Independent Projects in Biology, Environmental Science and Soil Science, 30 hp
(A1E or A2E – Magister or Master) Biology and Soil Science

NB! A1E can be written in Swedish or English, A2E must be written in English.

If you are interested in any of the suggested projects or just want more information please contact the supervisor.

Role of sulfide in regulation of the anaerobic digestion of lipids: Implications for increasing biogas production in municipal wastewater treatment plants
Mains subject: Biology and Environmental Science
Contact: Bettina Müller, bettina.muller@slu.se
For project description see below.

Characterization of mannoside binding to a cyanobacterial carbohydrate-binding protein by NMR spectroscopy
Main subject: Biology or Chemistry
Contact: Gustav Nestor, gustav.nestor@slu.se
For project description see below.

Unravel the competition between methane-producing microorganisms in biogas systems
Main subject: Biology
Contact: Maria.Westerholm@slu.se
For project description see below.

Microbial consortia in rewetted peatlands
Main subject: Biology or Soil Science
Contact: Bettina.Muller@slu.se, or Sabine Jordan@slu.se (Dept of Soil and Evironment)
For project description see below.

Purification and properties of artificially matured [Fe-Fe] hydrogenases from syntrophic acetate oxidation bacteria
Main subject: Biology
Contact: Bettina.Muller@slu.se, or Livia.Meszaros@kemi.uu.se (Dept of Chemistry, Uppsala University)
For project description see below.

How plant cells upcycle their own organelles: physiological roles of selective autophagy
Main subject: Plant cell and molecular biology
Contact: Sanjana Holla, sanjana.holla@slu.se
For project description see the text below or follow this link.

Novel aspects of autophagy in plant stress response: the path to developing better crops
Main subject: Plant molecular biology
Contact: Alyona Minina, alena.minina@slu.se
For project description see the text below or follow this link.

Exciting search for Chlamydomonas metacaspase interactors
Mains subject: Biology
Contact: Yong Zou, yong.zou@slu.se

Exploring autophagy-mediated stress granule degradation in plants
Main subject: Biology
Contact: Adrian Dauphinee, adrian.dauphinee@slu.se

PROJECT DESCRIPTIONS

Role of sulfide in regulation of the anaerobic digestion of lipids: Implications for increasing biogas production in municipal wastewater treatment plants.

Background

Anaerobic digestion (AD) of lipid-rich wastes with sewage sludge enables a utilization of the excess capacity at existing municipal anaerobic digesters for biogas production, contributing to an energy efficient wastewater treatment process. However, lipids tend to degrade slowly during AD causing process disturbances, associated with accumulation of their intermediate degradation products (long chain fatty acids, LCFA) and the resulting microbial toxicity. In a laboratory trial, we observed that an increase of the sulfide level in a digester treating municipal sewage sludge resulted in a faster conversion of LCFA to biogas, by favoring growth of the LCFA-degrading microorganisms. To our knowledge, this was the first time that such a connection between sulfide level, growth of LCFA-degrading microorganisms, and LCFA turnover kinetics was reported, which provided evidences for potential regulatory role of sulfide in degradation of lipids.

In the next part of the project, we studied the degree and kinetics of the degradation of saturated and unsaturated LCFA at different sulfide levels in six laboratory anaerobic digesters, treating primary and secondary sewage sludge. In particular, the microbial community response to saturated and unsaturated LCFA loads was studied in relation to sulfide level in the digesters. The outcomes of this study are found in the following publication:

Literature

Shakeri Yekta S, Liu T, Axelsson Bjerg M, Šafarič L, Karlsson L, Björn A, Schnürer A (2019). Sulfide level in municipal sludge digesters affects microbial community response to long-chain fatty acid loads. Biotechnology for Biofuels, 12, 259 (Link)

Aim

The aim of the proposed master's thesis is to further assess the differences in microbial metabolic functions during anaerobic digestion of saturated and unsaturated LCFA at different sulfide levels. For this purpose, the overall project includes the following steps:

1) To perform DNA purification for Nanopore and Illumina sequencing of selected samples
2) Perform Nanopore sequencing
3) Perform protein and peptide purification for Ms/Ms analysis
4) Apply MAFIN (a bioinformatics pipeline) for assembly, binning and annotation
5) Analyze and evaluate retrieved data by using MaxQuant and Perseus software

Complementary information

The candidate may only perform some of the steps. Which steps the project will include, depends on the study program of the candidate. The project is suitable for master degree programs related to Bioinformatics, Molecular Biology, Biochemistry and Microbiology.

The project will be performed at SLU, Department of Molecular Sciences, SLU, Uppsala and supervised by Bettina Müller (Bettina.Muller@slu.se) and Sepehr Shakeri Yekta (sepehr.shakeri.yekta@liu.se) from Linköping University, Department of Thematic Studies-Environmental Changes. Please contact us when you are interested in a master thesis.

Characterization of mannoside binding to a cyanobacterial carbohydrate-binding protein by NMR spectroscopy

Cyanovirin-N (CV-N) is an antiviral lectin (a carbohydrate-binding protein) from the cyanobacterium Nostoc ellipsosporum. CV-N binds to oligosaccharides composed of mannose sugar residues (mannosides), which are present on the surface of several viruses, such as HIV, Ebola, and influenza virus. For example, the HIV-1 envelope glycoprotein gp120 is heavily glycosylated with mannosides. These sugars can serve as targets for antiviral compounds, which bind to the glycans and interfere with the viral entry into the target cell. CV-N has shown antiviral activity against HIV type I and II and other enveloped viruses like Ebola and influenza at nanomolar concentrations. At present, CV-N is tested for therapeutic use as topical applications in microbicides. The characterization of CV-N/mannoside interaction is important for the development of new microbicides, but also for the general understanding of lectin/mannoside interactions, which may be used as a target to block viral infection when no vaccine is available.

The specific aim of this project is to investigate the binding of different mutants of CV-N to certain mannosides by using nuclear magnetic resonance (NMR) spectroscopy. The mutants will be expressed in E. coli with a medium that contains sources of 15N and/or 13C, which is a

requirement for the NMR analysis. Proteins will be purified with ion-exchange chromatography, size-exclusion chromatography and dialysis, and then subject to NMR analysis. Mannosides will be labelled with 13C to facilitate the analysis and new NMR experiments that are designed for such compounds will be utilized.

Skills that will be developed within this project:
- Expression of isotopically labelled proteins in E. coli
- Protein purification
- Protein and carbohydrate analysis by NMR spectroscopy

Unravel the competition between methane-producing microorganisms in biogas systems

We are looking for a highly motivated student who is interested in joining our group to investigat the competition between different methane-producing microorganisms in order to find ways to predict biogas production rates.

Biogas production is a waste-to-energy technology with outstanding climate, environmental and societal benefits. Biogas is produced when organic materials are broken down by microorganisms in an anaerobic environment that proceeds in a series of steps divided into hydrolysis, acidogenesis, anaerobic oxidation and methanogenesis. The last and methane-producing step is extremely important for efficient biogas production. This is also the step that easily gets restricted by toxic compounds or during changes in process operation. A restricted methanogenic step will cause severe process disturbance and decrease the biogas production.

In this project we aim to study the competition between different microorganisms that perform the methane-producing step. Two main pathways for biogas production are aceticlastic methanogenesis (performed by acetate-utilizing methanogens) and syntrophic acetate oxidation (performed by acetate-utilizing bacteria and H2-utilizing methanogens). Cultivation studies with these two groups of methane-producers will be set up and the impact on the methane production rate by ammonia (a toxic compound formed in the degradation of proteins) and temperature will be investigated. The interplay of the microorganisms will be followed by molecular approaches. The result from the study can be used to predict consequences on methane production rates in biogas processes operating at different conditions.

You will acquire skills in:
1. Anaerobic cultivation techniques
2. Analytical analyses using high-performance liquid chromatography (HPLC), gas chromatography (GC), H2-measurement
3. Molecular techniques including DNA extraction, agarose gel electrophoresis, quantitative PCR (qPCR), RNA extraction, conversion to complementary DNA (cDNA)
4. Performance of a design of experiment approach.

<u>Complementary information</u>

The project is suitable for master degree programs related to Bioinformatics, Molecular Biology, Biochemistry and Microbiology and will be performed at SLU, Department of Molecular Sciences, SLU, Uppsala. Please contact Maria Westerholm (Maria.Westerholm@slu.se) if you are interested in this project.

Purification and properties of artificially matured [Fe-Fe] hydrogenases from syntrophic acetate oxidation bacteria

Background

The reversible conversion of protons and electrons into molecular hydrogen represents the simplest possible redox reaction in nature, and provides potential in supporting a future society free from fossil fuels. Molecular hydrogen can be used as a renewable fuel, as chemical feedstock to produce methane, methanol, and other hydrocarbons, or in industrial processes such as the Haber-Bosch process for producing ammonium fertilizers. At present hydrogen is mostly produced non-renewably from steam reformation of fossil fuels. The only current efficient renewable way is the electrolysis of water, for which rare noble metals such as platinum need to be used as catalysts.

However, nature is capable of catalyzing hydrogen conversion reversible and efficiently by employing enzyme catalysts, so-called HYDROGENASES, under ambient temperatures and pressures, using one of the most abundant metals on earth, iron. [Fe-Fe] hydrogenases are the champions in frequencies H2 production with reported turnover of 10,000 per sec and occur both in prokaryotes and eukaryotes (single cellular algae). The biosynthesis requires a complex insertion machinery for incorporating the redox active [Fe-S] clusters and the [2Fe] subsite what impedes cloning, modifications and mechanistic studies. However, [Fe-Fe] hydrogenases have been successfully overexpressed recombinantly in Escherichia coli as pro-enzymes in high yields and with high purity. Then, the active enzymes have been produced by adding an artificially produced inorganic [2Fe] active site variant circumventing any species-specific maturation machineries.

[Fe-Fe] hydrogenases are the engine of so-called syntrophic consortia, where one species lives from the hydrogen, which has been produced by another species. In syntrophic acetate oxidizing bacteria, acetate is oxidized to hydrogen, which is consumed by methane producing archaea. Thus, syntrophic [Fe-Fe] hydrogenases might be potential candidates for future bio-hydrogen production.

Project description

Within the 30-credit degree project corresponding to 20 weeks of education, the candidate will investigate the suitability of overexpressing syntrophic [Fe-Fe] hydrogenases in E. coli and reconstitute and compare their activities by adding an inorganic [2Fe] active site mimic. These include cloning of the hydrogenase genes using a well-established pET vector system; overexpression in E. coli, purification with metal-affinity chromatography, anaerobic work and different spectroscopical methods (UV-Vis and EPR spectroscopy) The candidate student will acquire fundamental understanding of experimental design in molecular biology and obtain

competence in laboratory work, data handling, data analysis and manuscript preparation. The candidate is expected to write and present the master thesis in English.

How plant cells upcycle their own organelles: physiological roles of selective autophagy

Background

Autophagy, which translates to "self-eating", is the clean-up machinery in all eukaryotes. In plants, this mechanism is increasingly recognized for its paramount role in development, immunity and fitness. Autophagy can function in a selective manner, wherein specific components of the cell (cargo), including protein aggregates and organelles are recycled in response to stress. Currently, a major gap exists in understanding the sequential targeting of the cargo to be degraded, and its impact on plants.

Project goals

In this project we will study selectivity of autophagy in plants and its physiological relevance for plant fitness and stress tolerance.

Skills that will be acquired through this project:

- Working with transgenic plants
- Advanced fluorescence microscopy
- Processing large data sets using ImageJ
- Immunoblotting
- Handling of Arabidopsis thaliana seedlings and plants

Novel aspects of autophagy in plant stress response: the path to developing better crops

Short description
Autophagy is an extremely interesting catabolic pathway that allows cells to upcycle their own content. Similarly to a trash recycling system, autophagy converts damaged or superfluous components into energy and building blocks. In our group we are investigating how this process helps plants to cope with stress conditions. This knowledge will eventually allow us to improve crops and make them better fitted for the changing climate.

In this project you will help to optimize our non-invasive bioluminescence-based advanced approach for quantifying autophagic activity in different organs of living plants and use it to reveal the specific roles autophagy plays in the stress response of plant organs.

Project goals:

1. Cloning constructs encoding novel molecular reporters of plant autophagic activity
2. Verifying/optimizing the constructs using transient expression in plants and advanced fluorescence microscopy
3. Initiating stable transgenic lines expressing the new constructs
4. High-throughput phenotyping of transgenic plant seedlings using our new robotic system SPIRO

You will acquire skills in:

- Genetic engineering and cloning
- Advanced fluorescence microscopy
- Working with one of the most popular plant model organism *Arabidopsis thaliana*
- Transient expression in plants
- Working on stable transgenic plant lines
- Use of automated assays for plant phenotyping
- Working in a research team

Exciting search for Chlamydomonas metacaspase interactors

Project description

Caspases are **c**ysteine-dependent **asp**artate-directed prote**ases** found almost exclusively in animals, with a crucial role in programmed cell death (PCD) and inflammation among other fundamental processes. There are no direct sequence homologs of caspases in the genomes of organisms from other kingdoms of life than animals. However, they have distantly related class of proteases named metacaspases and sharing caspase fold and conserved catalytic dyad of Cysteine and Histidine (Uren *et al*., 2000). Notably, there are key biochemical differences between caspases and metacaspases. While caspases are active as Ca^{2+} independent dimers, active metacaspases are monomers and their activation usually requires millimolar concentrations of Ca^{2+}. Furthermore, caspases cleave their substrates after Aspartate, whereas metacaspases after Arginine or Lysine residues. Great efforts have been made in the last two decades to understand caspase regulation and function, but metacaspase research remains in its infancy. In most higher plants including *Arabidopsis* there are several members of the two major structural types (I and II) metacaspases, hampering understanding of non-redundant functions of individual family members. In contrast, haploid genome of green algae *Chlamydomonas reinhardtii* encodes only one member of either type of metacaspases, providing powerful paradigm for the metacaspase structure-function research.

Yeast two hybrid (Y2H) is a useful molecular tool to detect protein-protein interactions (PPI). The premise behind the test is the reconstitution of a functional transcription factor (TF) when two proteins or polypeptides can interact in genetically modified yeast strains. In Y2H, the TF is split into two separate parts, the DNA-binding domain (BD) and activating domain (AD), which are responsible for binding the upstream activating sequence (UAS) and initiating transcription of downstream reporter genes, respectively. The proteins of interest are fused to BD (referred as “bait”) and AD (referred as “prey”) separately. When interaction occurs, BD and AD are recruited in close proximity and a functional TF is reconstituted, promoting the expression of the reporter gene and then resulting in a specific phenotype, such as growth on a selective medium or color changes of the yeast colonies.

In this project, we plan to construct a Y2H library with catalytic active or catalytically dead mutant metacaspases as baits, and *Chlamydomonas* transcriptomes in different growth conditions as prey candidates. We aim to find proteins interacting with metacaspases, including their substrates. The preselected interaction partners will be re-validated using Y2H or other PPI methods (e.g. co-immunoprecipitation).
We are looking for a highly motivated student with a burning interest in laboratory work and passion for molecular biology.
You will acquire skills in:

1. Molecular techniques including protein extraction, Western blot, DNA/plasmid extraction, agarose gel electrophoresis, RNA extraction, qPCR
2. Chlamydomonas culture
3. Yeast two hybrid test and library construction

Exploring autophagy-mediated stress granule degradation in plants

Background

All cells require efficient mechanisms to cope with stress in order to survive. Autophagy ("self-eating") is a major catabolic process in eukaryotes that allows for the targeted or bulk removal and recycling of cytoplasmic components. Upon the induction of autophagy, cytoplasmic contents are sequestered into double membrane vesicles known as autophagosomes, which are then delivered to the lytic vacuole for degradation. The process is critical for maintaining cellular homeostasis and it has profound impacts on cell death, stress responses and longevity. Another vital mechanism for cell survival is stress granule formation. Stress granules are membraneless organelles comprised of RNA and proteins that aggregate to form dense cytoplasmic granules. These stress granules allow for the rapid shutdown of protein synthesis that is no longer conducive to cellular function when confronted with challenging conditions.

It is now apparent that the regulation of autophagy and stress granule formation have significant impacts on the fitness and health of organisms. In animals, these processes have been linked to several conditions including diabetes, cancer and neurodegenerative diseases. In plants, autophagy impacts several agronomically important traits such as growth, yield and disease tolerance. Stress granules are relatively unexplored in plants, however they are formed in response to a plethora of stressful stimuli and warrant further investigation. Unravelling these critical biochemical pathways and gaining insight into how we can regulate these processes is of great interest to the agricultural and biomedical fields.

Aims

The purpose of this work is to investigate the autophagy-mediated degradation of stress granules within the model plant *Arabidopsis thaliana*. Previously established Arabidopsis suspension cultures created from fluorescent tagged stress granule marker lines will be employed. Stress granule degradation will be assessed after treatment of the cultures with various autophagy

modulating compounds and protease inhibitors. In addition, Arabidopsis suspension cultures expressing fluorescent protein tags for both stress granules and autophagosomes will be developed and evaluated over time following treatment using advanced microscopy techniques.

Skills that will be developed within the project:

1) Advanced microscopy skills including confocal and super resolution structured illumination microscopy (SIM)
2) Plant genetic transformation
3) Molecular biology techniques
4) Plant and cell culture establishment and maintenance
5) Experimental design and data analysis

Complementary information

This project is suitable for master's degree programs related to molecular biology and biochemistry. The 30 credit project, corresponding to 20 weeks of education will be carried out in the Plant Catabolism Laboratory in the Department of Molecular Sciences, SLU, Uppsala. Please contact Adrian Dauphinee (adrian.dauphinee@slu.se) if you are interested in this project.