarrays are special arrays that are prepared by using stamps made in NTNU Nanolab facilities. Arrays are formed by simple stamping on treated glass surfaces, which leaves defined small adhesive islands, where bacteria can adhere to. These stamps can be designed in such a way that the spacing between the stamp pillars can be optimized for studies of bacterial conjugation.

Goal of thesis projects

The goal is to study the intrinsic details of conjugation, HGT, by the use of bacterial microarrays.

Techniques

Bacterial microarrays with different pillar spacing will be designed and events of conjugation will be followed under fluorescent microscopy. The work will involve molecular biology techniques such as, PCR, cloning, fluorescent protein measurements, transcript analysis, fluorescent microscopy and sterile culturing techniques.

Literature

1. Skippington, E. & Ragan, M. A. Lateral genetic transfer and the construction of genetic exchange communities. *FEMS Microbiol. Rev.* **35,** 707–735 (2011).

2. Babić, A., Lindner, A. B., Vulić, M., Stewart, E. J. & Radman, M. Direct Visualization of Horizontal Gene Transfer. *Science* **319,** 1533–1536 (2008).

Title
Sulfur metabolism in cyanobacteria

Advisors

Martin Hohmann-Marriott – Department of Biotechnology, NTNU
Niels-Ulrik Frigaard – Department of Biology, University of Copenhagen

Goal

Identify genes involved in sulfur metabolism in cyanobacteria

Introduction

Although sulfur is an essential element for life, many sulfur components are toxic, but play important roles as electron donors and acceptors in microbial ecosystems. In this Master project you will investigate sulfur metabolism in cyanobacteria.

We have observed that in the absence of oxygen, cyanobacteria can utilize thiosulfate to produce hydrogen sulfide and sulfur. This process is anticipated to have significance when cyanobacteria experience anaerobic conditions in their environment such as in sediments and biofilms. The genes and enzymes that are involved in this reaction are unknown in cyanobacteria.

Project

The project will investigate the sulfur metabolism in cyanobacteria. The student will use bioinformatic approaches to identify genes involved in sulfur metabolism. Identified genes will by inactivated by genetic manipulation of the cyanobacterium. Analytical methods will be used to characterize the growth of the constructed mutants under different environmental conditions. his will result in a better understanding of how cyanobacteria survive and behave under anaerobic condition..

Methods

The student will use bioinformatics and access databases. To genetically manipulate bacteria the student will perform molecular biological approaches (e.g. PCR-amplification, plasmid construction and transformation). Sterile culturing techniques will be used to grow bacterial cells and to select transformants. Analytical techniques (imaging and statistical techniques for the determination of growth parameters, HPLC & mass spectrometry for pigment analysis) will be used to characterize mutants and wild type.

References

Jorgensen B B (1990) Sulfur cycle of freshwater sediments: role of thiosulfate. Limnol Oceanogr 35: 1329–1342.

Jorgensen B B, Bak F (1991) Pathways and microbiology of thiosulfate transformations and sulfate reduction in a marine sediment (Kattegat, Denmark) Appl Environ Microbiol 57: 847–856.

Jorgensen B B (1994) Sulfate reduction and thiosulfate transformations in a cyanobacterial mat during a diel oxygen cycle. FEMS Microbiol Ecol 13: 303–312.

Sheridan RP, Castenholz RW (1968) Production of hydrogen sulphide by a thermophilic blue-green alga. Nature 217: 1063-1064.

Sheridan R.P. (1973) Hydrogen sulfide production by Synechococcus lividus Y52-s. J Phycology 9: 437–445

Masteroppgave

Industriell bruk av nye bakterier for produksjon av terapeutiske proteiner.

Industrial use of new bacteria in the production of therapeutic proteins.

Faglærer: Trygve Brautaset
Veiledere: Anne Krog (anne.krog@vectronbiosolutions.com), Tlf: 98404313,
Trond Erik Vee Aune (trond.aune@vectronbiosolutions.com), Tlf: 95027449

Belastning: 15stp

Mål:
I denne oppgaven er målet å videreutvikle Vectron Biosolutions sin teknologi til produksjon av kommersielt interessante proteiner i alternative bakteriearter.

Delmål:

- Klone gener som koder for terapeutiske proteiner inn i Vectron's vektorer.
- Teste ekspresjonen av valgte terapeutiske proteiner i alternative bakteriestammer.
- Sammenligne og evaluere produksjonen av løselig versus uløselig fraksjon av proteinene fra de ulike vetsbakterier.

Bakgrunn:

Det finnes et stort marked for rekombinante proteiner. Disse blir i dag nesten utelukkende produsert i *E. coli*. Et stort problem i produksjonen av rekombinante proteiner er at store deler av proteinfraksjonen ofte foreligger som uløselig. Et større utvalg av produksjonsorganismer vil øke muligheten for å finne en vert som egner seg til produksjon for et langt høyere antall rekombinante proteiner enn det vi ser i dag.

Vertron Biosolutions teknologi innehar i dag en teknologi som har vist seg å være konkurransedyktig for produksjon av rekombinante proteiner i *E. coli*. Det er ønskelig å bruke vår ekspresjonsteknologi til produksjon av rekombinante proteiner i en rekke andre bakteriearter, for bedre å kunne skreddersy en produksjonsprosess som passer det enkelte protein som skal uttrykkes. I denne studien skal det uttrykkes rekombinante poteiner i en annen bakterieart enn *E. coli* og ekspresjonen skal deretter sammenlignes med eksisterende teknologi.

Referanser:

Brautaset, T., R. Lale, and S. Valla, *Positively regulated bacterial expression systems.* Microb Biotechnol, 2009. **2**(1): p. 15-30.

Improved microbial safety and shelf life of fish

Faglærer: Turid Rustad

60 credits

Background

Seafood deterioration is mostly governed by microbial and biochemical activities which are influenced by temperature and storage conditions. The main cause of bacterial contamination of fish processing line is due to rapid bacterial proliferation on the skin during early storage which spreads during filleting and by post-contamination during processing. Reducing the microbiota before process and preventing its development during storage will extend shelf life.
The main objective of the SAFEFISHDISH project is to improve the microbial and sensory quality and safety of fish from harvest to consumer. The project will focus on farmed salmon and wild cod, which are the major species traded in Europe. Novel handling techniques and combination of innovative preservation technologies involving biopreservatives (protective cultures and chitosan), superchilling and modified atmosphere will be evaluated. Treatment well ahead of the food chain (on the skin upon harvest and on flesh just after filleting) may maximize its efficiency and will be explored. Combination of these preservation techniques is innovative and needs to be tested. Bacterial ecosystem and their metabolism profile will be explored via modern tools such as new generation sequencing (NGS) and various chromatographic methods.

The work in the master thesis will be focused on studying the effect of protective cultures on the shelf life and quality changes during storage of salmon. Methods used will be determination of microbial growth (TPC), quality (water holding, colour, protein properties, texture, flavour substances..)

Reducing salt content in fish products

Faglærer: Turid Rustad
60 credits

Background

Salt (sosium chloride, NaCl) is one of the most used food additives, both because it is a preservative and because it has a positive influence on the technological properties and the sensory quality of food. Because of this salt is used in higher concentration in most industrially produced foods. The need for salt to maintain body functions is 1-3 g per day. In most European countries, the salt intake is 3 times as high as this (8-12 g/day). A high intake of sodium is related to increased risk of high blood pressure which again is an important factor in development of cardiovascular disease and stroke. Due to this there is a large interest in developing products with reduced salt content without changing the properties of the products (water holding, yield and shelf life). For processed fish products, the solubility properties of the muscle proteins have been shown to be related to the other functional properties. Increased knowledge about how different ions influence solubilisation of proteins. In addition the effect of different ions will be studied also in heat treated fish products.

"Protein from seaweed" – Extraction processes and functional properties /

Protein fra brunalger – Ekstraksjonsprosesser og funksjonelle egenskaper

Faglærer: Turid Rustad

Veiledere: Inga Marie Aasen SINTEF Materialer og Kjemi,
Ana Karina Carvajal SINTEF Fiskeri og havbruk

Background

Cultivated seaweed is a biomass resource that can be utilized for food, feed, chemicals and energy. The seaweed protein is interesting as an ingredient in animal and fish feed, but is yet poorly characterized. Processes for extraction of the protein, or at least to remove components that may have a negative effect in feed, are required.

Content and tasks

Evaluation of processes for extraction of the protein

Use of enzymes as processing tools (alginate lyases, cellulases, proteases etc.) for high protein yields

Characterization of the protein, including:

- Binding to other components (polyphenols, membrane lipids etc.)
- Molecular weight distribution
- Solubility of different fractions
- etc.

The extent of the work can be adapted to project – diploma or to a 2-year Master.

Prosjektoppgave høst 2015

Bestemmelse av kvalitetsendringer i fisk ved bruk av ny metodikk

Faglærer: Turid Rustad

Veileder: Ulf Erikson (SINTEF fiskeri og havbruk)

Bakgrunn

Det er et økende salg av frossen tint fisk. Denne selges også som fersk. Det er derfor behov for målemetoder som kan skille mellom fersk og frossen tint fisk. Noen målemetoder finnes – men disse er tidkrevende og lite presise. For å kunne forbedre og utvikle nye og bedre prosesseringsmetoder for bla fisk er det behov for gode målemetoder for å bestemme endringer i råstoffet som funksjon av prosessbetingelser. Dette vil for eksempel være å bestemme når proteindenatureringen begynner – bla ved varmebehandling – hvis vi ønsker mildere varmebehandling – hva skjer med proteinene (og tekstur med mer). Det er også mulig å studere effekt av ulike tinemetoder osv.

Oppgaven vil gå ut på å bruke måle endringer i proteiner ved hjelp av endringer i overflatespenning ved hjelp av et nytt utviklet instrument og koble dette til målinger med konvensjonelle metoder.

Det vil bli gjort målinger på fersk fisk som er behandlet på ulik måte, fersk, lagret og frossen tint. Prøvene vil bli analysert ved hjelp av overflatespenningsmetoden og ved metoder slik som endringer i proteinløselighet, vannbindingsevne, tekstur og evt endringer i enzymaktivitet.

Prosjektoppgave høsten 2015 15 stp.

Enzymatiske prosesser i spekeskinke

Faglærer: Turid Rustad

Veileder: Inga Marie Aasen, SINTEF Materialer og Kjemi

Bakgrunn:

Spekeskinke er et tradisjonsrikt produkt i Norge, men det foregår stadig produktutvikling, og det har også blitt utviklet «nye» norske høykvalitets, langtidsmodnede spekeskinkeprodukter. Det er et ønske om å øke kunnskapen knyttet til tradisjonsmat. Dette vil gjøre det mulig å øke verdiskapningen på mat basert på norske råvarer, inkludert fenalår og spekeskinke. I løpet av salte- og modneprosessen skjer det mange endringer i råstoffet. Disse er særlig knyttet til enzymatiske prosesser, og det er et stort behov for å øke kunnskapen om hvilke parametre (pH, temperatur, vannaktivitet, saltinnhold, alder på dyret..) som er viktige for de biokjemiske endringene som skjer.

Oppgave

Tradisjonelt måles enzymaktiviteter i kjøtt og fisk i "ekstrakter" framstilt ved å homogenisere kjøttet i vann, sentrifugere, og måle aktiviteten i vannløsningen. Dette gir imidlertid andre forhold enn i kjøttet, særlig mhp. vanninnhold.

Oppgaven vil gå ut på uttesting av en ny metode for å bestemme enzymaktiviteter i spekeskinke "*in situ*", bl.a. for å kunne studere modningsprosessene under tørking av skinke.

Dette vil bli gjort ved injeksjon av substrat som er spesifikke for forskjellige kjente enzymer, i kjøttbiter, som så inkuberes ved forskjellige betingelser. Enzymaktivitetene måles ved å ekstrahere spaltningsproduktene og måle disse fluorometrisk eller spektrometrisk.

Metoden vil bli benyttet til å følge aktivitet av 3-4 utvalgte enzymer som funksjon av temperatur, salt- og vanninnhold under lagring ved konstante betingelser. Det er gjort noen innledende forsøk slik at vi vet at metoden fungerer, men metoden trenger videre utvikling bla for å hindre mikrobiell vekst i prøvene under lagringen.

Disse forsøkene kan følges opp videre med tørkeforsøk, dvs. å følge aktiviteten under tørking ved forskjellige betingelser.

Oppgaven kan videreføres i form av masteroppgave våren 2016.

Bacterial microarrays for bacterial gene expression studies

Relevant literature: http://www.sciencedirect.com/science/article/pii/S0006349508701681
http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0089532

Background: Bacteria respond to environmental changes by turning functional genetic modules on or off. Examples for such modules include cell-to-cell communication systems and stress response systems. Owing to recent technological advances, the gene expression dynamics of functional modules in single cells of bacterial populations can be monitored in real time while controlling their environments. Results obtained using this approach yield unprecedented insight into the strategies for cellular decision making.

We have developed a procedure for preparing highly ordered arrays of immobilized bacteria. Time series imaging of genetically altered bacteria revealed that the immobilized bacteria both divided and expressed the fluorescent protein GFP (Fig 1). Through the topics described below you are invited to contribute to the further optimization and use of these arrays.

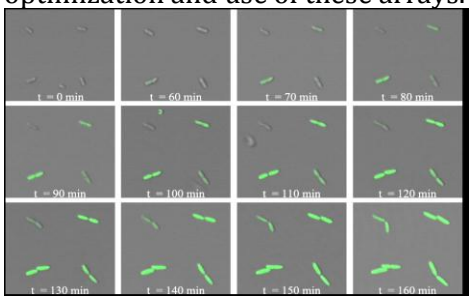

Figure 1. Time laps overlays of transmission light and fluorescence images of *P. putida*. obtained 0 to 160 minutes after adding an inducer promoting the expression of GFP.

Topic 1: Induction kinetics of promoter systems: The aim is to study induction characteristics of two promoter systems, Pm and PBAD, inserted into *Pseudomonads Putida,* using quantitative time-lapse fluorescence microscopy of bacterial arrays. Upon addition of an inducer, a characteristic time delay is expected before the cells switched from low to high expression of the promoter controlled genes. The duration of this delay is expected to exhibit a systematic dependence on the externally supplied inducer concentration. Questions we would like to address through statistical analysis of images obtained using fluorescence microscopy:

- *At a saturating inducer level, is a rapid induction occurring within all cells of the culture, or do the cells behave differently?*
- *How is the level of gene expression reduced when decreasing the inducer levels? Do the cells respond differently to this reduction?*

Topic 2: Bacterial arrays for studies of horizontal gene transfer: Horizontal gene transfer (HGT) is a process where transmission of DNA occurs between organisms. Bacterial conjugation is one of the mechanisms for gene exchange. HGT play a significant role in bacterial evolution including the spread of antibiotic resistance.

The details of conjugation are yet to be understood, and a technology allowing continuous tracking of high numbers of single bacterial cells would be advantageous. We propose to, by the use of bacterial microarrays with dimensions of adhesive islands and inter island spacing optimized for studies of bacterial communication, address the influence of the following variables on the conjugation process:

- the distance between donor and recipient cell
- the size and location of the genes that are to be transferred (on a chromosome or on a plasmid)

The array preparation requires PDMS stamps prepared in NTNU Nanolab.

Contacts: Associate Prof. Mari Sletmoen, (marit.sletmoen@ntnu.no), Researcher Rahmi Lale (topic 1 and 2) Martin F. Hohmann-Marriott (topic 2).

Bacterial microarrays for high throughput analysis of bacterial heterogeneity

Background: Antibiotic resistance is increasingly common in dreaded pathogens like *Mycobacterium tuberculosis* (Mtb), that causes Tuberculosis (TB), and a high percentage of hospital-acquired infections are caused by highly resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) or multi-drug resistant Gram-negative bacteria. The identification of ways to prevent acquisition of drug resistance and transmission of drug resistance traits has therefore received increased focus. Emerging antibiotic resistance has also urged the need for an improved understanding of the mechanisms underlying the formation of persister cells, defined as the subpopulation of cells displaying tolerance to high doses of bactericidal antibiotics, of which much is still to be discovered.

Through your involvement in the projects described below you will contribute to the development of new technology which can form the basis for efficient studies of bacterial populations. You will be part of a multidisciplinary academic team ensuring expertise related to nano- and microfabrication, surface functionalization as well as bacterial pathogens and infectious diseases. Each of the topics described below identify challenges that due to their complexity cannot be tackled by one student alone. However, we invite you to address chosen questions related to these overall challenges. Which questions you contribute to addressing will be defined based on your interests and scientific background.

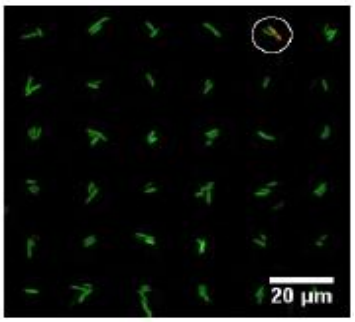

Figure 1: Bacterial microarrays prepared using µCP and observed using confocal microscopy.

Topic 1: Novel drug targets and persistence in pathogenic mycobacteria: Drug resistant Mtb strains are common (6-10% of new cases), emphasizing the need for new drugs and thus discovery of novel drug targets. Our approach for finding novel drug targets is based on identification of proteins responsible for essential biological processes in the pathogen. We have identified hundreds of essential genes and we will utilize the microarray technology (Fig 1) to evaluate our proposed protein targets level of essentiality by performing growth competition experiments on the microarrays in combination with high throughput imaging to measure growth and colony development. The studies will be performed by immobilizing Mtb mutated in potentially essential genes on µCP surfaces and follow and compare their colony formation abilities using microscopy.

Topic 2: Development of persistent cells under antibiotic treatment: If a treatment with antibiotics is discontinued before the whole population of bacteria is killed the chance of resistance development is high. Persistence development is today usually studied using traditional growth methods and flow cytometry. We propose an improved approach based on the microarray technology, enabling us to follow the growth of thousands of individual bacteria as they develop into colonies. Using this approach we will identify and quantify persister cells in a population of bacteria (e.g. clinical isolate). The quantification will be performed both prior to and after exposing the population to antibiotics. Such studies are important since the accepted notion that persisters are present in a given population prior to antibiotic treatment recently was challenged by experimental evidence revealing induction of persistence by antibiotic exposure.

The array preparation requires PDMS stamps prepared in NTNU Nanolab. Contacts: Associate Prof. Marit Sletmoen, (marit.sletmoen@ntnu.no), Senior Research Scientist and Co-director of CEMIR Trude H. Flo.

Glycans and recognition

Glycosylation is one of the most widely found and complex post-translational modifications, and the glycome encompasses a vast and extensive repertoire of sugars covalently linked to proteins, glycolipids or proteoglycans. Nearly all proteins that are expressed on the cell membrane, or are secreted, carry glycans and these are involved in cell adhesion, recognition, molecular trafficking, clearance and signaling. Indeed, the recognition of specific carbohydrate chains (glycans) by carbohydrate-binding proteins (lectins) is an important regulatory mechanism in healthy and diseased immune physiology. You are welcome to contribute to our ongoing activities related to the study of glycan interactions using atomic force microscopy (AFM) or optical tweezers (OT) (Fig 1). These techniques allow manipulation at the single molecule or cell level while determining intermolecular interaction forces with picoNewton resolution. They are thus powerful tools to provide new information concerning specific biological interactions.

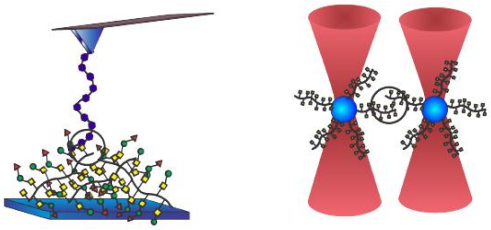

Figure 1: Biomacromolecules to be investigated are immobilized on the AFM tip and a sample surface (left) or on two polystyrene beads trapped using the optical tweezers (right).

Topic 1: Yeast surface molecules in adhesion, communication and microbial infection: The surface of yeast cells is decorated with proteins that have a role in adhesion, communication and microbial infection. Understanding how these cell surface proteins contribute to sensing the external environment is a challenging open question. The answer to this question is relevant for understanding several physiological and biotechnological processes, such as molecular recognition and cell adhesion, aggregation and flocculation, biofilm formation, resistance to antifungal drugs and barrier for mycotoxins.

As a first step towards an improved understanding of this system, we have probed the interactions between the yeast *Saccharomyces cerevisae* mutated for cell wall genes and the lectins ConA and WGA. However, several questions still remain to be investigated and you are invited to contribute the further work.

Topic 2: Carbohydrate antigens in human health and disease: We are investigating the effect of the aberrant glycosylation occurring in the majority of human cancers. We have shown that certain carbohydrate based tumor-associated antigens can bind the C-type lectin MGL, found on antigen presenting cells. This interaction has consequences for the further progression of the cancer. Additionally, we have documented that these antigens self-interact, and that a GalNAc group is essential for these interactions.

This new insight leads to new questions which will be addressed through studies of interaction abilities of synthetic polymers displaying mono or oligosaccharides.

Contacts: Associate Prof. Marit Sletmoen, (marit.sletmoen@ntnu.no). Topic 1 will be performed in collaboration with Etienne Dague at LAAS, Toulouse, France.

General information concerning all the topics proposed: The detailed content of the projects will be decided together with the student. The amount of work will be adjusted to fit the requirements of a project, or master thesis.

Expression of heterologus proteins in eukaryotic host cells

Faglærer: Trond E. Ellingsen, trond.e.ellingsen@sintef.no

Veileder: Anne Tøndervik, anne.tondervik@sintef.no

Background and aim of the work

Production of recombinant proteins for various applications (industrial, pharmaceutical, research) can be achieved by using both prokaryotic and eukaryotic expression host. When the target proteins are of eukaryotic origin, mammalian or yeast cells are frequently used to obtain e.g. post translational modifications necessary for activity. SINTEF and NTNU have ongoing projects were eukaryotic polysaccharide modifying enzymes are expressed in yeast and mammalian cells, specifically *Pichia pastoris* and HEK-293. Currently production conditions are established in small scale shake flasks with culture volumes in the range of 50-100 ml. However there is a need to establish also conditions for larger scale lab production using fermentor systems with cultivation volumes up to 1000 ml. Initially, existing expression vectors will be used, but depending on the choice of target proteins there will be a need for constructing new vectors. The work will include the following tasks:

- Establish cultivation conditions for HEK-293 and CHO cells in fermentor systems
- Establish conditions for transfection of mammalian cells in large scale volumes
- Optimization of production of target proteins in fermentors
- Comparative production studies with HEK-293 and CHO
- Construction of expression vectors for new target proteins
- Comparative studies using *Pichia pastoris* and mammalian cells
- If relevant, also prokaryotic expression systems (*E. coli*, *P. putida*) will be included

Master thesis in Biotechnology, 30 or 60 sp

Limitations of Microbial Control in Marine Aquaculture

Goal

- Set up an experimental systems suitable for studying processes that determine community assembly (i.e. community composition) in tanks for rearing of marine fish larvae
- Do experiments aiming at disentangling the relative importance of the four ecological processes that determine community assembly: speciation, selection, dispersal and drift.

Background

Our research group has shown that detrimental fish larvae-microbe interactions are a main reason for low survival and growth in cultivation of marine fish and shellfish. As a consequence we have had focus on microbial management strategies, and the establishment of research based methods for microbial control. A significant part of this work has been on steering the composition of the microbial community in the rearing water and of the gut microbiota of the larvae.

We have, however, seen that also for replicated tanks the composition of the microbial community of the rearing water and of the larvae may drift apart, and differences between replicates may be comparable to the differences between different treatments. As the input of microbes to the tank via water and feed, is the same in replicated units, other processes than input must contribute to the observed differences.

Ecological theory proposes that four ecological processes determine community assembly (i.e. community composition): speciation, selection, dispersal and drift. Speciation adds new species diversity, whereas selection alters the relative abundance of species based on their competitive ability. Dispersal is the movement (import) and successful establishment of a species in a new location, and drift is changes in relative abundance of species in a location due to random processes.

Work description

In a small scale (250 – 1000 ml) experimental system which maintains the structural similarity to a fish tank, it is possible to control and vary selection and dispersal, and this can be used experimentally to disentangle the relative importance of the four ecological processes determining community assembly. Experimental variables could be dilution rate which affects selection, or the degree of dispersal. Variability between identical treatments should be attributed to drift. The composition of the bacterial community will be characterized by a PCR/DGGE strategy. The species composition (i.e. the DGGE band pattern) will be analysed by multivariate ordination and multivariate statistics. Samples could also be analysed by high throughput Illumina sequencing. The extent of the experimental work will depend on whether this is a 30 or 60 stp thesis.

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Master thesis in Biotechnology, 60 sp

Gnotobiotic Salmon: Development of a Method to Establish Bacteria Free Salmon Larvae

Goal: Given by the title of the thesis

Background

The relationship between hosts and their microbes is an example of coevolution where the normal situation is that the relationship is mutually beneficial. So far focus has been on negative disease causing microbes both for humans and for fish. However, during recent years the beneficial host-microbe interactions have received more attention, and there is a rapid growth in new knowledge within this area. This is particularly the case for studies of mammals, but our research group has been instrumental for such studies of young stages of fish. Model systems were it is possible to establish microbe free individuals have served an important research tool to study the importance of microbes for a host, as they serve as a negative reference. As examples such studies have shown that a microbe free individual needs to eat 40% more to have the same weight gain as a conventional animal, and that a large number of host genes are regulated by the microbiota.

We have already developed a method for obtaining microbe free Atlantic cod larvae (Forberg et al. 2011), and have had success using this as a model system. Now we want to establish a protocol for microbe free Atlantic salmon.

Work description:

The main goal of the thesis is to develop a disinfection and antibiotic protocol that results in microbe free yolk-sac larvae without harm to the larvae. We will aim at establishing a protocol that does not require antibiotic treatment after hatching.

The student will do does-response experiments with 2-3 different disinfectants with and without addition of an antibiotics mixture. Presence of microbes will be quantified by both culture dependent and independent methods (including flow-cytometry), and effects on the salmon eggs and yolk sac larvae will be evaluated by registration of survival during both the egg and the yolk sac stage, hatching success, egg yolk conversion efficiency and possibly quantification of expression of a stress related gene by qPCR.

Details in the goals/sub-goals and in the experimental setup will be planned together with the student.

Forberg, T, Arukwe, A and Vadstein, O. 2011. A protocol and cultivation system for gnotobiotic Atlantic cod larvae (*Gadus morhua* L.) as a tool to study host microbe interactions. Aquaculture 315: 222–227. DOI: 10.1016/j.aquaculture.2011.02.047

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Molecular interactions and new gelling concepts between chitosans and fucoidan from the Norwegian seaweed *Laminaria hyperborea*

(Norsk tittel: Molekylære interaksjoner og nye gel-konsepter mellom kitosaner og fukoidan fra stortare (*Laminaria hyperborea*)

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Medveileder: Georg Kopplin (stipendiat)

Background:
Fucoidan is a new and interesting polysaccharide with a very high charge density and a large number of branching points. Several bioactivities of fucoidan have been reported, such as anticoagulant, antithrombotic, anti-inflammatory, antiviral and immune stimulating activity. Furthermore, it is already used for medical purposes to stimulate wound healing and prevent scarring after surgeries. A fucoidan gel (instead of a solution) could provide beneficial properties for medical applications.

Chitosan-Alginate gelling systems have been studied in our group. Such gels form through ionic interactions between the positive charges on the chitosan molecules and the negative charges on the alginates, and the gelling system is controlled by changes in the pH-value. Like alginate, fucoidan also consist of negatively charged sugar units (highly sulfated fucose units), and would form gels with the positively charged chitosan through electrostatic interactions.

The source for our fucoidan is the Norwegian brown seaweed *Laminaria hyperborea.* This algae is the most important raw material for production of alginate in Norway, and about 160 000 metric tons are harvested along the Norwegian coast annually, from which about 6 000 metric tons of alginate is produced. In addition to alginate, there are also other valuable components in the alga, such as fucoidan, proteins and pigments.

This project is a part of the “Marine polysaccharides” project financed by Biotech 2021 through the Norwegian Research Council and industry. Gelling experiments will be performed through rheological methods and Young's modulus measurements, further characterization will be performed by NMR, FTIR and other methods.

Goal:
Characterizing interactions between fucoidan and chitosan, particularly fucoidan-chitosan gels.

Alginate – chitosan hybrid gels

This project is relevant for 60 sp Master Students in Biotechnology and Nanomedicine finishing spring 2016 or parts as project (15 sp) for students in Chemical Engineering and Biotechnology (2014) that will continue with a master project (30 sp) spring 2015.

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Alginates are linear polymers of 1→4-linked α-L-guluronate (G) and β-D-mannuronate (M). They are considered as block polymers with homopolymeric blocks of M and G, as well as blocks with an alternating sequence. Alginate forms hydrogels with the crosslinking of G-blocks and to some extent MG-blocks with divalent cations such as calcium. The mechanical properties of the hydrogel can be tuned by the composition and molecular weight of the alginate as well as the choice of gelling ions. Alginate composition can be tailored by using mannuronan C-5 epimerases that convert M to G in the polymer [1]. By introducing short G-blocks alginate gels are formed with calcium that are initially strong but very unstable. We have recently shown that alginates can be crosslinked with positively charged chitosan oligomers, and that this crosslinking is stronger between M-sequences (as compared to G-sequences) [2]. By using both calcium and chitosan oligomers as cross-linkers, a hybrid hydrogel can be formed that is relevant both for mechanical tuning of alginate gels but also as potential materials in tissue engineering applications.

The aim of the project is to develop novel calcium-alginate-chitosan hydrogels based on both natural and alginates with G- and M-blocks solely (AlgE6 epimerised mannuronan) and evaluate the mechanical properties of the gel (elasticity/stiffness, resistance to breakage, stability).

References:

1. Morch, Y.A., et al., *Mechanical properties of C-5 epimerized alginates.* Biomacromolecules., 2008. **9**(9): p. 2360-2368.
2. Khong, T.T., et al., *Gelling concept combining chitosan and alginate-proof of principle.* Biomacromolecules., 2013. **14**(8): p. 2765-2771.