Leibniz
Bioactive
Compounds

# Abstract Book

## Leibniz Conference on Bioactive Compounds

**April 9-10. 2025**

IPB Leibniz Institute of Plant Biochemistry

## *It is a great pleasure…*

to welcome you to the Leibniz Conference on Bioactive Compounds. We meet again, this time in Großbeeren, at the Leibniz Institute of Vegetable and Ornamental Crops: scientists from various disciplines present and discuss their latest research related to the topics of drug discovery, (non-)medical applications of bioactive compounds, method development, novel targets and biotechnology. We hope you enjoy the program and we thank all for their contributions to this conference!

*The organizing committee*

Prof. Ludger A. Wessjohann (IPB)and Dr. Anna Rusznyak (IPB)

*And the speakers of the alliance*

Prof. Ludger A. Wessjohann (IPB), Dr. Pierre Stallforth (HKI), and Prof. Yvonne Mast (DSMZ)

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## LEIBNIZ RESEARCH NETWORK BIOACTIVE COMPOUNDS

Involving 17 institutions, the Leibniz Research Network Bioactive Compounds bundles the Leibniz Association's broadly-based research on molecules with biological effects.

Speaker:
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# Conference Program

| Wednesday, April 9, 2025 | |
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| 12:00 | **Registration** |
| 12:30 | **Welcome addresses**<br>**Nicole van Dam**<br>Scientific Director, Leibniz-Institut für Gemüse- und Zierpflanzenbau IGZ, Großbeeren |
| **Session 1** | **Bioactive phytochemicals: from ecological interactions to human consumption**<br>**Chair: Franziska Hanschen (IGZ)** |
| 13:00 | **PLENARY TALK**<br>**Niels Agerbirk**<br>*Glucosinolate structures in evolution*<br>University of Dänemark, Copenhagen |
| 13:45 | **Maik Behrens**<br>*On the intricacies of individual bitter taste perception*<br>Leibniz Institute for Food Systems Biology at the Technical University of Munich, Freising |
| 14:05 | **Vanda Púčiková**<br>*Utilizing Brassica oleracea biodiversity to elucidate the regulation of the formation of health-promoting metabolites from glucosinolates and S-methyl-L-cysteine sulfoxide*<br>Leibniz Institute of Vegetable and Ornamental Crops (IGZ), Großbeeren |
| 14:25 | **Franziska Beran**<br>*How flea beetles use plant glucosinolates for defense*<br>Leibniz Institute of Vegetable and Ornamental Crops (IGZ), Großbeeren |
| 14:45 | **Poster session & Coffee break** |
| **Session 2** | **Young researchers´ session**<br>**Chair: Pierre Stallforth (HKI)** |
| 16:15 | **Kudzai G. Mbudu**<br>*Functional characterization of nitrile-specifier proteins which promote nitrile formation at the expense of bioactive isothiocyanates in red cabbage and kohlrabi*<br>Leibniz Institute of Vegetable and Ornamental Crops (IGZ), Großbeeren |
| 16:35 | **Nabanita Hazra**<br>*Bio-based microgels as containers for plant protection*<br>Leibniz-Institute for Interactive Materials (DWI), Aachen |
| 16:55 | **Valeriya Denisova**<br>*Ulva biomass production in an innovative brine-based cultivation system: choice of cultivar and nutritional profile*<br>Leibniz Institute of Vegetable and Ornamental Crops (IGZ), Großbeeren |
| **Session 3** | **Award Ceremony, Chair: Ronald Frank (EU-OPENSCREEN)** |
| 17:00 | **Leibniz Drug of the Year 2025**<br>*Discovery of a highly selective molecule acting against resistant (triple-negative) breast cancer, and characterization of its auto-activating prodrug mode of action"*<br>Leibniz Institute of Plant Biochemistry (IPB), Halle (Saale) |
| 18:00 | **Dinner** |

| Thursday, April 10, 2025 | |
|---|---|
| **Session 4** | **BIOACTIVE COMPOUNDS**<br>**Chair: Robert Rennert (IPB)** |
| 09:00 | **Research Award 2025**<br>*New cytokinin biostimulants for agriculture and horticulture*<br>**Miroslav Strnad**<br>Laboratory of Growth Regulators, Institute of Experimental Botany, Czech Academy of Sciences and the Faculty of Science, Palacký University |
| 09:30 | **Charlotte Wit**<br>*Advancing drug discovery: EU-OPENSCREEN's resources for bioactive compound screening*<br>EU-OPENSCREEN |
| 09:50 | **Jasmine Tauchelt**<br>*Antimicrobial potential of Pimpinella saxifraga seeds for controlling Rhizoctonia*<br>Leibniz Institute of Vegetable and Ornamental Crops (IGZ), Großbeeren |
| 10:10 | **Roman Makitrynskyy**<br>*Activation of secondary metabolite biosynthetic gene clusters in Streptomyces spp. using SARP regulators*<br>Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig |
| 10:30 | **Sven Heiles**<br>*Revealing bioactive compounds in tissues with mass spectrometry imaging*<br>Leibniz-Institut für Analytische Wissenschaften - ISAS - e.V, Dortmund and<br>University of Duisburg-Essen, Essen |
| 10:50 | **Coffee break** |
| **Session 4**<br>**11:30** | **Award Ceremony: Young Researcher´s talk and Poster Prize 2025**<br>**Chair: Ludger A. Wessjohann (IPB)** |
| 12:00 | **Closing remarks** |
| 12:10 | **Lunch** |
| 13:15 | **Member Assembly**<br>**conference room**<br>IGZ Theodor-Echtermeyer-Weg 1, 14979 Großbeeren |
| 13:15 | **Guided tour IGZ (participants)**<br>Theodor-Echtermeyer-Weg 1, 14979 Großbeeren |
| 16:00 | **Guided tour IGZ (LRN assembly members)**<br>Theodor-Echtermeyer-Weg 1, 14979 Großbeeren |

# ORAL PRESENTATIONS

# Glucosinolate structures in evolution

Nils Agerbirk*

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# On the intricacies of individual bitter taste perception

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Soon after the discovery of human bitter taste receptors, researchers noticed the high number of non-synonymous polymorphisms in TAS2R genes. This immediately raised the idea that human bitter taste perception must be highly individual. While several functional polymorphisms in TAS2Rs were discovered rather quickly and thus, supported the assumed individuality in human bitter perception, the observation of rather broadly tuned receptors with pronounced overlaps in their agonist profiles seemed to limit the suspected high individuality again considerably. The best investigated variant human bitter taste receptor is the TAS2R38. The discovery of its impact on the perceptual variability of humans for the synthetic compounds phenylthiocarbamide (PTC) and 6-n-propyl-thiourea (PROP) dates back almost a century and has, after the identification of the responsible receptor, enabled to pinpoint the underlying molecular mechanism for the observed individuality. This historical example will provide the basis for the discussion of less frequent and/or more subtle examples for individual bitter taste variabilities discovered by our lab in collaboration with other groups. The presentation will include data explaining the remaining variability of PTC/PROP tasting in individuals that are homozygous for the TAS2R38 non-taster variant by the existence of TAS2R4 variants1. Further, the impact of copy number polymorphisms in the TAS2R43 gene for the perception of the potent coffee bitter compound mozambioside and derivatives thereof is being presented2,3. Finally, evidence for somewhat superior bitter tasting abilities of pygmies, which harbor a functional variant of the segregating pseudogene TAS2R2 is shown4. In summary, the combination of functional calcium-mobilization assays with sensory experiments and the discovery of novel agonists allows to extend previous limitations in the assessment of individual human taste abilities.

## References

1. Nolden A. A., Behrens M., McGeary J. E., Meyerhof W., Hayes J. E. (2024) Differential Activation of TAS2R4 May Recover Ability to Taste Propylthiouracil for Some TAS2R38 AVI Homozygotes. Nutrients.16:1357.
2. Bichlmaier C., Fröhlich S. M., Brychcy V., Graßl A., Behrens M., Lang R. (2025) Contribution of mozambioside roasting products to coffee's bitter taste. Food Chem. 469:142547.
3. Czech C., Lang T., Graßl A., Steuer A., Di Pizio A., Behrens M., Lang R. (2024) Identification of mozambioside roasting products and their bitter taste receptor activation. Food Chem. 446:138884.
4. Lang T., Di Pizio A., Risso D., Drayna D., Behrens M. (2023) Activation Profile of TAS2R2, the 26th Human Bitter Taste Receptor. Mol Nutr Food Res. 67:e2200775.

# Utilizing *Brassica oleracea* biodiversity to elucidate the regulation of the formation of health-promoting metabolites from glucosinolates and S-methyl-L-cysteine sulfoxide

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*Brassica oleracea* vegetables are of great importance for human nutrition. Their sulfur-containing compounds, especially certain metabolites of glucosinolates (GLS) and *S*-methyl-l-cysteine sulfoxide (SMCSO), are associated with many health benefits, such as antimicrobial, anticarcinogenic, antioxidant, anti-inflammatory, and neuroprotective effects. Therefore, understanding the regulatory mechanisms of the formation of these health-promoting compounds could enhance the selection of *B. oleracea* genotypes with improved nutritional value for cultivation and market. 282 genotypes representing seven *B. oleracea* varieties (white, red and savoy cabbage, kohlrabi, kale, cauliflower, and Chinese broccoli), provided by a gene bank (IPK), were grown in the field. The majority of the genotypes were of European origin, but also genotypes originating from the Middle East, China, Japan, and the USA were included in the screening. The oldest of these were entered into the gene bank in the 1950s, while the other genotypes were added in the later decades of the 20$^{th}$ and 21$^{st}$ century. GLS were analyzed using UHPLC-DAD-ToF-MS, SMCSO by UHPLC-FLD, and GC-MS was used to analyze GLS and SMCSO metabolites in the edible parts of the plants. Nano-LC-MS/MS was used for proteomics to gain a deeper understanding of the regulation of GLS and SMCSO degradation. Subsequently, multivariate data analyzes were applied to explore and integrate the analytical datasets. In general, Chinese broccoli genotypes showed the lowest GLS as well as SMCSO levels, whereas savoy cabbage accumulated the highest GLS and SMCSO amounts. The computational analyzes showed that the profiles and levels of GLS and their metabolites are homogeneous in kohlrabi and cauliflower genotypes. In contrast, a strong heterogeneity of these compounds was found between the white cabbage genotypes. The highest potential to form health-promoting isothiocyanates (ITC) upon GLS degradation was found in kohlrabi genotypes. Rather less bioactive GLS metabolites were found in cauliflower genotypes. *S*-methyl methanethiosulfinate, showing antimicrobial and chemopreventive properties, was the most common metabolite of SMCSO in almost all genotypes. The relevance networks and functional enrichment analysis help to further elucidate the regulation of the formation of health-promoting metabolites in *Brassica oleracea*. Our results show that the health-promoting potential of different cabbage genotypes may differ greatly due to their varying degradation patterns. Multivariate analyzes of the data sets contribute to our aim of harnessing *Brassica oleracea* biodiversity for health-promoting plant-based foods.
Funding: This study received funding from the German Federal Ministry of Education and Research (16LW0389 and 16LW0554K) and the Leibniz Research Network Bioactive Compounds.

# How flea beetles use glucosinolates from *Brassica* plants for defense

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Glucosinolates, the characteristic specialized metabolites of Brassicales, are precursors of several bioactive metabolites formed upon enzymatic hydrolysis by so-called myrosinase enzymes. The negative impact of glucosinolate hydrolysis products, especially isothiocyanates, on non-adapted insect herbivores as well as microbes is well documented, as is an impressive diversity of strategies used by adapted insect herbivores to cope with this plant defense. Probably the most advanced strategy is the sequestration of ingested glucosinolates in insects combined with the convergent evolution of insect myrosinase enzymes, as demonstrated in both cabbage aphids and *Phyllotreta* flea beetles. The aim of our research is to understand the molecular evolution of sequestration and defense mechanisms in *Phyllotreta* flea beetles and the consequences for ecological interactions relevant for the development of sustainable pest management strategies. Here we present the identification, characterization, and evolution of myrosinases in *Phyllotreta* flea beetles and the impact of variation in myrosinase activity on the interaction with a generalist predator. Our results show that the duplication of myrosinase genes in *Phyllotreta* is an important adaptation to glucosinolate variation between plant tissues and species, driven by selection pressure from natural enemies.

# *Functional characterization of nitrile-specifier proteins which promote nitrile formation at the expense of bioactive isothiocyanates in red cabbage and kohlrabi*

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*Brassica oleracea* vegetables such as red cabbage and kohlrabi are glucosinolate (GLS)-rich and their incorporation in the diet is linked to a decreased risk of cancer[1]. It is not the GLS itself but the bioactive isothiocyanates (ITCs) formed from enzymatic hydrolysis that exert multiple human health-beneficial properties[1]. The formation of health-promoting ITCs from enzymatic hydrolysis of GLSs by myrosinase in vegetables is complex and depends on many factors including specifier protein activity[1,2]. Specifier proteins such as epithiospecifier proteins (ESPs) and nitrile-specifier proteins (NSPs) promote the formation of less bioactive hydrolysis products and impact the proportion of ITCs formed in vegetable tissues which are commonly consumed[3]. The function of specifier proteins is not yet clear. Understanding specifier protein function in GLS hydrolysis is one of the key factors in optimizing ITC formation in *B. oleracea* vegetables. The *B. oleracea* ESPs have been characterized[4] but the functional characterization of the BoNSPs is still missing. To investigate NSP function, we cloned, expressed and characterized two putative *B. oleracea* NSPs, putative BoNSP1 (isoform XP_013587057.1) and BoNSP2 (isoform XP_013609641.1). The substrate specificity, dependency on ferrous and ferric ions and optimal pH were investigated *in vitro* in assays containing myrosinase, pure GLS standard and 0.5 µg/µl of purified BoNSP1 or BoNSP2. The NSP activity was assessed as the proportion of nitriles formed from each GLS standard in the presence of BoNSP1 or BoNSP2. Further, using an LC-MS based approach, the protein abundance patterns of the *B. oleracea* NSPs in red cabbage and kohlrabi were determined at different ontogenic stages (seed, day 5, day 9 and day 42). The GLSs and GLS hydrolysis products in all tissues were also measured by GC-MS and LC-MS, respectively. We confirmed that *B. oleracea* has with BoNSP1 and BoNSP2 at least two functional NSPs, as they reduced the proportion of ITCs formed from GLS hydrolysis in favor of nitriles. Both BoNSPs had broad substrate specificity, their activity was affected but not strictly dependent on the presence of ferrous and ferric ions and their optimal pH was between pH 7 and 8. Further, proteome analysis revealed six putative BoNSPs, including the two NSPs we characterized *in vitro*, in the red cabbage and kohlrabi tissues analyzed and at all ontogenic stages. The BoNSPs had different protein abundance patterns in different tissues. To assess the impact of BoNSPs on ITC formation, the protein abundance patterns were correlated to the GLS hydrolysis products level and profile at each ontogenic stage and tissue. Our study adds to the understanding of specifier protein function *in planta*. Also, results from characterization of the BoNSPs further support the enhancement of bioactive ITC content in *Brassica* vegetables through acidification during food preparation[5].

**References**

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2. Oliviero et al. (2018). Molecular Nutrition & Food Research, 62(18), e1701069.
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# Bio-based microgels as containers for plant protection

Nabanita Hazra[1], Tuvshinjargal Budragchaa[2], Nikola Mijailovic[1], Ulrich Schwaneberg[1], Ludger Wessjohann[2], and Andrij Pich[1]*

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Sustainable agricultural production with less use of herbicides, pesticides, and fungicides to decrease drought stress is the main need to preserve our livelihoods. In this case, Biocompatible and biodegradable polysaccharide-based microgel (pectin) as containers play a crucial role in loading the plant protectants and delivery systems and to a sustainable release. [1] Herein, Pectin is readily available from citrus, sugar beet, and inexpensive raw material. We synthesized a wide-size range of citrus- based Pectin microgels via inverse mini-emulsion polymerization allowing flexible variation of the chemical structure, size, and crosslinking degree of microgels. The chemical modification of polysaccharides [2] will help the integration of reactive groups able to generate covalent/cleavable or supramolecular crosslinks by controlling the swelling degree and programming the degradation profile of microgels. Our synthesis methodology will also allow programming the release kinetics of the bioactives from microgel containers (Fig 1) triggered by the variation of swelling degree a response to the humidity variations, UV-triggered cleavage of phenacyl crosslinks, or enzymatic degradation of polysaccharide chains.

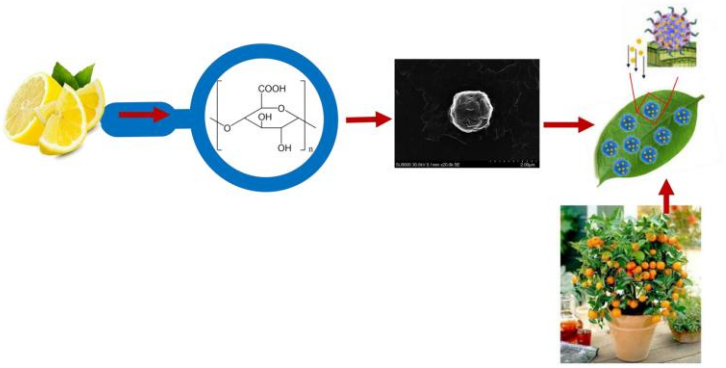


*Figure 1. Bio-based microgels for plant protection*

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# *Ulva* biomass production in an innovative brine-based cultivation system: choice of cultivar and nutritional profile

Valeria Denisova[1,2,3], Wichard T.[4], Rawel H.[3], Förster T.[1,5], Dreyer C.[1,5,6], van Dam N.[7], Schreiner M.[1,2], Fricke A.[1,2]*

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Research on new food sources that are nutritious, healthy, and sustainable is an objective of the federally funded project "food4future." Within this project, a land-based system for macroalgae cultivation using regional brine as cultivation media was developed. *Ulva compressa*, a green macroalgae, is the first species demonstrated to be suitable for cultivation using this approach. To enhance the production capacity of the innovative systems, the current research focuses on investigating and evaluating the quantity, nutritional value of the produced algal biomass of two different algal cultivars: the recently isolated strain of *Ulva compressa* (by Fricke in 2019) and a descendant of the isolate of B. Føyn (aca *Ulva mutabilis*), serving as a model organism for macroalgae. For this purpose, a multivariate experiment was repeatetly (two runs) conducted over a period of 10 weeks including step-wise acclimatization of the different strains to the cultivation media. Here spores of the different strains were seeded on different settelement materials and gradually transferred to the cultivation media under controlled conditions before transferring the seeded material in the cultivation units (>100L). Algae were frequently investigated for biomass growth, evaluating differences between materials and final biomass was analysed for nutritional composition at the end of the experiment (e.g. amino acids, monosaccharides, carotenoids, chlorophylls). Both *Ulva* harvested from the cultivation tanks were detected to produce all essential amino acids, with variations depending on environmental conditions. Organic sulfate content as well as rhamnose and uronic acids associated with polysaccharides such as Ulvan were also detected in the land-based cultivated biomass. Health-promoting compounds such as the carotenoids lutein, violaxanthin, ß-Carotene were measured on levels comparable to spinach. As apart from abiotic factors (e.g., cultivation media, settlement substrates), direct interactions with the associated microbiome is known to affect the phytomorphology of *Ulva*, (e.g. phytohormone-like compound thallusin produced by the bacterium *Maribacter* sp), samples for microbiome analyses were frequently taken and thallusin content will be measured in the cultivation units at experimental end.

## Leibniz Drug of the Year 2025

# Discovery of a highly selective molecule acting against resistant (triple-negative) breast cancer, and characterization of its auto-activating prodrug mode of action

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Selective and efficient targeting of cancer cells remains a major challenge in anticancer drug development. In this study, we identified and characterized SelectAHRyl A as a highly selective prodrug activated in certain cancer cells, especially in TNBC but also liver cancer, for instance. Through a series of biochemical and cell-based assays, we elucidated its mode of action, revealing activation via the aryl hydrocarbon receptor (AHR) pathway. Upon AHR-mediated metabolism, SelectAHRyl A is converted into SelectAHRyl B, a hydroxylated and cytotoxic derivative that remains trapped within cancer cells. This selective auto-activation mechanism not only enhances tumor specificity but also minimizes off-target effects. Furthermore, SelectAHRyl B inhibited a family of crucial enzymes rather than a single target, reducing the likelihood of resistance development. These findings position SelectAHRyl A as a promising therapeutic candidate for AHR-expressing cancers.

## Research Award 2025

# New cytokinin biostimulants for agriculture and horticulture

Miroslav Strnad*, Doležal K., Koprna R., Plíhalová L., Zatloukal M., Nisler J., Novák O.

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Alternate wetting (irrigation) of plants with phytohormone solutions can improve yield, and is correlated with altered phytohormone (including cytokinins) metabolism and signaling. The main aim of the development was to explain molecular mechanism of function of novel cytokinin prodrug(s) on plant development. Precise quantification of cytokinin content and profiling of genes involved in cytokinin metabolism and perception in treated plants revealed several aspects of different action of m-methoxytopolin base and its substituted derivatives on plant development. In contrast to standard cytokinins. N9-tetrahydropyranyl derivative of *meta*-topolin and its methoxy-counterpart showed the negative effects on root development only at three orders of magnitude higher concentrations. Moreover, the methoxy-derivative demonstrates a positive effect on lateral root branching and leaf emerging in a nanomolar range of concentrations, in comparison with untreated plants. Tetrahydropyranyl substitution at N9-position of cytokinin purine ring significantly enhances acropetal transport of a given cytokinins. Together with the methoxy-substitution, impedes accumulation of non-active cytokinin glucoside forms in roots, allows gradual release of the active base, and has a significant effect on the distribution and amount of endogenous isoprenoid cytokinins in different plant tissues. The utilization of novel aromatic cytokinin derivatives can distinctively improve expected hormonal effects of plant production techniques in the future.

We also explored new cytokinin urea derivatives. The most potent compound named ASES (Anti-Senescence Substance, currently produced STATUS, U.K.), strongly inhibited dark-induced senescence in leaves of wheat and *Arabidopsis thaliana*. In vivo, ASES also improved the salt tolerance of young wheat plants. Interestingly, ASES did not affect root development of wheat and Arabidopsis, and molecular and classical cytokinin assays demonstrated that ASES exhibits very low cytokinin activity. A proteomic analysis of the ASES-treated leaves further revealed that the senescence-induced degradation of photosystem II had been very effectively blocked. Taken together, our results including data from cytokinin content analysis demonstrate that ASES delays leaf senescence by mechanism(s) different from those of cytokinins and, more effectively. It has also unique properties which can be effectively used in agriculture to increase stress tolerance.

# Advancing drug discovery: EU-OPENSCREEN's resources for bioactive compound screening

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EU-OPENSCREEN is a non-for-profit European research infrastructure for chemical biology with the aim to support chemical probe and early drug discovery projects. Our infrastructure unites over 30 scientific partners across 9 European countries, offering access to cutting-edge technologies, access to small molecule libraries, expertise, and technology platforms for the global Life Sciences community.

Discover in this presentation how EU-OPENSCREEN supports researchers screen the bioactives compound collection for their research, translating novel biological insights into potential drug candidates. We will provide examples of successful screening campaigns, outline key resources available to the scientific community, and discuss opportunities for collaboration. By lowering barriers to advanced screening technologies and promo□ng data-sharing, EU-OPENSCREEN enables researchers to accelerate their discovery efforts and contribute to the development of new bioactive compounds. We invite interested partners to explore how our resources and expertise can support their research goals.

# Antimicrobial potential of *Pimpinella saxifraga* seeds for controlling *Rhizoctonia*

Jasmin Tauchelt[1], Mohamed R. Abdelfadil[1], Melanie Hanser[1], Cheng Han Hsieh[1], Leonard L. Brunner[1], Sumin Han[1], Franziska S. Hanschen[1,2], and Katja Witzel[1]

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Wild plants have evolved defense mechanisms to adapt to their natural environments in the presence of diverse pathogens. These strategies involve the production of a complex volatile compounds and secondary metabolites, which serve as barriers against microbial attacks. Wild plant species extracts have previously demonstrated phytosanitary potential against plant pathogenic microbes. However, these plants have been rarely studied, particularly concerning their bioactive compounds and antimicrobial activities. In this study, we investigated the antimicrobial activity of crude extracts of *Pimpinella saxifraga* seeds from two different populations. The crude seed extracts from these two sources demonstrated significant antimicrobial activity against the pathogenic fungi *Rhizoctonia solani* in a direct bioassay, with differences observed among the germplasms. Additionally, seed meals with different concentrations derived from these two sources exhibited inhibitory activity against *R. solani* in a biofumigation assay. Metabolite analyses revealed significant variations in the profiles of antimicrobial compounds among these two seed sources. The current phase of this study evaluates the efficacy of these two seed meals in mitigating *R. solani* infection in lettuce in a pot experiment under controlled conditions. Different concentrations of seed meals will be incorporated into the growth substrate to assess their potential for *R. solani* control. The findings from this study contribute valuable information to the field of natural product research and to developing environmentally sustainable products for mitigating soil-borne plant pathogens.

# Activation of secondary metabolite biosynthetic gene clusters in *Streptomyces* spp. using SARP regulators

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The emergence of antibiotic resistance constitutes a significant and imminent global health crisis. New antimicrobial agents that are effective against antibiotic resistant pathogens are urgently needed to relieve the burden on the world's healthcare systems. Natural products (NPs) isolated from actinomycetes have historically played a key role in the development of pharmaceutical drug leads for the treatment of various infectious diseases, as well as found their application as herbicides, fungicides, and insecticides.[1] In particular, the genus *Streptomyces* is the primary source of antimicrobials, accounting for nearly two-thirds of all clinically used antibiotics[2]. On average, *Streptomyces* genomes harbour 20–30 NP biosynthetic gene clusters (BGCs)[3]. Typically, only a small subset of these BGCs is actively expressed under laboratory conditions, while the majority is either poorly expressed or remains silent due to the lack of activation signal(s). The biosynthesis of NPs is tightly controlled by a complex regulatory system involving both, global and pathway-specific regulators. The *Streptomyces* Antibiotic Regulatory Protein (SARP) family of regulators includes many pathway-specific regulators and some global regulators, which generally act as direct transcriptional activators of NP biosynthesis.[4] The expression level of SARP regulatory genes is a key factor that determines the production yield of many NPs in actinomycetes. In *Streptomyces pristinaespiralis*, the biosynthesis of pristinamycin is initiated by the SARP PapR2. Heterologous expression of *papR2* led to the activation of the silent undecylprodigiosin BGC in *Streptomyces lividans* and also induced the expression of a nucleoside antibiotic BGC in the novel Indonesian strain isolate *Streptomyces* sp. SHP 22-7.[5] In the current project, we employ a SARP-based activation strategy to enhance the expression of BGCs in a panel of poorly explored and phylogenetically unique actinomycetes from the DSMZ collection.[6] To achieve this, we combine bioinformatic approaches to pre-select the most suitable candidates, followed by the heterologous expression of SARP genes in the target strains. To date, we have generated more than sixty genetically modified strains harbouring the SARP overexpression constructs. Here, we present results from the SARP-guided activation strategy for the discovery of novel NPs from streptomycetes.

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# Revealing bioactive compounds in tissues with mass spectrometry imaging

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Detection and localization of bioactive compounds within tissues remains challenging. Often labeled compound is required for the ex vivo detection and quantitation of one or a few drug candidates. One potential alternative method that does not require labelling methods or use of derivatives of bioactive molecules is mass spectrometry imaging (MSI). In particular, matrix-assisted laser desorption/ionization (MALDI) MSI enables untargeted detection of hundreds endogenous and exogenous low-molecular weight biomolecules within tissues, including bioactive substances and their metabolites. For atmospheric-pressure MALDI MSI and tandem MSI (MS2I) experiments, matrix solutions were pneumatically sprayed with a SMALDIPrep sprayer (TransMIT GmbH, Giessen, Germany) or sublimated onto samples. MALDI MS2 / MS2I experiments were performed, employing an AP-SMALDI5 AF ion source (TransMIT GmbH, Giessen, Germany) coupled to a Q Exactive HF (Thermo Fisher Scientific GmbH, Bremen, Germany). In some experiments, a low temperature plasma (LTP) ion source was combined with our MALDI MSI source in order to boost overall signal intensities. In this contribution I will highlight the potential of MALDI-MSI for the detection and identification of bioactive compounds within tissues. Therefore, I will outline our endeavors to a) locally track exogenous administered biomolecules, b) reveal endogenous substance distributions with known bioactivity or compounds speculated to trigger immune responses, and c) improve the overall coverage of small molecular weight compounds in MALDI MSI experiments. By showcasing various case studies, I will describe how MALDI-MSI a) can reveal the distribution of known kinase inhibitors, explored for drug-repurposing within parasitic organisms, b.1) MALDIS-MSI is used to localize glycosphingolipids that are formed as a response to parasitic infection and hold the potential to be used to activate the immune system, b.2) MALDI-MSI tracks heart glycosides down to the single cell scale within butterfly larvae hinting towards a potential sequestration mechanism of this substance class, and c) how different phytochemicals can be visualized in plants using our prototype LTP-MALDI MSI setup. Finally, I will outline how MALDI-MSI can be used for the detection of bioactive compounds within cells and describe necessary steps required to achieve this goal routinely in the future.

# POSTER PRESENTATIONS

# *Identification and evaluation of anti-infective compounds in human breast milk*

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Respiratory syncytial virus (RSV) and *Staphylococcus aureus* (*S. aureus*)-induced sepsis represent significant global health burdens, particularly affecting vulnerable populations such as infants and the elderly. The urgent need for effective treatments and preventive strategies is underscored by the lack of accessible treatments for RSV and the rise of antimicrobial resistance.

Human breast milk is a key source of nutrition and immunity for newborns and infants. Its individual bioactive components are increasingly recognized for their protective effects against infections. Compounds such as human lactoferrin and human milk oligosaccharides (HMOs) exhibit potent antimicrobial, anti-inflammatory, and immunomodulatory properties both *in vitro* and *in vivo*. Emerging evidence suggests HMOs may inhibit pathogen attachment by serving as decoy receptors or altering receptor binding through structural changes.

This study investigates the potential of recombinantly produced human milk compounds to inhibit RSV and *S. aureus* infection *in vitro*. Preliminary results from the *in vitro* screening indicate that certain human milk compounds lead to a dose-dependent reduction in RSV replication, a slight decrease in *S. aureus* proliferation, and show trends of inhibiting biofilm formation.

These findings highlight the potential of these human milk compounds as promising therapeutic candidates. Future research of this project will focus on investigating the compounds' effects on host cell viability and elucidating their mode of action (MoA). Additionally, the impact of these compounds on *S. aureus* cell invasion and the bacteria's cytotoxic effects will be evaluated using live-cell imaging and flow cytometry. Compounds demonstrating significant potential will undergo further evaluation *in vivo* using mouse models of RSV and *S. aureus*-induced sepsis, providing critical validation of their efficacy under physiological conditions.

# Investigating of cultivable macroalgae and their associated fungi in land-based cultivation under different light regimes

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Macroalgae are considered as valuable sea-vegetables, which are increasingly consumed worldwide. Able to be grown without the requirement of arable land or valuable drinking water, controlled land-based cultivation of certain macroalgae is a promising approach for food production. As primary producers the chemistry and thus nutritional quality of cultivated macroalgae strongly depend on the different cultivation factors, including their light regime. Supporting a diverse and partly obligate microbiome, macroalgae provide a host for different biota, including endophytic fungi. Living in close association with their host, endophytic fungi can provide metabolites that are not only important for the nutrition of their host, but also of interest for various commercial applications. To study marine endophytic fungi in controlled macroalgal cultivation and investigate how light quality in respect of UVB radiation affect the host-fungal system and thus the nutritional quality of the harvested crop, different in vivo UVB exposure experiments were conducted with three different edible host macroalgae (*Ceramium virgatum*, *Ulva compressa* and *U. fenestrata*). Before and during the experimental exposure the host algae were screened for the presence of endophytic fungi. Two of the identified fungi grown in axenic culture and exposed on their own to the given experimental conditions. Growth and vitality of the host algae was documented over experiment and fungal presence were investigated via laser scanning microscopy (CLSM) at the end of study in the algal material. To identify potential changes in the nutritional profile samples of exposed algal biomass as well as isolated fungi were analysed for differences in their metabolic profile. Two different species of filamentous fungi were found: *Purpureocillium sp*. in *U. fenestrata* and *Aspergillus sp*. in *Ceramium* sp. Additional two yeasts were observed in *Ceramium* sp. and *U. compressa*. Some preliminary results show (i) presence of isolated fungi in host macroalgae, ii) differences in the colonization of fungi depending on host specifity, ii) differences in the response of host and isolated macroalgae towards UVB.

# Study on the diversity of endophytic algicolous fungi in cultivated marine macroalgae

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With the global need for alternative nutritional sources and growing interest in marine derived crops, the cultivation of macroalgae (sea vegetables) in coastal but also controlled land-based systems faces a growing interest. Known to be associated with a variety of microorganisms, macroalgae host complex interspecific relations, which can significantly affect the health and chemistry of the algal host. Here fungal endophytes show a high potential not only to affect the biochemistry of their host but to provide themselves with a valuable source of different bioactive substances. Yet, their presence in land-based macroalgal cultivation systems remains largely unexplored. In this context, the present work studies the diversity of fungal endophytes associated with different macroalgae cultivated under controlled laboratory conditions. For this purpose, samples were taken from the Chlorophytes *Ulva compressa*, *U. fenestrata* and the Rhodophyte *Acrochaetium sp.*, surface sterilized, placed onto potato dextrose agar plates supplemented with 2% artificial sea salt and kept under controlled laboratory conditions. A total of 32 distinct morphospecies were observed growing from the samples, that were then isolated and kept under controlled conditions. *Ulva compressa* and *Acrochaetium sp.* exhibited the highest diversity (12 morphospecies respectively), followed by *Ulva fenestrata* (8 morphospecies). Variations in fungal endophyte identity across species suggest potential differences in host specificity and environmental adaptability. This study highlights the potential of land-based macroalgal cultivation as a reservoir for fungal biodiversity, providing insights into host-endophyte interactions and their possible applications in biotechnology and aquaculture. The presented morphospecies serves as a valuable reference for future studies on fungal-macroalgal associations and their functional roles in marine and cultivated ecosystems.

# Land-based macroalgal cultivation: microalgal contamination or enrichment?

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With the recognized value of marine macroalgal biomass for several commercial applications, there is a growing interest in the use and cultivation of these sea vegetables. Overcoming natural fluctuations, by controlling the environmental conditions responsible for the growth and chemistry of the cultivated crops, controlled land-based cultivation is on the rise. In this context, the food4future project is developing innovative brine-based systems to enable macroalgal cultivation in urban areas. During several cultivation trials, the presence of non-targeted but persistent microalgae have been recognized. Based on the need to evaluate this biological factor and the need to either counteract or promote its appearance, the following study was initiated. For this purpose, microalgae, presumably *Bracteacoccus*-like strains, were isolated from the food4future cultivation systems at IGZ and cultured under controlled conditions. To study and optimize the growth conditions for both micro- and macroalgae, different commercial (e.g., Bold' basal media (BBM), Tropic marine, Algal fertilizer) and alternative (e.g., regional brine) media were applied. In a controlled in vitro study, the *Bracteacoccus*-like strains were exposed to cultivated macroalgae, e.g., *Ulva compressa*. The aim was to study the potential impact on the survival, growth and nutritional profile of the sea vegetable but also on the growth and metabolites of the accompanying microalgae, as *Bracteacoccus* species are known for their ability to produce bioactive compounds making them particularly relevant for biotechnological applications. Algae growth were assessed through multiple approaches, e.g., biomass weight (macroalgae), cell counts and optical density(microalgae), while microscopic analyses (e.g., LM, CLSM) supported the examination of cell morphology and interactions. For nutritional profile, bioactive compounds like chlorophylls and carotenoids were extracted using solvent-based methods and quantified via high-performance liquid chromatography (HPLC) to determine potential changes in pigment composition resulting from co-cultivation. The present study aims to clarify whether and to what extent the aimed co-cultivations affect the growth and nutrional profile of the involved algal species. The outcome will provide an insight in the biology and (allelo)chemistry of the two targeted taxa and shall support the further development of the envisaged urban algal cultivation approaches for healthy bioactive compounds.

# Identification of antimicrobial epoxy-phenylpropanoids isolated from burnet-saxifrage (*Pimpinella saxifraga*)

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Burnet saxifrage (*Pimpinella saxifraga*) is a perennial umbellifer with antimicrobial and antioxidant effects reported from its seeds and essential oil [1]. This herbaceous plant also has been traditionally applied in folk medicine to treat respiratory and digestive indications with its expectorant and anti-inflammatory effects [2]. We aimed not only to identify the chemical constitution that contributes to the antimicrobial potential of burnet saxifrage, but also to further specify the distribution of the bioactive phytochemicals in different plant organs for the future agricultural and pharmaceutical applications. The bioactivity-driven approach was applied on isolation of burnet saxifrage plant secondary metabolites. The ethanolic and watery extracts from various plant tissues were screened and selected for further purification based on the results of antimicrobial assays against phytopathogenic bacterium *Xanthomonas campestris* and fungus *Rhizoctonia solani*. The antifungal ethanolic seed and root extracts were fractionated by solid-phase extraction (SPE). Candidate compounds were further purified via preparative RP-HPLC and were selected based on their antimicrobial efficacy. 1D and 2D NMR spectroscopy revealed the epoxide-bearing structures of purified phenylpropanoids. The exact molecular mass of the antifungal expoyphenylpropanoids was confirmed by high resolution mass spectrometry (HR-MS). Quantitative analysis parameters of the bioactive index phenylpropanoid compounds were also developed and optimized for plant quality control and future studies.

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# Drought stress and oenothein B accumulation in *Oenothera biennis*: adaptive responses and antioxidant potential

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Wild plant species are a valuable source for natural products with chemopreventive activities. Oenothein B, a phenolic compound found in Oenothera and other species, has been extensively studied for its antioxidative, antitumor, immunomodulatory, and anti-inflammatory properties. However, the effects of abiotic stress factors, especially drought, on the accumulation of Oenothein B in plant tissues have been studied only to a limited extent. To fill this knowledge gap, we characterized the antioxidant potential of Oenothein B extracted from field-grown *Oenothera biennis* in different plant organs as well as during the vegetation period.

Furthermore, we conducted a controlled drought stress experiment on *O. biennis* to investigate the physiological responses, antioxidant potential and tissue-specific accumulation of Oenothein B under varying water availability. Our results demonstrate that *O. biennis* exhibits remarkable tolerance to drought conditions, maintaining survival and physiological activity even at significantly reduced (30%) soil moisture levels. Interestingly, we observed a differential response in Oenothein B accumulation across plant organs. While the roots of *O. biennis* displayed a significant decline in Oenothein B levels under drought stress, the shoots maintained consistent concentrations of this phenolic compound, irrespective of water availability. This finding suggests a possible tissue-specific regulation of Oenothein B biosynthesis and allocation, potentially linked to distinct protective roles in roots and shoots.

The study contributes to a better understanding of the adaptive mechanisms employed by *O. biennis* under drought stress and highlight the dynamic regulation of bioactive secondary metabolites in response to environmental challenges.

# *Arabidopsis thaliana vtc2-1* mutant reduces aphid survival via metabolic shifts

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Vitamin C serves multiple roles in plants, functioning as an antioxidant and a modulator of stress response. The vitamin C deficient *vtc2-1* mutant of *Arabidopsis thaliana* has been shown to exhibit resistance to the generalist aphid *Myzus persicae*. Here, we investigated its impact on the specialist aphid *Brevicoryne brassicae* and explored potential underlying mechanisms on how reduced vitamin C levels may affect aphid performance. Our findings demonstrated that *vtc2-1* plants similarly impaired the survival of *B. brassicae*. Untargeted metabolic profiling revealed significant changes in both primary and secondary metabolites in the *vtc2-1* mutant compared to the wild type, Col-0. These metabolic differences were further enhanced by aphid infestation. Specifically, *vtc2-1* exhibited elevated sugar levels. This can be linked to upregulation of upstream genes in the vitamin C biosynthesis pathway, which overlap with processes involved in polysaccharide synthesis. In addition, an increase in phenylpropanoids, particularly sinapic acid was associated to reduced aphid survival. We propose that the enhanced sugar flux in *vtc2-1* has a potential to induce osmoregulatory stress in aphids, exacerbating the effects of heightened defense metabolites. Together, the herewith described metabolic alterations could provide a mechanistic basis for the increased aphid resistance observed in the *vtc2-1* mutant.

# Identifying a candidate for isothiocyanate hydrolase activity in kohlrabi and Brussels sprouts

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Brassicaceae vegetables are a primary dietary source for isothiocyanates (ITC), which are known to have health-promoting effects, e.g., antimicrobial, anti-inflammatory and chemopreventive properties.[1] However, ITC are highly reactive due to the electrophilic carbon atom and can therefore react with nucleophiles, resulting in the loss of their bioactivity.[2] Besides this non-enzymatic degradation of ITC, recent studies concluded that a uncharacterized enzymatic pathway is responsible for the conversion to amines in *Brassica oleracea*.[3,4] Thus, the identification of the postulated ITC degrading enzyme (ITCase) is important to understand the role of the glucosinolate metabolism pathway as well as in the context of ITC-related health effects. The ITCase activity was studied in four different ontogenetic stages of kohlrabi tissues (seed, day 5, day 9 and day 42) by adding 3-butenyl ITC and quantifying the corresponding amine after 1 h at room temperature. Also, a proteomic approach using nanoLC-HRMS was used to study protein abundance patterns. The ITCase activity was correlated with the protein abundances for the ontogenic stages and tissues to identify possible candidates for the putative ITCase. Also, freeze-dried Brussels sprouts were used for protein isolation. A protein extract was used in different protein purification approaches, e.g., ammonium sulfate precipitation and preparative ion-exchange chromatography. The enzymatic activity of isolated fractions was tested, and proteins were identified as described above. In the ontogeny experiment, enzymatic activity was high in seeds and in the whole above-ground plant at days 5 and 9. However, by day 42, enzymatic activity shifted: high enzymatic activity was observed in the roots, while amine formation in above-ground tissues decreased. Correlating protein abundances with enzyme assay data identified six candidate proteins, all of which exhibited high abundance in tissues with elevated enzymatic activity. Comparable enzyme activity was observed in ammonium sulfate fractions precipitated at 20-30% and 30-40% saturation at 0 °C, suggesting that the ITCase likely precipitates within these saturation ranges. For preparative ion-exchange chromatography, only anion-exchange chromatography successfully fractionated active enzymatic fractions. However, the combination of ammonium sulfate precipitation and anion-exchange chromatography was not successful. Our results suggest a strong candidate for ITCase activity that was consistently detected by both purification approaches. This protein is likely responsible for ITC degradation to amines, thereby enabling subsequent functional validation.

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Funding: Funded by the German Research Foundation (DFG) – 524096542.

# Isolation of new bacteriophages against rare actinomycetes with a focus on vB_AmeS_Stercus, an *Actinomadura* phage

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Actinomycetes are a heterogenous group of Gram-positive bacteria belonging to the phylum Actinomycetota. They constitute one of the largest bacterial phyla and are common in terrestrial and aquatic ecosystems, mainly in soil. They are also well-known as producers of natural compounds. The well-known genus *Streptomyces* is a major source of novel antibiotics, insecticides and other secondary metabolites that could be useful in medicine, biotechnology and agriculture, making them valuable bacteria for research and applications [1]. In addition, rare actinomycetes such as *Kitasatospora*, *Actinomadura*, *Kibdelosporangium* or *Lentzea* are increasingly considered to be another source of novel secondary metabolites. However, the phylum also includes pathogenic species, and rare actinomycetes are no exception. Some species can cause disease in humans and animals, such as *Nocardia brasiliensis* or *Actinomadura madurae*, which causes nocardiosis and mycetoma (e.g. Madura foot) [2]. The use of bacteriophages could be one approach to control these pathogens. Our aim was to isolate new bacteriophages against rare actinomycetes from the DSMZ collection as an alternative control agent against infections with these bacteria. In total, a set of 22 phages was isolated against seven different strains of actinomycetes. In particular phage vB_AmeS_Stercus against *Actinomadura meyerae*, was characterized more thoroughly. Its characterisation revealed a narrow host range, genomic analysis identified genes for an integrase and a transcriptional repressor, pointing to a temperate phage. Further experiments are in progress to express the endolysin of vB_AmeS_Stercus as an alternative to control pathogenic *Actinomadura* species such as *Actinomadura madurae* or *Actinomadura latina*.

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# Optimizing the bioactive profile of edible macroalgae in controlled inland cultivation: adapting protocols for fatty acids

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With an increasing world population and decreasing availability of arable land, there is a dire need for the availability of sustainable healthy food sources. Providing a valuable nutritional profile of healthy bioactive compounds and being able to be cultivated without extensive use of fresh water, macroalgae offers great potential as a healthy sea vegetable. Besides finding "new" food sources, new cultivation methods have to be developed to allow a sustainable production of the required saline crop. To overcome the increasing issues of environmental pollution (e.g., heavy metals, iodine) and allow a stable production, controlled land-based cultivation provide a promising approach for the production of high qualitative and healthy biomass. With targeted cultivation, adjusting environmental parameters, the change of the bioactive compounds and therefore nutritional profile of the macroalgae, can be controlled. In this context the food4future project developed an innovative brine-based cultivation approach, which foster the use of regional brine sources, instead of commercial sea salts as cultivation media. Moreover, the project aims to interconnect the side streams of the envisaged alternative food sources (macroalgae, halophytes, crickets, jellyfish) to foster a sustainable use for integrative inland cultivation. The main aim is the provision of fresh and healthy food in the urban realm. Apart from developing a suitable cultivation approach, there is also the urgent need to adapt the analytical protocols for the investigation and control of the produced algal biomass. With the aim to optimize the bioactive profile of the produced biomass, e.g., content of polyunsaturated fatty acids (PUFA), we approach in the present study the analytical methods for their determination. PUFAs are very susceptible to thermal and chemical oxidation as well as isomerization. Therefore, the effects of the extraction and especially derivatization methods, turning fatty acids into fatty acid methyl esters (FAMEs) for analysis in GC/MS, have to be looked upon. For this, we alter heating temperatures, reaction times and chemicals using a standardized lipid sample with a known fatty acid composition containing PUFAs also to be found in alga tissue. The method delivering the closest results to the original composition will them be used to track the effects of changing environmental variables on the nutritional value of the algae.