9th

SYMPOSIUM

on

MICROALGAE AND SEAWEED PRODUCTS IN PLANT/SOIL-SYSTEMS

25-26 June 2019
Mosonmagyaróvár – Hungary

Book of Abstracts

Organiser

Department of Plant Sciences
Department of Food Science
Faculty of Agricultural & Food Sciences
Széchenyi István University
Mosonmagyaróvár – Hungary

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Széchenyi István University

Participants of the 8th Symposium held in 2017

Editors:

Prof. Dr. Vince Ördög

&

Dr. Zoltán Molnár

Scientific Programme

SCIENTIFIC PROGRAMME

MONDAY, JUNE 24TH

16:00-20:00 **Registration and Poster Mounting** (at the Venue of the Symposium, Lucsony Str. 2., H-9200 Mosonmagyaróvár)

19:00 **Welcome – Dinner** (at the Venue of the Symposium)

TUESDAY, JUNE 25TH

8:00-10:00 **Registration and Poster Mounting**

Opening, General Introduction

10:00-10:20 *Ördög V.* (Mosonmagyaróvár)
Greeting of Participants and
Opening the 9th Symposium on Microalgae and Seaweed Products

10:20-10:50 *Molina, E.* (Spain)
Microalgae biotechnology – past present and future (tentative)

10:50-11:20 *Tőkés G.* (Budapest)
New situation in the authorization of yield enhancers in the EU

11:20-11:30 **Coffee Break**

Session 1: New Tools for Microalgae Biotechnology

Chairperson: *Strnad, M.* (Czech Republic)
Co-Chair: *Balázs E.* (Martonvásár)

Plenary Lectures

11:30-12:00 *Strnad, M.* (Czech Republic)
Plant and algae hormonomic – profiling on tissue and cellular level

12:00-12:30 *Maróti G.* (Szeged)
Exploitation of algal-bacterial consortia in combined resource recovery and biohydrogen generation

12:30-13:10 **Buffet Lunch** (snacks, sandwiches, refreshments)

Oral Presentations

13:10-13:30 *Hódi B.* (Szeged)
Effect of singlet oxygen on gene expression profile in *Synechocystis* PCC6803

13:30-13:50 *Patil, P.* (Szeged)
Establishment of a simple screening system for photosynthetic traits of microalgae and cyanobacteria

13:50-14:10 *Patyi G.* (Szeged)
Assessment of intracellular singlet oxygen by GFP fluorescence in *Synechocystis* PCC 6803

14:10-14:30 *Szabó M.* (Szeged)
Algal phenotyping – agricultural and industrial applications

14:30-14:50 **Coffee Break**

Session 2: Plant Bio-stimulants

Chairperson: *Van Staden, J.* (South Africa)
Co-Chair: *Ördög V.* (Mosonmagyaróvár)

Plenary Lectures

14:50-15:20 *Van Staden, J.* (South Africa)
Role of smoke- and seaweed-derived compounds in improving growth and biochemical composition of spinach and cabbage crops

15:20-15:50 *Mógor, Á.F.* (Brasil)
Microalgae as biostimulant: field results and biochemical approach

15:50-16:50 **Round Table Discussion and Poster Session**

18:00-18:30 **Folklore Show** (at the Venue of the Symposium)

18:30-(22:00) **Dinner** (at the Venue of the Symposium)
Sorry to inform that alcoholic beverages will not be served free of charge

WEDNESDAY, JUNE 26TH

8:00-9:00 **Poster Session**

Session 2: Plant Bio-stimulants (continuation)

Chairperson: *Van Staden, J.* (South Africa)
Co-Chair: *Ördög V.* (Mosonmagyaróvár)

Oral Presentations

9:00-9:20 *Bojtor Cs.* (Debrecen)
Application of microalgae-containing bio-fertilizer in maize

9:20-9:40 *Horváth N.* (Mosonmagyaróvár)
Extracellular polysaccharides in twenty *Chlamydomonas* strains of the Mosonmagyaróvár Algal Culture Collection

9:40-10:00 *Ravirajan, M.* (India)
Seaweed bio-stimulants for plant growth

10:00-10:20 *Takács G.* (Mosonmagyaróvár)
Plant biostimulating effects of the green alga *Tetracystis* sp. on winter wheat in field experiments

10:20-10:40 *Tóth J.* (Mosonmagyaróvár)
Effect of *Arthrospira platensis* on the growth and condition of nursery plants

10:40-11:00 **Coffee Break**

Session 3: Antimicrobial Compounds and Bio-pesticides from Microalgae

Chairperson: *Acien, G.* (Spain)
Co-Chair: *Molnár Z.* (Mosonmagyaróvár)

Plenary Lectures

11:00-11:30 *Ördög V.* (Mosonmagyaróvár)
Microalgae against necrotroph and biotroph plant fungal pathogens

11:30-12:00 *Fu, P.* (China)
Microalgae for heavy metal removal

Oral Presentations

12:00-12:20 *Horváth Á.* (Budapest)
In vitro antifungal activity of selected freshwater and marine cyanobacteria strains

12:20-12:40 *Malec, P.* (Poland)
Myxoxanthophylls – carotenoid glycosides from cyanobacteria and their specific optical and antioxidant properties

12:40-13:20 **Buffet Lunch** (snacks, sandwiches, refreshments)

Session 4: Photobioreactors for Agricultural Purposes: Pilot and Large-scale Construction and Operation

Chairperson: *Masojidek, J.* (Czech Republic)
Co-Chair: *Maróti G.* (Szeged)

Plenary Lectures

13:20-13:50 *Acien, F.G.* (Spain)
Challenges in the production of microalgae in large scale

13:50-14:20 *Masojidek, J.* (Czech Republic)
Outdoor pilot-scale cultivation of selected microalgae in Třeboň:
thin-layer cascade vs. raceway pond

Oral Presentations

14:20-14:45 *Branyikova, I.* (Czech Republic)
Electrocoagulation – a smart way to minimize microalgae harvesting cost

14:40-15:00 *Kanna, S.D.* (Hungary)
Salt stress adaptation of biotechnologically important microalgal strains

15:00-15:30 *Ördög V.* (Mosonmagyaróvár)
Molina, E. (Spain)
Round Table Discussion
Closing of the 9th Symposium on Microalgae and Seaweed Products

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Lectures & Oral Presentations

Microalgae biotechnology – past present and future

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New situation in the authorization of yield enhancers in the EU

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Products made of algae can be used for yield enhancement or plant protection. The registration process and conditions depend on the categorization of the product. Yield enhancers are regulated by national rules, in Hungary by the decree 36/2006 FVM. Biostimulants (as we call plant conditioners) are belonging to this group. The requirements for authorization in the EU are differing very much among the member states. The European Commission decided to create a new system for yield enhancers, that would allow to trade these products without authorization, by a special preliminary investigation process.

After many discussion rounds, the European Parliament accepted the modified proposal on 27th March, that was confirmed by the Council on 21 May 2019. The new regulation is published on 25 June and to be applied after 3 years (from July 2022).

The background idea behind the regulation is the principle of circular economy. It is a system aimed at minimizing waste and making the most of resources. In a circular system resource input and waste, emission, and energy leakage are minimized by slowing, closing, and narrowing energy and material loops.

According to the new regulation, national authorizations will be substituted by a standardization method. All products and all component materials to be marketed will be put to categories, and investigated by 'notified bodies'. If the product fulfills the requirements, it can be marketed in all EU member states without further authorization process. National authorization can be applied on voluntary basis, but it can not be obligatory. Approximately 80-90% of the present products will not need authorization in the future.

It will make easy to put products onto the market, nevertheless such system can cause serious consumer protection problems and can undermine the status of good quality products.

Algae based products as biostimulants can be marketed in the EU according to the new regulation from 2022. On the other hand, products with plant protection effect or declared hormonal properties fall under the 1107/2009 EC regulation and need very complicated dossier.

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Plant and algae hormonomic - profiling on tissue and cellular level

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Phytohormones are highly physiologically important signalling molecules divided into several structurally different groups, such as purine and indole derivatives, plant steroids, lipid-based substances and terpenoid carboxylic acids. They play a crucial role in many physiological and developmental processes at the levels of individual plant components (cells, tissues, and organs) and by coordinating activities across these parts. High-resolution measurements of intracellular CKs in different plant tissues can therefore provide insights into their metabolism and mode of action. In the last decade, procedures described for phytohormone analysis as well as the available hardware have improved substantially, resulting in rapid, efficient, and highly sensitive methods that allow the analysis and identification of different phytohormones starting from a minimal amount of plant tissue often as little as 10 – 50 mg. The above mentioned approaches have also been used for detailed analyses of phytohormone metabolites in (micro)algae. In conclusion, there are several excellent procedures available for analysis of plant hormones, which we hope, will be further improved by introduction of very sophisticated methods preferentially based on combination of immunoaffinity chromatography and LC-MS. We have also recently developed fluorescence-activated cell sorting of green fluorescent protein (GFP)-marked cell types, combined with solid-phase microextraction and an ultra-high-sensitivity mass spectrometry (MS) method for analysis of cytokinin (CK) biosynthesis and homeostasis at cellular resolution. Application of targeted metabolomics shows an optimal method for phytohormonal screening. This method was validated by series of control experiments, establishing that protoplast isolation and cell sorting procedures did not greatly alter endogenous CK levels. The MS-based method facilitated the quantification of all the well-known CK isoprenoid metabolites in four different transgenic Arabidopsis thaliana lines expressing GFP in specific cell populations within the primary root apex. Our results revealed the presence of a CK gradient within the Arabidopsis root tip, with a concentration maximum in the lateral root cap, columella, columella initials, and quiescent centre cells. This distribution, when compared with previously published auxin gradients, implies that the well-known antagonistic interactions between the two hormone groups are cell type specific.

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Exploitation of algal-bacterial consortia in combined resource recovery and biohydrogen generation

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Microalgae cultivation in municipal, industrial and agricultural wastewater is an emerging, highly effective approach for resource recovery and concomitant bioenergy generation. Wastewater effluents represent ideal sources of nutrients (especially nitrogen and phosphorous) for eukaryotic green algal species. However, the recovery performance of photosynthetic green algae is strongly dependent on the associated bacterial partners present in the effluents. Algal microbiome is a pivotal part of the algae holobiont and has a key role in modulating algal growth and functions in nature. There has been no comprehensive study on the importance of microbial communities supporting the algal hosts for the bulk of the time wastewater treatment methods have been in use. Our proposed approach applies a green microalgae-based photoheterotrophic degradation using dark fermentation effluent as substrate. The results showed that condition-dependent mutualistic relationships between the microbial and Chlorella algae populations had direct impact on the biodegradation efficiency and also on algal biohydrogen production. The genome level analysis of the novel hybrid biodegradation system provided important clues for the primary importance of the green algae partner in nitrogen and phosphorous removal. With further development and optimization this new approach can lead to a highly efficient simultaneous organic waste mitigation and renewable energy production technology.

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Effect of singlet oxygen on gene expression profile in *Synechocystis* PCC6803

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$^1O_2$ is one of the most harmful type of ROS, which is produced as a byproduct during photosynthesis. Under high light conditions triplet chlorophyll is formed, which is able to interact with molecular oxygen and gives them energy to form singlet oxygen.

The intracellular effects of $^1O_2$ is very well known, it causes DNA damages, lipid peroxidation and inhibits the function of PSII in photosynthetic microorganisms through D1 protein degradation.

Nowadays it is proved that singlet oxygen has an effect on gene expression and it might have a signal transduction pathway. In plants and higher eukaryotic organisms genes that are involved in singlet oxygen generated intracellular signal transduction pathway have been identified, but are not known in cyanobacteria.

We aimed to find and identify the elements of singlet oxygen specific signal transduction pathway. To generate intracellular singlet oxygen, we used photosensitizer dyes. We treated the wild type *Synechocystis PCC6803* cells and carried out a whole transcriptome analysis to find genes with increased expression level. Hundreds of genes showed some changes in their expression. Based upon the extent and specificity of the overexpression, we selected two genes, constructed insertion mutants and analysed the gene expression profiles of the latter ones as well. We examined the morphological, biophysical properties, gene expression and the pigment composition of the strains under high light and singlet oxygen stress conditions.

We observed that the singlet oxygen mediated overexpression rate of several genes was different in the mutant from the wild type. It proves that these two genes take part in the singlet oxygen transduction pathway. The exact roles of the genes/proteins are not known yet and are under further investigation.

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Establishment of a simple screening system for photosynthetic traits of microalgae and cyanobacteria

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Microalgae and cyanobacteria are considered as important model organisms to investigate the biology of photosynthesis, moreover, they are valuable sources of biomolecules for several biotechnological applications. Understanding the species-specific traits of photosynthetic electron transport is extremely important, because it contributes to the regulation of ATP/NADPH ratio, which has direct/indirect links to carbon fixation and other metabolic pathways and thus overall growth and biomass production.

In this project, we investigated the photosynthetic efficiency and operations of photosynthetic electron transport pathways in cyanobacteria and microalgae undergoing inorganic carbon limitation. We developed a noninvasive controlled setup, in which combination of measurements for photosynthetic processes, such as electron transfer kinetics measured by chlorophyll and NADPH fluorescence, plastoquinone pool redox state by flash induced fluorescent decay kinetics and OJIP transient curve, along with other physico-chemical parameters, dissolved oxygen and pH, could be performed without disturbing the ongoing inorganic carbon status of the sample.

Our data showed that photosynthetic activity (measured by effective quantum yield of PSII and oxygen evolution) and NADPH uptake in the light (indicative of the operation of Calvin-Benson cycle) was down-regulated in Ci deplete phase, but it was largely regained after addition of $NaHCO_3$. Cyclic electron flow was indicated by post-illumination chlorophyll fluorescence rise when the PSII activity was downregulated during Ci limitation, species-specific chlorophyll fluorescence pattern was observed due to different alternative electron flow pathways. Flash-induced fluorescence decay and polyphasic OJIP transients showed highly reduced plastoquinone pool in the carbon deplete stage.

We propose that the fluorescence data reveal the species specific changes of photosynthetic electron transport and function at different stages onset of inorganic carbon limitation, which could be used as photophysiological marker in different species and mutants of cyanobacteria and microalgae.

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Assessment of intracellular singlet oxygen by GFP fluorescence in *Synechocystis* PCC6803

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Singlet oxygen ($^1O_2$) is a very important reactive oxygen species (ROS), it can damage a wide range of macromolecules, like lipids, carotenoids and proteins. Its formation takes place in the second photosynthetic reaction centre (PSII), during the photosynthetic reactions. In high light condition the generated triplet state chlorophyll can interact with the molecular oxygen leading to $^1O_2$ formation via energy transfer.

This highly reactive ROS, besides its degradation effects can take part in signal transduction mechanisms and other intracellular reactions. This importance is the reason why we investigate the $^1O_2$ intracellular mechanisms. There are various $^1O_2$ detection methods, such as His mediated O_2 uptake, which allows to calculate the rate of $^1O_2$ generation by the rate of O_2 consumption. However, still there is a lack in detection methods, that could be used to detect the spatial distribution of $^1O_2$ generation inside intact cyanobacterial cells.

The Green Fluorescent Protein (GFP) is a very commonly used reporter protein in biological research nowadays. $^1O_2$ can damage this protein, hence quenching its fluorescence. We treated GFP producing *Synechocystis* PCC6803 cyanobacterial mutant cells with the $^1O_2$ sensitizer Rose bengal (Rb) and Methylene blue (Mb) dyes under high light conditions. We observed that the GFP fluorescence decreased suggesting that $^1O_2$ mediated degradation of GFP can be utilized for *in vivo* $^1O_2$ detection.

We investigated the specificity and sensitivity of the quenching reaction and established experimental parameters for a widely applicable *in vivo* $^1O_2$ assessment method protocol.

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Algal phenotyping - agricultural and industrial applications

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A rapidly emerging field in plant and agricultural sciences is precision phenotyping or plant phenomics, which utilizes non-invasive, high throughput measuring and data recording and systems that monitor growth and environmental responses of plants. Despite the rapid development of plant phenomics in the past decade, this approach is far less advanced and utilized in case of microalgae, which therefore represents a significant basic and applied research potential to address. Until now, studies that link light energy utilization and metabolism are only available in a few model algal species, such as the green alga *Chlamydomonas reinhardtii*, but these connections are largely unknown in species that have high relevance in industrial applications.

Phenomics requires approaches that are able to identify characteristic phenotypes of photosynthetic processes of microalgae using bio-optic sensors in a matrix array to allow screening for light use efficiency, growth and metabolic status under dynamic and fluctuating environments, e.g. under field conditions. Chlorophyll fluorescence is a widely applied non-invasive biophysical tool to investigate photosynthetic efficiency of plants, microalgae and cyanobacteria. Besides the commonly applied parameters of chlorophyll fluorescence, other non-invasive biophysical methods such as transient absorbance changes in the near-infrared region have a great potential for investigating bottlenecks and potential regulatory mechanisms of electron transport circuits that may have implications in primary productivity, such as the operation of Photosystem I reaction center, acceptor and donor side limitation, and kinetics of NAD(P)H production and consumption e.g. via Calvin-Benson cycle. However, these methods are seldom applied for algal screening, usually due to their generally lower sensitivity and limitations in geometry.

The presentation aims to give an overview about the current progress in the field of algal phenotyping, and a simple microwell plate method that is suitable for monitoring the growth and general photosynthetic capacity of microalgae and cyanobacteria is presented. This platform allows a semi-high throughput characterization of regulatory mechanisms of the photosynthetic electron transport processes. Advantages, limitations and potential applications of the matrix array-based screening platform for microalgae that are relevant in agricultural and industrial applications are discussed.

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Role of smoke- and seaweed-derived compounds in improving growth and biochemical composition of spinach and cabbage crops

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Consumption of leafy and cruciferous vegetables has increased four to five times since 1972 as consumers have recognized the value of vitamins and minerals present in these vegetables. Substantial quantities of chemical fertilizers, herbicides and pesticides are used to meet the increasing market demand for vegetables. This may lead to the accumulation of chemical residues in crops. Thus, growers are opting for alternative plant growth supplements which are organically enriched, affordable and eco-friendly. Natural based biostimulants are therefore becoming popular in agriculture. The bioactive molecules karrikinolide (KAR_1) and eckol, derived from smoke and seaweed *Ecklonia maxima* respectively, have recently demonstrated growth-promoting effects on several important crops. These biomolecules along with crude extracts of smoke-water and *Ecklonia maxima* (Kelpak®) were tested on spinach and cabbage crops. Foliar application of eckol at 10^{-6} M concentration showed a significant increase in growth and biochemical parameters compared to the control both in cabbage and spinach. Alpha-amylase activity, photosynthetic pigments and protein content of spinach leaves were enhanced by smoke-water and eckol treatments. Endogenous c*is*-Zeatin, dihydrozeatin and isopentenyladenine type of cytokinins were promoted by both the plant biostimulants. Sinapic acid, which is known for its antioxidant, antimicrobial, anti-inflammatory, anticancer and anti-anxiety activities, was higher in the biostimulant-treated spinach plants. Growth-promoting and insect-repelling effects were observed in cabbage plants treated with Eckol.

Microalgae as biostimulant: field results and biochemical approach

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The search for new natural sources for plant growth promotion and yield gains can contribute to ensuring safe and sustainable vegetable production. The potential of microalgae as a plant biostimulant source has been investigated in recent years. The plant growth promotion effect of some microalgae species is related to a range of bioactive compounds released by these organisms, such as hormones, polyamines, and amino acids that are able to act on the plant metabolism. At a step-by-step approach, to identify microalgae bioactivity the *Vigna radiata* model plant was used for rooting bioassay, the selected strains were applied by foliar sprays to seedlings, to plants on pot trials in greenhouses, and in the field on organically grown vegetables. The results indicated that the foliar sprays of *Scenedesmus subspicatus* lyophilized biomass improved tomato yield as a consequence of greater leaf expansion and improvement on free amino acids on leaves and fruits, indicating interaction with nitrogen metabolism. Likewise the *Arthrospira platensis* lyophilized biomass foliar sprays improved red beet yield gains also related to increase in amino acid contents in plants. The lettuce growth and yield were also improved by foliar sprays of hydrolyzed *A. platensis* biomass related to nitrogen compounds, the polyamines, molecules derived from amino acid ornithine. The hydrolysis improved spermine concentration in cyanobacteria biomass, and its application improved spermine concentration in lettuce leaves, suggesting that this polyamine could be a metabolic indicator and also a bioactive compound. Moreover, the release of free amino acid by *A. platensis* biomass was extraordinarily improved by hydrolysis, depending on the enzyme and the reaction time. These results allow us to suggest that the free amino acids or related compounds released by lyophilization or hydrolysis, are active ingredients of some microalgae strains, improving the plants endogenous synthesis of compounds of amino acid metabolism, and as a consequence, improving plant growth and yield.

Application of microalgae-containing bio-fertilizer in maize

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In sustainable agriculture, the role of biological products is gradually increasing, replacing currently used chemicals. An important group of biological substances are algae-containing biostimulants that can directly affect metabolism processes of the plant. The purpose of their application is to improve the efficiency of nutrient uptake for achieving better yield quality and quantity (Garcia-Gonzalez and Sommerfeld, 2016). Algae are primarily valuable for agriculture because of their plant growth regulating and plant protection materials (Ördög, 2015). Also dry green algae contain high percentage of macronutrients, considerable amounts of micronutrients and amino acids (El-Fouly et al., 1992).

The objective of our trial was to examine the effect of a *Chlorella vulgaris* microalgae-containing complex bio-fertilizer with optimal micro and macronutrient supply on yield quantity and on the nutrient status of the plants with different nitrogen levels. The experiment was carried out at the Látókép Experimental Station of the University of Debrecen in the scope of a long-term experiment with a maize hybrid (FAO 490) as test crop. In the long-term field experiment, started in 1983, 6 different nitrogen levels were applied (Control: 0 kg*ha^{-1}, for 35 years, 1st: 60 kg*ha^{-1}, 2nd: 120 kg*ha^{-1}, 3rd: 180 kg*ha^{-1}, 4th: 240 kg*ha^{-1} and 5th: 300 kg*ha^{-1}), with 4 randomized repetitions of each one. Total algae content of the applied product was > 3*10^{7} (pcs.*ml^{-1}) and it was discharged at a dose of 5l*ha^{-1}, with 400 l*ha^{-1} of water at the 8-leaf stage of the plants. The amount of micro and macro nutrients in the applied microalgae-containing product were the following: 150550 mg*l^{-1} N; 1400 mg*l^{-1} P_2O_5, 38645 mg*l^{-1} K_2O; 5170 mg*l^{-1} MgO; 5259 mg*l^{-1} S; 745 mg*l^{-1} CaO; 3790 mg*l^{-1} Fe; 2550 mg*l^{-1} Mn; 1520 mg*l^{-1} Cu; 1945 mg*l^{-1} Zn; 2550 mg*l^{-1} B; 125,004 mg*l^{-1} Mo; 62,502 mg*l^{-1} Co; 56 mg*l^{-1} Na and 27 mg*l^{-1} C.

The results of the experiments were statistically analysed with three-factor variance analysis and Duncan test, and significant effect was measured in every different nitrogen levels with the applied bio-fertilizer on the yield quantity. In the plot 0, which had no nitrogen fertilization for 35 years, 1,12 t*ha^{-1} higher yield quantity was measured as the effect of the bio-fertilizer. The mayor effect of the treatment was realized in the 2nd level of nitrogen, with 3,44 t*ha^{-1} higher yield quantity than the non-treated 2nd level experimental plots.

The results indicate that the *Chlorella vulgaris* microalgae-containing bio-fertilizer with optimal micro and macronutrient supply had significant effect on the crop yield of the maize, and the scale of the significant difference was also affected by the different nitrogen fertilization levels.

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Extracellular polysaccharides in twenty *Chlamydomonas* strains of the Mosonmagyaróvár Algal Culture Collection

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Extracellular polysaccharides (EPS) are high-molecular-weight polymers of carbohydrates. Many microorganisms secrete extracellular polymeric substances during their life cycle. Some species of the genus *Chlamydomonas* EHRENBERG nom. cons. (1833) and other green algae secrete EPS under specific conditions. For the first time, strains of the Mosonmagyaróvár Algal Culture Collection (MACC) were investigated for EPS production. In this study, twenty Chlamydomonas strains were analysed using the Phenol-Sulphuric Acid method (DuBois et al. 1956). Three strains produced more than 2 g/L and seven strains more than 1 g/L soluble EPS (sEPS). Strain MACC-398 was the highest sEPS producer (2763 mg/L) after 30 days of incubation. This study highlighted promising strains for application in soil conditioning.

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Plant biostimulating effects of the green alga *Chlamydopodium fusiforme* on winter wheat in field experiments

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There are increasing evidences that the effects of environmental stress in plants can be decreased with microalgae treatments. The aim of the present work was to investigate the plant biostimulanting effects of the green alga *Chlamydopodium fusiforme* on winter wheat in field conditions.

The wheat variety cv. „Bőség" was treated with the MACC-430 *Chlamydopodium fusiforme* green alga obtained from the Mosonmagyaróvár Algal Culture Collection (MACC). The experiment was carried out in three experimental years and included 20 plots of 10 m^2 (1,2 x 9,34m) size, 5 treatments in 4 replications. The plants were treated at tillering and at ear emergence, with microalgae in dosages of 120 g/ha (0.03% suspension) or 400 g/ha (0.1% suspension). The root dry weight was determined 2 days before and 10 days after the first treatment. The proline concentrations of the flag leaves were measured by the method of Bates et. al. (1973) and the relative water content (RWC%) according to Cabreara-Bosquet et al. (2009) once a week. The chlorophyll content was characterised with SPAD-units, measured with the portable device: SPAD 502 Plus Chlorophyll Meter. Ear number per m^2, grain number per ear, ear length and thousand grain weight of the control and treated plants were detected at or after the harvest.

The 120 g/ha treatment with the MACC-430 at tillering was the most economic, which gave an average yield increase of 31.4%. Mainly the ear number and ear length, and partly the grain number per ear and the thousand grain weight contributed to the increased yield. All microalgae treatments increased significantly the root dry weight after the first treatment, with special regards to the 1 g/l treatments. The microalgae treatments drastically increased the leaf proline content, which could also explain the increased leaf RWC.

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Effect of *Arthrospira platensis* on plant growth parameters of nursery plants

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The extreme weather changes (e.g. drought, high temperature) are becoming major challenges for plant breeders. There are experimental evidences that plants treated with microalgae reply with higher tolerance to environmental stress. The aim of the present experiments was the plant treatment with microalgae to stimulate the growth and development of purple chokeberry (*Aronia prunifolia* 'Viking'), some varieties of gooseberry (*Ribes uva crispa*), red currant (*Ribes rubrum* 'Rovada, Rolan Jonkheer van Teet's '), black currant (*Ribes nigrum* 'Titania'), white currant (*Ribes sativum* 'Weisse Versailler'), blueberry (*Vaccinium corymbosum* 'Goldtraube and Emblue') and some varieties of hortensia (*Hydrangea arborescens Annabelle*, *Hydrangea paniculata*). The cyanobacterium *Athrospira platensis* L. was tested in 2017 and 2018 in an Austrian nursery on some varieties of berries and other nursery species. The nursery young plants were transplanted in 2-5 L containers. At the time of potting 2, 4, or 6 g dried *A. platensis* were added to the substrate of each container of 2 litre volume before planting the 10–20 cm plants. The results showed, that soil treatment with *Athrospira platensis* increased the (1) shoot thickness 6-92%, (2) shoot number 10-93%, (3) number of leaves 17-138%, (4) plant height 2-32% and other parameters of different nursery plants.

Microalgae against necrotrophic and biotrophic plant fungal pathogens

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Plants can develop resistance against pathogens by hormone signaling mechanisms. Salicylic acid signaling triggers resistance against biotrophic pathogens, while jasmonic acid and ethylene signaling activates resistance against necrotrophs. However, without application of synthetic or natural pesticides, plants cannot survive a massive infection. Researchers from several countries joint forces in the SABANA H2020-project to identify freshwater and marine microalgae with antimicrobial activity. *In vitro* agar diffusion bioassay against necrotrophic fungi and leave disc or entire leave bioassay against the biotrophic pathogen *Plasmopara viticola* were used to check the bioactivity of microalgae. In a preliminary screening of 239 freshwater microalgae strains, approximately half the extracts demonstrated fungistatic activity and 20 strains in a concentration of 10 g/l showed a fungicide effect against necrotrophic pathogens. Three microalgae strains with a broad spectrum of antifungal activity were selected and cultivated in different outdoor culture systems either in artificial nutrient medium or in diluted sewage. The bioactivities of the harvested biomass samples were highly variable, but showed some tendencies: (a) the type of culture system did not significantly affect the bioactivity; (b) biomass samples harvested from diluted pig manure or centrate were more active than those produced in nutrient medium; (c) SAB-M1 inhibited *Fusarium oxysporum*, while SAB-M677 and SAB-M612 inhibited *Phytophtora capsici*; (d) the marine strain SAB-GC-866B demonstrated the highest bioactivity against the investigated fungal pathogens. Fifteen freshwater strains with fungicide effect on necrotrophic pathogens were tested against the biotrophic *P. viticola* and 3 of them were very effective when tested at 10 g/l. It was established that grape can be protected against *P. viticola* with the strain SAB-M14 if the microalgae suspension can penetrate through the stomata of the lower leaf surface.

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Microalgae for heavy metal removal

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In modern world of biology, microalgae play an important role in the environmental science and engineering for wastewater and soil bioremediation due to their sustainable production and eco-friendly nature. Heavy metal removal from soil and water and degradation of toxic chemicals are critical, as these pollutants may not only kill the aquatic & non aquatic species, but also enter the food web to cause severe disasters for human beings. Bioremediation of contaminated environment has attracted increasing interests in the research community for environmental biotechnology. In this work, we have evaluated twenty two stains of microalgae for removal of heavy metals (Mercury (Hg2+), Cadmium (Cd2+), Lead (Pb2+), Copper (Cu2+), Aluminum (Al2+), and Iron (Fe2+) in aqueous solutions as a single metal species at concentration of Hg, Cd, 5 ppm, Pb, Cu, 10 ppm Al and Fe 30 ppm, respectively. Five out of 22 strains were selected for their comparatively tolerance to one or more of the heavy metals. Further characterizations and heavy metal removal efficiency were successfully measured using the selected strains for afore mentioned heavy metals by different technique like OD, ICP Mass, SEM, and Confocal Microscopy etc.

It was found that the 5 selected stains could maintain normal growth under 10 ppm of (Hg, Cd), on 20 ppm of (Pb, Cu), and on 50 ppm of (Al, Fe) , the growth became inhibited when the concentration of heavy metals went higher than 15 ppm for (Hg, Cd), 20 ppm for (Pb, Cu), and 50 ppm for (Al, Fe), respectively. Removal efficiencies of Hg, Cd, Pb, Cu, Al and Fe on afore mentioned concentrations were 92, 97, 94, 98, 97, and 99.5 %, respectively, while those for soil was 68, 71, 68, 73, 70 and 75 %, respectively. A slight increase was observed in leakage of protein and nucleic acid with the passage of time. With short time on desired concentration 2nd stage morphology are same like control and 3rd stage with long time compared little effect occur on morphology compared with untreated First stage. From above evidence is cleared that these heavy metals are toxic like as Hg > Cd >Pb> Cu > Al > and Fe.

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In vitro antifungal activity of selected freshwater and marine cyanobacteria strains

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Fungal plant pathogens cause serious crop losses year by year worldwide. Some fungal pathogens (e.g. *Botrytis cinerea*, *Fusarium graminearum*) infect a wide range of commercially important plant species, thereby jeopardizing food security. Although the number of licenced antifungal compounds has decreased over the past few years, protection of agricultural and horticultural plants still relies mainly on fungicide use. For this reason, alternative plant protective techniques are needed in the near future to supplement the use of fungicides. Since microalgae, like cyanobacteria were found to be a rich source of various bioactive secondary metabolites (with antibacterial, antifungal and even anticyanobacterial effects), their use in plant protection might be a possible alternative to complement fungicide based plant protection. In the present work an *in vitro* agar-well-diffusion method was successfully applied to investigate four, economically important plant pathogenic fungal species (*Alternaria alternata*, *Botrytis cinerea*, *Fusarium graminearum* and *Rhizoctonia solani*). Water extracts of 15 freshwater and 15 marine cyanobacteria strains were tested, as potential fungal growth inhibitors. The extent of inhibition was established by measuring the hyphal growth of the fungal strains towards 9 mm wells cutted in the agar plate and filled with freeze-dried cyanobacterial biomass re-suspended in distilled water (10 g/l). The results of these tests showed that 5 of the freshwater and 9 of the marine cyanobacteria strains inhibited hyphal growth of more than one plant pathogens. However, there were only 3 freshwater and 1 marine cyanobacteria strains with broad spectrum of inhibition effect against at least 3 of 4 fungal strains. Based on the obtained results cyanobacteria strains with antifungal effect has been selected and are ready for subsequent tests and applications in plant experiments.

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Myxoxanthophylls – carotenoid glycosides from cyanobacteria and their specific optical and antioxidant properties

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Myxanthophylls are carotenoid glycosides occuring in various genera of cyanobacteria. They are derivatives of the terpenoid myxol ((3S,4E,6E,8E,10E,12E,14E,16E,18E,20E,22E,24E)-25-[(4R)-4-hydroxy-2,6,6-trimethylcyclohexen-1-yl]-2,6,10,14,19,23-hexamethylpentacosa-4,6,8,10,12,14,16,18,20,22,24-undecaene-2,3-diol) bound with sugar moiety.

Particularly, in unicellular cyanobacterium *Synechocystis* sp. PCC6803 myxol 2'-dimethyl-fucoside has been identified and its structure has been characterized at the molecular level by NMR spectroscopy (Takaichi et al., Plant Cell Physiol., 2005, 46(3), 497). However, chemical properties of this compound were not a subject of systematic study. A simple, inexpensive, easily-scalable method of myxoxanthophyll purification from cyanobacterial biomass has been developed. In this work we show that in *Synechocystis* myxoxanthophyll is present in four isomeric forms, with a dominant *all-trans* isomer that consists c.a. 70% of total myxoxanthophyll pool. Purified myxoxathophyll shows solvatochromic shifts in Vis region of its absorption spectra. These shifts are promoted by the polarity of the solvent. In organic solvents, myxoxanthophyll shows the absence of any specific solvent-solute interactions, as its energy of electronic transitions increases with solvent polarizability. On the contrary, aqueous environment promotes intermolecular interactions, which are directly manifested by changes in the absorption and circular dichroism spectra of myxoxanthophyll. Preliminary results from ORAC (oxygen radical absorbance capacity) and DPPH-based methods show that myxoxanthophyll antioxidant capacity seems to be comparable with other xanthophylls. Environment-dependent specific optical and antioxidant properties of myxoxanthophyll, in combination with its uncommon amphiphilic structure make this compound a cyanobacterial product potentially applicable in industry and agriculture.

Challenges in the production of microalgae in large scale

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In spite of applications of microalgae biomass includes from pharmaceuticals, to cosmetics, human nutrition, animal feeding, biofertilizers, biofuels and including wastewater treatment, the reality is that today no more than 35.000 t/year are produced it being mainly focused in food industry. The raison for that is that still there is two major problems limiting the microalgae production capacity in worldwide: (i) the excessive production cost of actual technologies, and (ii) the large amount of resources required to produce the microalgae biomass in large amounts. Thus, the biomass production cost of current technologies ranged from 5-50 €/kg depending of the utilization of raceway or closed systems (Acien et al., 2013). In terms of consumption of resources, the production of large amounts of biomass requires enormous amounts of fertilizers, so competing with agriculture. The alternative to solve these problems is to combine the production of microalgae biomass with the treatment of effluents, both liquid and gases, thus at the same time saving resources by improving the sustainability of conventional treatment systems. In this sense, it has been widely demonstrated that microalgae biomass production can be coupled with the capture of CO2 from industrial flue gases, at the same time that with the recovery of N and P from wastewaters (Acién et al., 2012; Acién et al., 2016). Opposite, when producing microalgae biomass using effluents the resulting biomass cannot be used for human related applications, but it can be used to produce biofertilizers or in aquaculture, among others applications.

In this work real figures from a demonstration plant producing microalgae for biofertilizers and aquaculture are showed. The reliability of the proposed technologies has been demonstrated at real outdoor conditions, accomplishing industrial standards, always operating in continuous mode all the year around. The processing of the biomass to obtain valuable fertilizers and aquafeed has ben also studied. Business plan demonstrate that this process is profitable, it sustainability being mainly a function of the type of resources involved on the production step. In this case the utilization of wastewater and flue gas provide the best scenario of large-scale production of microalgae biomass.

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Outdoor pilot-scale cultivation of selected microalgae in Třeboň: thin-layer cascade vs. raceway pond

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Several freshwater microalgae strains (e.g. *Nostoc, Chlorella* and *Scenedesmus*) – filamentous as well as single celled – were selected for their biostimulating and biopesticide activities. Prior to outdoor trials the strains were characterised in laboratory experiments. Outdoors pilot cultivation trials were carried out in a thin-layer cascade (TLC) and a thin-layer raceway pond (TL-RWP) in order to test their suitability for cultivation of selected strains. The cultivation units were placed in greenhouses to avoid cross-contamination and the effect of unfavourable environmental conditions.

We have focused on several tasks: (ii) growth of selected strains in inorganic media vs. centrate obtained from municipal wastewaters, (ii) selection of monitoring techniques to follow culture performance and a choice of marker variables; and (iii) technical improvements of cultivation units (mixing/circulation, sensor positioning, cooling, CO_2 supply).

At present selected microalgae strains are being cultured in TLC and TL-RWP using centrate from municipal wastewater as a nutrient source to produce biomass which bioactivity is further tested.

One of the tasks (6.4) of the SABANA project is to develop fast and robust monitoring techniques to estimate the performance of large-scale outdoor microalgae cultures. Various *in-situ* and *off-situ* measuring techniques (e.g. Chl fluorescence, photosynthetic oxygen production, etc.) were used to monitor photosynthetic activity of cultures as to correlate them with growth (and productivity) in laboratory and outdoor units. These data make it possible to work-out suitable culturing regimes for a selected microalgae strain in a particular cultivation system and optimise biomass production.

Pilot trials have been important to test the culturing of particular strains and monitoring techniques (physiological and photosynthesis variables) before DEMO cultivation plants are built and operated to produce microalgae for agricultural use (biostimulants and biopesticides).

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Electrocoagulation – a smart way to minimize microalgae harvesting cost

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Harvesting densities of microalgae suspensions in large scale cultivation devices are most often in the range between 0.5 and 5 g/L of dry biomass. Nevertheless, the biomass concentration suitable for drying (the most usual biomass processing) are around 150 g/l. It is well know that microalgae like e.g. chlorella, which has small cells, are impossible to be separated from the cultivation media by low cost methods as sedimentation or filtration. They are usually separated by energetically demanding centrifugation, which energy cost was estimated to be around 2 kWh/kg of dry biomass. The microalgae cells have negative surface charge, so they repulse each other by electrostatic forces and because of that form suspension, which is stabile and do not tend to sediment of flocculate. To destabilize the suspension it is possible to apply a process of coagulation (flocculation) when flocculating agent is added and cell aggregates (precipitates) are formed. Flocculating agent is suitable chemical compound, which has positive surface charge and enables crosslinking between negatively charge microalgae cells. Unfortunately to find a flocculating agent, which is effective and food-grade in the same time, is almost impracticable. To overcome this difficulty, we employed electrocoagulation with iron electrodes in order to destabilise a microalgae suspension and form aggregates, which can be easily separated by sedimentation. During electrocoagulation, iron cations are released from the sacrificial anode and acts as the flocculating agent. When process parameters are set optimally, separated microalgae biomass contains very low amount of this biogenic element and remains suitable for human consumption. In our work, we focused on deep examination of various parameters influencing the separation efficiency of the electrocoagulation and iron content in the separated biomass of *Chlorella vulgaris*. Influence of current/voltage, electrodes distance, agitation, culture media components, pH, temperature, initial microalgae suspension density, agitation etc. was tested in a laboratory apparatus operated in a batch mode. The obtained knowledge was applied in construction and operating settings of a pilot scale continuous device (volume of 50 L). We were able to separate *Chorella vulgaris* from suspension with 98% efficiency and decrease the biomass separation costs to 0.15 kWh/kg, which was 7.5 % of the energy consumed by centrifugation.

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Effect of salt stress on a biotechnologically important microalgal strain

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Growth and productivity of photosynthetic organisms are significantly affected by stress factors. Photosynthesis is very sensitive to changes in the environmental conditions because it balances the absorbed light energy with the energy consumed by metabolic processes of the organisms. In general, photosynthetic functions are controlled by multilevel regulatory mechanisms and depend largely on the molecular composition and (macro-) organization of the thylakoid membranes, which contain virtually all protein complexes that are involved in the light reactions of photosynthesis. The stress signal is first perceived at the thylakoid membrane level. Adjustment of the characteristics of membrane structure enables the membrane components to maintain and perform their physiological functions upon changes in the environmental conditions. The understanding of adaptation to higher salt concentration of biotechnologically important microalgal strains is highly important since every continent is affected by salinized soil and water, and because of the possible use of water sources of higher salt concentrations. We have investigated the adaptation of the membrane organization and photosynthetic performance of *Euglena gracilis* to salt stress.

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Poster Presentations

P1

Protoplast technology as an experimental platform for characterizing oxidative stress in *Symbiodinium* sp. and other microalgae

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Symbiodinium is an important dinoflagellate which lives in endosymbiosis with reef invertebrates, including corals, making them central to the health of corals. With coral reefs currently under extreme threat from climate change, there is a pressing need to improve our understanding on the stress tolerance and stress avoidance mechanisms of *Symbiodinium* sp. Protoplast technology provides the platform for a wide range of experimental techniques including oxidative stress studies along with genetic transformation and protoplast fusion. The cell wall of the dinoflagellates is composed of cellulose-enforced pellicle and cellulosic thecal plates positioned between membranous layers. The membranous outer layer and thecal vesicle membrane serve as potential obstacles in dinoflagellate protoplast generation by reducing access to the internal cell walls. Previous studies have struggled to remove the cell wall from armored dinoflagellates, potentially due to the internal placement of their cell walls.
Here, we produced the protoplast from physiologically distinct clades of *Symbiodinium* via incubation with cocktail of enzymes (cellulase, macerozyme and lysozyme) and osmotic agents. Digestion of the cell walls was confirmed by a lack of Calcofluor White fluorescence signal and by bursting of protoplast due to osmotic shock in distilled water. Following digestion and transfer to regeneration medium, protoplasts remained photosynthetically active, regrew cell walls, regained motility, and entered exponential growth. We also investigated intracellular localization of the singlet oxygen specific dye Singlet oxygen sensor green (SOSG) in protoplasts, which cannot be introduced in the intact cells due to the complex cell wall. Uptake of antisense oligonucleotides was observed for the first time in the *Symbiodinium*, as they are only well studied in the plants indicating the potential for the application of oligonucleotide technology in *Symbiodinium*. Generation of *Symbiodinium* protoplasts opens potentially new avenues for researching these crucial symbiotic dinoflagellates, including the possibility for genetic modification. We expanded protoplast technology to other microalgal species, *Chlorella* and *Nannochloropsis*, which have a high potential in industrial applications.

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P2

Macro-organization of the photosynthetic membranes in isolated microalgal strains

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Microalgal biomass and algae-derived compounds have a very wide range of potential applications, from animal feed to human nutrition and health products. Microalgae, i.e. unicellular algae and cyanobacteria, are photosynthetic microorganisms. Photosynthesis is very sensitive to changes in the environmental conditions because it balances the absorbed light energy with the energy consumed by metabolic processes of the organisms. In general, photosynthetic functions are controlled by multilevel regulatory mechanisms and depend largely on the molecular composition and (macro-) organization of the thylakoid membranes, which contain virtually all protein complexes that are involved in the light reactions of photosynthesis. Changes in the membrane composition and macro-organization upon stress exposure are linked, via different feedback mechanisms, to changes in the photosynthetic performance. In our project we have investigated and compared the (macro-) organization of the thylakoid membranes of some newly isolated microalgal strains.

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P3

Measurement of brassinosteroid content of *Klebsormidium* sp. BEA IDA_0061B and the optimization of the rice lamina inclination bioassay

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The auxin-, and cytokinine-like phytohormone producing ability of various algal strains is well-known. Gibberellin activity has been demonstrated in brown algae. The presence of steroid plant hormones like brassinosteroids (BR) are essential for plant growth and development. Among lower organisms, algae are the primary producers of these molecules. 24-epi-castasterone and 28-homo-castasterone were detected from *Hydrodictyon reticulatum* green algae. Brassinosteroids promote cell elongation and proliferation, increase ethylene production, stimulate root growth, accelerate plant growth and increase yield and plant resistance against environmental impacts. Interaction with gibberellins and abscisic acid stimulates the germination of seeds. *Klebsormidium* species are considered as one of the most ubiquitous group of biological soil crust (BSC) organisms with high ecological value. These filamentous green algae can grow in the upper 5-10 cm layer of soil of both meadows and forests of Central Europe, between 5 - 30 °C. The strain *Klebsormidium* sp. BEA IDA_0061B is able to accumulate brassinosteroids that can be detected in algal cultures by extraction and HPLC analytical methods, and presumed with seedling bioassay methods. High performance liquid chromatographic separation with fluorimetric detection was used to detect the brassinosteroid concentration of microalgae cultures. During the evaluation of the chromatograms it was found that brassinolides can be detected in *Klebsormidium* sp. BEA IDA_0061B algal cultures, however, the method needs further development. In addition to instrumental analytical procedures, we also tried to detect the presence of brassinolides by rice leaf lamina inclination bioassay. Several varieties of rice were tested to investigate the relationship between brassinosteroid concentracion and leaf lamina inclination using an analytical grade brassinolide standard. On the basis of results, we are developing a method of detecting brassinosteroids from wet algal cultures of *Klebsormidium* sp. BEA IDA_0061B.

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P4

Investigating the interaction between *Azospirillum brasilense* soil bacteria and *Scenedesmus rubescens* algal strain by using microfluidic methods

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Nowadays the use of algae for industrial or agricultural purposes, including their use as foodstuffs, is spreading globally. *Scenedesmus* species are important for both the food industry and agriculture due ti its nutritional properties. Previous research and development on a *Scenedesmus*-based foliar fertilizer raised many questions on effectiveness of large-scale algal culturing and the potential effects of algae on soil bacteria. Co-cultivation with *Azospirillum* species is a way of optimalization of *Scenedesmus* culturing, however, the operation of co-cultivation system is not well-known. *Azospirillum brasilense* can produce indole-3-acetic acid, which being a plant hormone, could promote the propagation of algae. However, the effects of algae on *Azospirillum* cultures are not well known.

Azospirillum species are associative nitrogen fixing rhizobacteria, one of the most studied plant growth promoting barteria in the practise of agricultural microbiology. They can fix nitrogen under microaerophilic conditions. Under anaerobic or microaerophilic conditions they can denitrify, and they are capable to assimilate ammonia, nitrate and nitrite.

Replacing plants with microalgae is a good method for modeling bacteria-root interactions. According to recent literature motile bacteria may move in the direction of algae due to the presence of chemoeffectors – in a similar method they use to move towards root exudates (sugars, amino acids).

According to our previous experiments, the *Scenedesmus rubescens* BEA D01_12 can produce certain secondary metabolites that can be used by *A. brasilense* as a carbon source. In order to reveal what kind of interactions are present between these two strains we used a micorlfuidic set up. This device consists of two large reservoirs and an observation channel separated by a membrane. Chemical gradient forms in the channel within minutes, and lasts fot several hours. We studied the behaviour of *gfp*- labeled *Azospirillum brasilense* CdS strain in the microfluidic gradient generator under different chemical conditions (e.g. the presence of a neighbouring *Scenedesmus rubescens* BEA D01_12 strain). We followed the spatial distribution of bacteria by fluorescent time-lapse microscopy. With this method we are able to detect specific motility patterns, such as bacterial chemotaxis and the growth rate of bacteria in the channel can be analyzed as well. We performed qualitative and quantitative analyses of the images. Based on our results, the algal culture is likely to contain chemoattractant compounds.

P5

Genetically modified *Synechocystis* PCC 6803 strains for starch and bioethanol production

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The main objective of Algenetics project has been to establish a joint Czech-Austrian centre for microalgae biotechnology in order to strengthen research cooperation. To accomplish this objective, both partners have provided their knowledge - the University of Applied Sciences in Wels in the field of genetic engineering and Centre Algatech Třeboň in the field of microalgae growth optimisation. The project has been focused on basic research of genetically modified cyanobacteria which are considered as a sustainable and alternative source of carbohydrates due to their utilization of solar energy, CO_2 and water in the photoautotrophic carbon metabolic pathway. This represents one of the main challenges for cyanobacterial products as the strains have not yet been optimized as cell factories for industrial processes. Up to now they have been studied in basic research to construct genetically improved transformants as specific producers of starch and bioethanol as potential sources of biofuels.

The cyanobacterium *Synechocystis* PPC6803, f. NIX has been selected due to abundant knowledge available on this strain and easy manipulation by genetic engineering. To enhance production of glycogen, the overexpression of genes (*glgA1*, *glgA2*, *glgC*) involved in the synthesis pathway have been introduced. Overproduction of 1,4-alpha-glucan branching enzyme (*glgB*) leading to starch-like polysaccharide was successfully engineered and determination of carbohydrates content was carried out by FTIR spectroscopy. The selected constructs of *Synechocystis* are being cultured under normal as well as stress conditions (nitrogen limitation) in order to study the accumulation of required compounds. The bioethanol and starch produced by cyanobacteria in a well-controlled process in photobioreactors have a potential for alternative bioenergy sources (transport fuels) or raw materials for chemical or pharmaceutical industry. It might be a way to find its economically feasibility for the market and contribute to a future higher independence from fossil fuel sources while not competing with food crops as in case of the first-generation biofuels.

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P6

Establishment and the characterization of the biomass and astaxanthin production in the culture of microalgae *Haematococcus pluvialis*

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The purpose of the research was to establish the biomass and astaxanthin production in the culture of the microalgae *Haematococcus pluvialis*. To obtain as possible as high producing line for further studies we screened biomass and astaxanthin production of different microalgal strains, obtained from CCALA (Culture collection of Autotrophic Organisms, http://ccala.butbn.cas.cz/en). We observed significant differences in biomass production among different strains and not significant differences in the astaxanthin production. The highest yielding strain S4 produced 1462 mg L^{-1} of dry weight, while the lowest strain S2 1233 mg L^{-1} of dry weight. The strain S4 had the highest 41 mg L^{-1} astaxanthin content, which can be connected with the highest dry mass production. The strain S4 was determined as the most appropriate strain for further research of the influence of environmental factors on the biomass production and the influence of stress or elicitation on astaxanthin production.

Key words: algal culture, *Haematococcus pluvialis,* astaxanthin

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P7

Establishment of the bioreactor system for the astaxanthin producing microalgae *Haematococcus pluvialis*

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We designed a flexible laboratory bioreactor system for the cultivation of more growth demanding microalgae, such as microalgae *Haematococcus pluvialis*. For this purpose, we constructed a bioreactor system with the ability of automatic regulation of the environmental factors as CO_2 flow, pH, temperature, the intensity and wavelength of LED light sources. Our microalgae bioreactor system can be used for studying the influence of environmental factors on the biomass production and furthermore for studying the influence of stress or elicitation conditions on certain biochemical production.

Key words: microalgae, *Haematococcus pluvialis,* photobioreactor, cultivation

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P8

The reclassification of previously identified Anabaena (Cyanobacteria, Nostocaceae) strains from the Mosonmagyaróvár Algal Culture Collection, Hungary.

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A study on thirty-seven Mosonmagyaróvár Algal Culture Collection (MACC) isolates previously identified as Anabaena, a freshwater filamentous heterocytous taxon, were carried out using the 16S rRNA gene. The aim of this study was to amend the strain designation of the collection. The study found that most of the strains were misidentified at the genus level. Three clusters of phylogenetically and morphologically similar taxa were identified. The previous determinations were amended with their new taxonomic classifications (partly due to changes in cyanobacterial classification). Some morphological structures could not be found in the cultures (e.g. akinetes). Molecular data revealed that six of the thirty-seven strains are Desmonostoc, eight are members of the genus Nostoc, nineteen strains bear genetic resemblance to the genus Trichormus and four strains remain unresolved. Clades were established by 16S rRNA similarity and p-distances. This study reveals the necessity to revisit established culture collections that originally used only morphological classifications for species identification.

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P9

Reducing microalgae harvesting costs using electrocoagulation

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This study is focused on harvesting of *Chlorella vulgaris*, well known phototrophic single-celled microalga with wide range of use in nutrition, health care, cosmetics and potential in biofuels production. Due to low harvesting densities of phototrophic microalgae (in case of *Chlorella vulgaris* they are usually in range 0.5-5 g/L) is harvesting one of the most expensive parts of the *Chlorella* biomass production. It was estimated that harvesting costs make up approximately 40 % of the biomass production costs. The aim of this study is to evaluate a new pre-harvesting method for reducing the cost of *Chlorella* harvesting in large scale.

Nowadays centrifugation is the standard method of microalgae biomass harvesting, but it is economically inefficient due to its high electricity consumption and expensive equipment. Using of pre-concentration method is an option for reducing the volume of algal suspension which needs to be centrifuged. One of the possible ways of suspension pre-concentration is electrocoagulation method. Process of electrocoagulation helps to decrease the volume of the suspension up to 30 times. The principle of this method is forming of well-sedimentating flocks made up of algae cells and flocculant agents, which are easy to separate from the medium by sedimentation or flotation. The surface of microalgae cells has to be negatively charged to form aggregates with positivelly charged flocculants. In case of electrocoagulation, the flocculants are metal ions released to the suspension by a sacrificial anode (usually made from aluminium of iron). In our case, iron electrodes were used in order to keep the food grade quality of the separated *Chlorella* biomass as the biomass is always partly contaminated by the electrode material.

In laboratory scale experiments, an influence of the following operating parameters was studied: electric current, biomass concentration, pH of the algal suspension, temperature of the algal suspension, concentration of residual salts in the culture medium, agitation. Parameters of the process were optimised to achieve high biomass separation efficiency simultaneously with the low iron contamination of the biomass. Subsequently three types of continuous harvesting devices were constructed and tested for bench-scale electrocoagulation. Using channel flow reactor the efficiency of separation above 95 % was achieved while the iron content in biomass didn’t excess recommended daily intake of iron for human.

P10

Molecular taxonomic studies on cyanobacteria strains from the Mosonmagyaróvár Algal Culture Collection

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Culture collections are important resources of science, biotechnology and bioindustry all over the world. The studies on microalgae strains of the Mosonmagyaróvár Algal Culture Collection (MACC) focused primarily on hormone production and antimicrobial effects for agricultural purposes. The characterisation of the MACC strains was primarily carried out using morphological markers. However, the identification was limited mostly to genera level due to the small size and slight variety of the cells and the degradation of phenotypical characteristics during the breeding.

Our main task has been to develop a molecular taxonomy method which enables genotype- level identification, and thereby facilitate the selection of strains for special economic utilisation. 40 deposits of *Nostoc* and 40 deposits of *Anabaena* microalgae isolate were analysed in our laboratory using probes specific for the *16S rRNA* and *rbcLX* gene sequences. Complete gene sequences were used to build a phylogenetic tree.

The results highlight some discrepancies for several strains, based on which the accuracy of previous morphological genera determination have become uncertain. Furthermore, some strains appeared to be identical based on their sequences.

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P11

Photosynthetic performance of three microalgae strains grown in centrate at continuous illumination and light/dark regime

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The use of municipal wastewater (centrate) as a growth medium for microalgae has been found to be a promising alternative to expensive inorganic fertilisers. The nutrients contained in these waters can be directly re-used that is a sustainable alternative to usual, costly wastewater treatment costly and complicated processes.

The aim of these trials was to verify the photosynthetic and growth performance of three microalgae strains – *Nostoc, Chlorella and Scenedesmus* selected for their biostimulating and/or biopesticide activity that were cultured in centrate (after aerobic treatment of municipal wastewater). Two irradiance regimes were used in laboratory experiments – continuous light and light/dark 12/12. Photosynthetic activity was monitored using three techniques – oxygen production/respiration, saturating pulse analysis of fluorescence quenching and fast fluorescence induction kinetics.

Such monitoring techniques are quick to perform, making it possible to estimate/adjust a suitable growth regime for microalgae strains. Then, more precise growth trials can be performed to verify strain suitability for biomass production and bioactivity tests.
The data obtained are important from a biotechnological point of view for a potential large-scale production of bioactive compounds for agricultural use.

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P12

Effect of combined application of microalgae- and PGPR-based biostimulants on vegetative growth of strawberry

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Biostimulants are substances or microorganisms with positive effects on plant's growth, yield, and condition. Microorganisms-based biostimulants contain living cells, algae, bacteria, fungi. Their use is becoming more and more popular in order to improve soil life and increase yield. In our experiment, two microbial products were used in combination, and their effects were investigated under organic farming conditions on the strawberry variety 'Joly'. During the experiment, sizes of leaf areas, fresh and dry masses of the roots and foliage of treated and control strawberry plants were compared.

The experiment was set up in 2017 at the experimental garden of John von Neumann University (Kecskemét, Hungary). *Fragaria ananassa* x 'Joly' plants have been planted in twin-rows. Control plot was separated from the treated plot.

On the treated plot, PGPR-based product, was sprayed together with basic fertilization, and was also applied to planting pits during planting in 2017. In 2018, it was sprayed on soil after the spring fertilization. The other microbial product, a *Scenedesmus obtusiusculus*-containing biostimulant, was sprayed to the leaves of treated plants in both years at the beginning of the flowering.

Leaves of selected (simple random sampling) plants were removed, scanned and sizes of leaf areas were determined with ImageJ win32 software in both years.
Based on the average of the leaf surfaces, the treated plants had larger leaf areas in both years, and in 2017 the difference was statistically significant ($p = 0.040$).

When fresh and dry masses of treated and control plants were compared, we found that treated plants have larger weights compared to control on average, but the differences were not statistically significant. Further investigations are in progress.

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P13

Physiological performance and bioactivity of *Chlorella* grown outdoors in thin-layer raceway pond

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Microalgae can be grown either in closed cultivation systems – photobioreactors – with no direct contact between the culture and atmosphere or in open systems. Raceway ponds (RWPs) are most commonly used open systems for pilot and large-scale cultivation. The advantages of these units are cheaper construction and easier operation and maintenance compared to photobioreactors. On the other hand the disadvantage of RWPs is insufficient mixing causing poor light utilization due to long light/dark cycles of the cells (usual light path of the culture ranges between 10 and 30 cm). Thus, we tested a unique pilot system, the so-called *thin-layer raceway pond* (TL-RWP) with culture layer of 1.5 – 2 cm (cultivation area of 5 m^2; working volume 100 – 150 L).

The green microalgae strain *Chlorella* which was preliminary selected for its biostimulating and biopesticide activity, was cultured in TL-RWP placed in polycarbonate greenhouse to avoid cross-contamination and to control environmental conditions. In the cultures grown in batch and semi-batch growth regime we monitored photosynthetic activity and growth. The cultures were harvested at various daytimes (morning and midday) and freeze dried. Water extracts of the biomass were applied the seeds of lettuce (*Lactuca sativa*) at two different concentration (0.5 and 2 mg DW L^{-1}) to determine biostimulating effect. Antimicrobial activity of biomass was determined by dual culture assay. The extracts of the biomass harvested in the morning showed a partial biostimulating activity (105-115%). In parallel, antimicrobial activity against four bacteria (*X. campestris, P. carotovorum, P. syringae and C. michiganensis*) and four fungi strains (*P. ultimum, F. oxysporum, R. solani and P. capsisci*) was also determined. The highest antifungal activity up to 32 % was observed against *F. oxysporum*. Minimum inhibitory concentration (MIC) of lyophilised water extract against this pathogen was determined by broth dilution method at the concentration of 1 mg mL^{-1}. Water extracts of the biomass are being further analysed using HPLC/MS to determine the differences in sample composition in order to identify the nature of potential compound(s) responsible for bioactivity.

The results presented here show a potentially use of the *Chlorella* strain for environment-friendly biological protection of plants or crops cultured in the field or in greenhouses.

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P14

Effect of algae-suspension in KH Lilla and Rihane spring barley variety.

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Periodic or lasting water shortages affect production of cereal crops and therefore population growth. Drought-tolerant plants could provide a safe crop in a water-stress environment resulting in a more reliable food production.

In agriculture, more and more emphasis is placed on environmental-friendly farming, where the use of different plant conditioning preparations can increase the resistance of crops[1]. Sea algae extracts and microalgae formulations have a positive effect on the life processes of plants, it was shown to increase the quantity and quality of the crop[2]. In our experiment we used a drought-sensitive and a drought-tolerant spring barley variety. The seedlings were grown in liquid culture. Samples treated with control or algae containing suspension (MACC-612; MACC-430) following by 20% PEG6000 were examined. The algae (*Nostoc entophytum* (MACC-612) and *Tetracystis sp.* (MACC-430)) was obtained from the algae collection of Mosonmagyaróvár. A suspension of 10 g/L was prepared from distilled water with lyophilized algae. For the experiments a freshly prepared microalgae suspension was used each time on day 2 and day 8 of germination. The dehydration treatment was simulated with 20% PEG6000 solution on day 8 after germination for both control and algae treated samples. On the tenth day a representative sample of all plant shoots was taken to measure growth parameters (root and shoot length, root and shoot wet weight), proline content[3], hormone effect (citokinin, abcisic acid) and expression of the P5CS3 gene[4].

The applied treatments had an impact on both types of spring barley. *Nostoc entophytum* (MACC-612) affected root growth of the barley varieties differently: Not the water-intensive KH-Lilla, but the Rihane variety increased root mass growth as the root length increased. The proline content and the expression of the P5CS3 gene also decreased for *Nostoc entophytum* (MACC-612) treatment. *Nostoc entophytum* (MACC-612) condition the drought-sensitive barley varieties for later stress relief.

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P15

Differential expression of algal host under axenic and bacterial associated condition

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One of the foundational principles of community ecology is that productivity is enhanced when diverse organisms are grown together. It has been commonly observed that cultures containing contaminating bacteria grow better than axenic algal cultures. Majority of the algal species cannot produce vitamin B 12, instead obtain it from their bacterial mutualists in exchange for photosynthetically fixed sugars.

Biotechnological cultivation of algae, be it for biofuel or value-added products, usually focuses on monocultures. It is only recently that multi-species cultivation has received more attention. However, not much is known about the scope of molecular interactions that occur when algae are co-cultured with symbiotic bacterial partners.

In this project we explore the influence of an arbitrarily selected bacterial partner, E. coli BW25113 on algal growth over the course of a day through RNAseq analysis. Illumina sequencing was carried out on two different strains of algal species; *Chlamydomonas reinhardtii strain cc124* and a newly discovered species; Strain 549 at 4 and 24-hour time point. We used „Express" to map the sequenced reads and GFold to identify differentially expressed transcripts.

Our preliminary data reveals molecular elements that are differentially expressed at 4 hours and 24 hours of algal growth, during the presence and absence of the bacterial partner. We also present a draft genome assembly along with structural and functional annotation of this new species of algae.

Designing synthetic algal bacterial consortia is becoming increasingly sought contemporarily. Identifying the elements that are involved in algal host functions under axenic and bacterial associated conditions would allow us to target specific bacterial partners and design efficient algal-bacterial consortia aimed to improve algal host productivity. This project is a first attempt towards that direction.

P16

Application of microalgae in pea and faba bean

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The effect of selected MACC strains of microalgae (genus *Nostoc*) was tested on pea and bean seed in laboratory and field conditions (pea). Under laboratory conditions, the effect of algal emulsion on seed germination was assessed, the rate of germination and plant growth was evaluated in vessel experiments, including the evaluation of photosynthetic apparatus performance by measuring fluorescence and aphid incidence. In field conditions, the leaf application of microalgae was chosen in three terms of plant development and the growth, health status (aphid occurrence) and yield depending on the time of treatment, the type of microalga strain used and the untreated variant were evaluated.

The results show a positive effect on the number of germinated seeds, but the inhibitory effect of the development of germs under laboratory conditions, ie after direct contact of pea and bean seeds with microalgae emulsion. In vessel trials, although there were no statistically significant differences in the effect of individual strains on plants, growth was stimulated for the application of some of the strains (higher levels of dry matter above ground) and some strains were observed to have a small aphid attack (artificial inoculation).

In field trials, the number of nodes, plant heights, and other growth parameters (leaf area, chl*a b*, and total carotenoids, RWC) were evaluated, total carotenoids, number and FW of seeds were positive affected due to foliar application (2016), however no in aphid attack. The yield of seeds in the application of 2 strains (in 2016, 2018) was higher in relation to the higher seed dryness, the analysis of the content of N-substances in the seeds did not show increased values compared to the control. The results will be discussed.

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P17

High efficiency, sustainable and safe industrial microalgae cultivation complexes for plant/soil-systems, novel food, feed and multisectorial products

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Algae play an important role in modern agriculture as multifunctional options for increasing of crops and aquaculture production efficiency in consumer friendly, environment-climate protection, natural resources management and circular bioeconomical modes.

The immense potential is manifested by use of their or biorefined products as novel food and feed, aquafarming enhancers, bioenergetical, biofertilizer, microbiological, hormonal, enzymatical biostimulants, yield and composition enhancers, biopesticides, biopharmaceuticals, bioplastics and as well as integrated wastewater and organical waste treatment excipients.

Corresponding of the above needs we are developed an integrated intensive industrial cultivation and biorefinery high efficiency, secure, sustainable system with minimal land use, zero carbon emissions, low water footprint, corresponding of circular bioeconomical criterions with adaptability to specific geographical conditions and specific needs of products.

Our self-developed dynamic photobioreactors can be used to cultivate different strains simultaneously, the harvests formulating and/or biorefinering for above purpose. The cultivation line and biorefinery facilities is completed by own bioenergy and/or biofuel production integrating with agricultural waste management in frame of integrated bioindustrial farms, complexes or parks.

The aims of realization of above kind of facilities fulfilling sustainability, smart energy and bio-economic conditions is in conformity of the UN, OECD, EU and several country-programs, SDGs and roadmaps (2030, 2050).

This poster presents, our own-development of dynamic photocatalytic bioreactors and their integration in industrial complexes which can allows the necessary microalgae cultivation for the above purposes. The steps of development:

- Laboratory testing
- Pilot testing
- Simulation of upscaling to industrial capacity
- Technological flowchart of a case of 4-line factory
- Technological flowchart of a case of integrated complex

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