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.

^{13}C-labelled sugars for NMR studies to learn how key biomass degrading enzymes work
Subject: Biology or Chemistry
Contact: Piera Wiesinger, piera.wiesinger@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.

Expression, purification and Biochemical characterization of Lytic polysaccharide monooxygenases (LPMOs)
Main subject: Biology
Contact: Mats.Sandgren@slu.se
For project description see below.

How plant cells upcycle their own organelles: physiological roles of selective autophagy
Main subject: Plant cell biology
Contact: Alyona Minina, alena.minina@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.

Genomic knock-in by CRISPR/Cas9 in green alga Chlamydomonas reinhardtii
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

Exploring the diversity of methanol-assimilating yeasts in nature
Main subject: Biology
Contact: tomas.linder@slu.se
For project description see below.

Isolation of novel xenobiotic-degrading yeasts from soil
Main subject: Biology or soil science
Contact: tomas.linder@slu.se
For project description see below.

Microbes go electric
Main subject: Biology
Contact: Maria.Westerholm@slu.se
For project description see below.

Novel cultivation techniques to discover new microbes
Main subject: Biology
Contact: Maria.Westerholm@slu.se
For project description see below.

PROJECT DESCRIPTIONS

^{13}C-labelled sugars for NMR studies to learn how key biomass degrading enzymes work

Background

The demand to reduce the use of fossil resources is high, since they are limited and linked to contributing to global warming. An alternative is to use plant biomass, which is rich in carbohydrates and can be converted to an array of bioproducts by enzymatic conversion. However, the enzymatic processes still need further investigations to be fully understood and optimized for industrial purposes.

We study carbohydrate-active enzymes, such as cellulases and lytic polysaccharide monooxygenases (LPMOs), with nuclear magnetic resonance (NMR) spectroscopy. Moreover, we develop methods to study protein-carbohydrate interactions in atomic detail, i.e. to detect which atoms in the protein bind to which atoms in carbohydrates.[1] For such experiments, ^{13}C and

^{15}N labelled recombinant proteins are used. But we also need ^{13}C labelled carbohydrates, and are therefore working on establishing and developing smart procedures to produce and purify various oligosaccharides from cellulose and fungal cell wall by rational combination of polysaccharide-degrading enzymes.

[1] Nestor et al. JACS 2017, 139, 6210; JACS 2018, 140, 339; Glycobiology 2021, 31, 508.

Project description

In the proposed master project the aim is to produce both recombinant protein and purified carbohydrate oligomers to perform interaction studies with NMR spectroscopy. The enzymes are expressed recombinantly in the bacterium E. coli and/or the yeast Pichia pastoris, and purified with protein liquid chromatography. Carbohydrates are often either found as polymers or as complex glycans in plants, yeast, fungi or bacteria. To perform NMR spectroscopic experiments pure carbohydrate oligomers are needed. In this project, cellulose will be degraded by cellulase enzymes and the cellooligomer products will be separated by liquid chromatography. More complex mixtures of oligosaccharides will be obtained from the cell wall of the yeast Pichia pastoris, by different combinations of polysaccharide-degrading enzymes.

Skills that will be developed within this project:

- Expression and purification of proteins
- Enzymatic degradation of cellulose and separation of cellooligomers with ion and liquid chromatography
- Extraction of yeast carbohydrates, degradation and preparative separation
- Protein and carbohydrate analysis by NMR spectroscopy
- Method development

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 investigate 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.

Complementary information
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.

Expression, purification and Biochemical characterization of Lytic polysaccharide monooxygenases (LPMOs)

The study of Lytic polysaccharide monooxygenases (LPMOs) enzymes is of great importance due to their significant role in the breakdown of polysaccharides, such as cellulose and chitin. LPMOs are a class of enzymes that utilize molecular oxygen to catalyze the oxidative cleavage of glycosidic bonds in polysaccharides, making them crucial players in the degradation of complex carbohydrate structures. Investigating LPMOs and their mechanisms of action can provide valuable insights into the efficient conversion of biomass into biofuels and other valuable bioproducts.

The focus of this project lies on LPMOs derived from *Clonostachys rosea*, an ubiquitous fungus that colonizes living plants, digests organic material in soil and parasitizes on or kills other fungi or nematodes.

To conduct this research, we will employ a series of methods and techniques.

Firstly, we will utilize transformation techniques for *Pichia pastoris*, a well-established expression system, to produce the LPMOs enzymes.

Once the enzymes are successfully transformed and produced, we will proceed with their purification using various types of chromatography techniques, such as affinity chromatography, size exclusion chromatography, and ion exchange chromatography, will be employed to isolate and purify the LPMOs.

Next, we aim to determine the activity of the LPMOs on cellulose based on available transcriptomic data. Mass spectrometry techniques will be utilized to analyze the products generated by the LPMOs during cellulose degradation, providing a detailed understanding of their catalytic activity.

Finally, we aim to crystallize the LPMOs proteins. Crystallization is a crucial step in structural biology as it enables the determination of the three-dimensional structure of the enzymes. By elucidating the atomic-level details of the LPMOs, we can gain insights into their catalytic mechanisms, substrate-binding sites, and potential for interaction with other enzymes.

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

Genomic knock-in by CRISPR/Cas9 in green alga Chlamydomonas reinhardtii

Short description
The unicellular green algae *Chlamydomonas reinhardtii* has served as a model for over 70 years due to the remarkable tractability of its short generation time (8–10 h), haploid genotype, sequenced genome, simple transformation methods, and a plethora of resources, including the Chlamydomonas Resource Center (University of Minnesota) and Chlamydomonas Sourcebook. Recently, the genomic editing in this green alga is extensively documented and is optimized to be feasible. In this project, you will help to optimize the procedure of knocking-in of aimed DNA fragments after editing by CRISPR/Cas9 complex.

In our group, we are focusing on the characterization of type I metacaspase (CrMCA-I) in Chlamydomonas. Metacaspases share a structural similarity with caspases in animals, which are essential proteases with a well-documented role in programmed cell death. Here, we will add an OLLAS (SGFANELGPRLMGK), a highly sensitive epitope tag, at the end of CrMCA-I in Chlamydomonas. The advantage of the tag-adding strain will facilitate the detection of CrMCA-I-OLLAS (by OLLAS antibody) and circumvent the challenges associated with the time-consuming and unpredicted antibody generation process.

Project goals:

Obtain the strain with a knock-in of OLLAS tag at the end of CrMCA-I in 1) cell-wall deficient line (UVM4) and 2) line with intact cell wall (CC-4533)

You will acquire skills in:

- Molecular techniques including protein extraction, Western blot, DNA/plasmid extraction, agarose gel electrophoresis, RNA extraction, qPCR
- Chlamydomonas culture
- Genome editing in Chlamydomonas
- Screening of transformants by colony PCR or Western blot

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.

Exploring the diversity of methanol-assimilating yeasts in nature

Background
Methylotrophy – the ability to use one-carbon compounds such as methanol as a carbon source, has predominantly been observed in only a few lineages of yeasts that are moderately close relatives of the regular baker's yeast *Saccharomyces cerevisiae*. How widespread methylotrophy is among other, more distantly related yeasts is still unclear. Since methanol is a promising growth substrate for sustainable production of yeast protein and other yeast-based products, it is of interest to discover new lineages of methylotrophic yeasts with other potentially valuable traits e.g. heat- or cold-tolerance or the ability to produce oils or antioxidant pigments. The discovery of new methylotrophic lineages of yeast would also shed light on how this ability evolved – did this ability evolve once in the ancestor of all yeasts and was then lost in the majority of yeast lineages or did this ability evolve independently in different lineages?

Project description

Within the 30-credit degree project corresponding to 20 weeks of education, the candidate will isolate and characterize methylotrophic yeasts using both molecular and physiological methods. The student will acquire fundamental knowledge of experimental design, data interpretation, information retrieval and scientific writing.

Skills that will be developed within this project:

- Cultivation methods for microorganisms

- Physiological characterization of microorganisms
- Isolation of genomic DNA, PCR and cloning of target yeast genes
- Sequence analysis including phylogenetic analysis

Complementary information

The project will be performed at Department of Molecular Sciences, SLU, Uppsala. Please contact Tomas Linder (tomas.linder@slu.se) if you are interested in this project.

Isolation of novel xenobiotic-degrading yeasts from soil

Background

"Xenobiotics" is the name given to man-made chemical compounds such as pesticides, solvents, detergents, dyes and pharmaceuticals found in nature. Although man-made compounds are not per definition more hazardous to living organisms than "natural" compounds, one common concern is that man-made compound may take longer to degrade since the natural population of decomposers has not yet evolved the ability to recognize these compounds. However, the metabolic diversity of the soil microbiota is vast and a purported failure to degrade a certain xenobiotic compound may in fact not be due to the lack of metabolic ability of the microorganisms but rather due to other circumstances. Factors that can influence the degradation efficiency of xenobiotics include the nutritional status of the environment (are there other, better molecules to eat?) and the microbial population structure (are the capable degraders in a small minority?).

Xenobiotic-degrading microorganisms are of interest as they may be used to dispose of chemical pollutants in the environment in a "natural" way – a process known as bioremediation. Xenobiotic-degrading microorganisms are also of interest because their ability to degrade unusual compounds can lead to the discovery of novel enzyme activities and biochemical pathways.

Project description

Within the 30-credit degree project corresponding to 20 weeks of education, the candidate will isolate and then characterize xenobiotic-degrading yeasts from soil using molecular, physiological and chemical methods. The student will acquire fundamental knowledge of experimental design, data interpretation, information retrieval and scientific writing.

Skills that will be developed within this project:

- Cultivation methods for microorganisms
- Physiological characterization of microorganisms
- Basic methods for chemical analysis.

- Isolation of genomic DNA, PCR and cloning of target yeast genes
- Sequence analysis including phylogenetic analysis

Complementary information

The project will be performed at Department of Molecular Sciences, SLU, Uppsala. Please contact Tomas Linder (tomas.linder@slu.se) if you are interested in this project.

Projects in biogas production and anaerobic microbiology

Numerous exciting research projects within biogas production are constantly conducted at SLU, in which there can an opportunity for you to perform your master project. These projects can be tailored towards more applied or more fundamental research questions, depending on your interests.

Biogas is a renewable energy source that contributes to a number of positive effects and can play a significant role in the development of a sustainable and fossil-free society. The production of biogas has great potential to increase in the future and with that, many jobs will be generated. By doing a Master's project with us, you can collaborate and become part of our research group that strives to understand and improve microbial processes with the aim of increasing biogas production. Below you find a list of Master's projects available in our group:

Microbes go electric

Many microbes need to pass electrons to oxygen molecules to "breathe" – but the microorganisms in the biogas process thrive in oxygen-free (anaerobic) environments. These anaerobic microorganisms solve this by passing their electrons to other molecules or through fermentation. However, microbes involved in the last steps of the methane (biogas) production process instead rely on a close connection to other species to be able to proceed with their metabolism. Scientists have studied and debated the mechanisms behind this behavior for decades but a relatively newly developed theory is that they perform direct electron transfer between the cells. How they do that is currently not known but usage of nanowires (electric pili, nanotubes) or stacks of proteins (cytochromes) are some of the suggestions. Increased insight in this area will reveal fundamental knowledge of these microorganisms but also brings considerable potential for development of applicable solutions to improve the biogas process and other biotechnological processes used for production of green products.

The aim of this project is to take the first step to reveal if these methane-forming communities really do conduct electricity. You will be involved and supervised in 3D-printing to develop the new anaerobic cultivation systems to enrich conductive microorganisms from the biogas process. You will also obtain basic knowledge in anaerobic cultivation techniques and microscopy and

perform molecular analyses to study the microorganisms with potential to conduct electricity. The work involves laboratory work, collection and analysis of data and report writing.

Complementary information

The project is suitable for Master's 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.

Novel cultivation techniques to discover new microbes

Figuring out the composition of the microbial community in the biogas reactor can help optimize the process for increased production. However, a majority of the microorganisms in the biogas process are unknown, making it impossible to determine their role in the process. The biogas lab at SLU has long experience in isolation and characterization of new microorganisms. However, the methods currently used are extremely time consuming, hampering our work to map key microorganisms involved in the conversion of waste to renewable energy and green products.

In this project, you will development and evaluate a novel cultivation technique that will facilitate and speed up screening of substrate pattern and optimized conditions for growth of new species. You will obtain knowledge in anaerobic cultivation techniques, analytical analyses for liquids and gases and molecular analyses. You will also acquire basic knowledge and understanding of anaerobic degradation occurring in biogas processes. The work involves laboratory work, collection and analysis of data, and report writing.

Complementary information
The project is suitable for Master's 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.