BioTech 2020

and 8th Czech-Swiss Symposium with Exhibition

Book of Abstracts

Edited by Vojtěch Spiwok, Olga Maťátková, Michaela Rollová,Anna Miškovská, Markéta Kulišová, Leona Paulová, Jan Káš

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Vojtěch Spiwok, Olga Maťátková, Michaela Rollová,
Anna Miškovská, Markéta Kulišová, Leona Paulová, Jan Káš
Editors

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Welcome Message from Jan Káš

Dear symposium participants, sponsors and partners,

On behalf of the organizing committee I would like to thank you for participation at the symposium which had to be changed to virtual platform due to the continuing COVID pandemy. This biotechnological symposium has already long tradition. The first event was realized in our university building as joint Czech-Swiss symposium in September 4-7, 1999. Later on, the symposium has been changed to successful international meeting organized in a newly built National Technical Library located in closed proximity of two main buildings of the University of Chemistry and Technology. At the last 7th Biotech symposium participated over 200 scientists from 30 countries.

We were proud that the symposium participants appreciated not only the excellent scientific program but also the accompanying atmosphere in the course of the symposium (welcome party, refreshment during breaks, galla diner, Prague sightseeing) and whole day trip at the end of the symposium. There are some other advantages joint with the location of symposium place. It is located near airport, main railway station, a lot of accommodation facilities around and fast metro connection to town centre). We prepared organization of the BIOTECH 2020 in a reconstructed university building with nice 3 big lecture halls, plenty of small lecture rooms for seminars and meetings of “ad hoc” created groups, big room for welcome party and lunches, option to visit university brewery, university laboratories, etc. We hope that these symposium advantage will be utilized at some future event.

At present time, we would like to make you sure that we tried to prepare best conditions for successful performance of the virtual symposium platform. Of course, the symposium success depends also on yours kind collaboration a understanding. We believe that even under these condition you will find new friends and research partners.

Now, let me express thanks to professor Eva Zažímalová, the president of the Czech Academy of Sciences, and to professor Pavel Matějka, the rector of the University of Chemistry and Technology in Prague, who kindly accepted patronage of our symposium and supported its performance in many ways.

My thanks belong also to all members of the organizing committee, chairs of thematic sections, students and all active symposium participants who all had to face new approaches in the ways of presenting scientific discoveries.

Jan Káš

Welcome Message from Pavel Matějka, Rector of University of Chemistry and Technology, Prague

Distinguished guests, colleagues, ladies, and gentlemen,

As the rector of the University of Chemistry and Technology Prague, one of the five best Czech universities as demonstrated by the worldwide rankings, I would like to warmly welcome all the participants of the Biotech 2020 and the 8th Czech-Swiss Symposium in the virtual form. It is an honour for me to be one of the patrons of this event. The Biotech conferences and the Czech-Swiss Symposiums exhibit a long tradition of meetings scoped on all important fields of advanced biotechnologies which are reflected in the structure of multiple sessions of the current meeting from medical and pharmaceutical biotechnology over industrial and agricultural biotechnologies to environmental biotechnology.

Despite the exceptional situation of the pandemic disease COVID-19 causing the postponement of the conference and the change to an online meeting, I hope that it will be a great event of reunion of experienced scientists, pioneering engineers, young innovative researchers and enthusiastic students interested in biotechnologies coming from various countries, different universities, research institutes and industrial companies. I am sure that excellent lectures inspire you to fruitful discussion during the meeting and to subsequent exciting research in your research teams. I wish you a unique meeting as a starting point for new collaborations and, of course, for strengthening existing partnerships.

It is a great pleasure for me, as a representative of the University of Chemistry and Technology (UCT) Prague, that our university is one of the organizers of this event. I would like to thank the colleagues from the University of Applied Sciences and Arts Northwestern Switzerland, Czech Biotechnology Society and Swiss Biotech Association for co-organization of this brilliant event. A lot of work has been done both by the Organizing Committee (headed by prof. Jan Káš from the Department of Biochemistry and Microbiology, UCT Prague) and the Scientific Committee (headed by prof. Petra Patáková from the Department of Biotechnology, UCT Prague). Both the Department of Biochemistry and Microbiology, and the Department of Biotechnology are excellent research workplaces of the Faculty of Food and Biochemical Technology, UCT Prague. They include outstanding research teams in the fields of microbial ecology, animal biochemistry, molecular biology and virology, plant biochemistry, proteins with biotechnological potential, bio-affinity techniques, applied proteomics, microbial processes, bioengineering etc.; that means in the research areas within the scope of this conference. Nevertheless, I would like to notify you that various issues of biotechnology are important for researchers of other faculties of UCT Prague including colleagues from the Faculty of Environmental Technology, Faculty of Chemical Engineering and somewhat the Faculty of Chemical Technology considering the interdisciplinarity of current research and the advanced requirements of modern industrial technologies.

The marvellous advances in the field of biotechnology achieved worldwide exhibit a substantial impact on the sustainability of our civilization and the harmony of human society with nature.

I wish a great success of the Biotech 2020 and the 8th Czech-Swiss Symposium developing biotechnology research and the human community.

Pavel Matějka

Welcome Message from Eva Zažímalová, President of the Czech Academy of Sciences

Dear Ladies, dear Gentlemen,

Allow me first to thank the organizers of "BioTech 2020 and 8th Czech-Swiss Symposium" for the invitation to this important event, the aim of which is to promote an exchange of information among academic and industrial researchers. For me, it is an honour and pleasure to welcome on behalf of the Czech Academy of Sciences all of you, who have come to discuss the topic of advanced biotechnologies.

In accord with the focus of my short talk, I would like to recall the fact that current scientific and technological development entails an increasing expectation of society towards science. In addition, the COVID-19 pandemic has further increased these expectations. The main challenges in today's world include the sustainability of the quality of life and the environment, the rational use of natural resources, food security, social solidarity and control of the impact of rapid technological change on nature, society and the individual. The possibilities of resolving these challenges within nation-states are limited and determined by the means that the given state has available. At the same time, it is becoming increasingly evident that only large interdisciplinary teams, beyond individual countries and institutions, can find answers to a number of research problems. At symposia such as BioTech 2020, which create a unique network of researchers from many fields of biotechnology and related disciplines, the importance of international scientific collaboration is clearly demonstrated. Over the years, cooperation with our Swiss colleagues proved to be especially advantageous and fruitful. I am therefore pleased to state that the Czech Academy of Sciences as a long-term partner of the University of Chemistry and Technology, Prague, is a regular guest at this event.

In this regard, I would like to mention that the basic feature of the institutional arrangement of science and research in the Czech Republic is the coexistence and cooperation between universities and research institutes established by the Czech Academy of Sciences. The Academy with Higher Education Institutions and applied research organizations form a functional complex, each component of which fulfills its irreplaceable role. This is particularly important and significant in the case of genuinely inter- and multidisciplinary topic - such as biotechnology. Synergistic effects of this cooperation facilitate the augmentation and application of new discoveries and knowledge, increase the level of education and create conditions for increasing

competitiveness of any country, including the Czech Republic.

It is beyond any doubt that scientific communication and exchange of information and ideas are among the basic features of the academic profession; therefore, I wish the "BioTech 2020 and 8th Czech-Swiss Symposium" many more successful years in its activities.

Eva Zažímalová

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Program at Glance

Wednesday 16 June 2021
Hall 1

9:00-9:10 – Official opening
9:10-9:30 – Welcome by officials
9:30-10:30 – **Plenary lecture:** Marek Basler *Biozentrum, University of Basel, Switzerland*
10:50-12:20 – **Environmental Biotechnology I** (Chair: K. Demnerová, P. Dürre)
13:00-14:30 – **Environmental Biotechnology II** (Chair: K. Demnerová, P. Dürre)
14:50-16:10 – **Environmental Biotechnology III** (Chair: K. Demnerová, P. Dürre)

Wednesday 16 June 2021
Hall 2

10:40-12:20 – **Plant Biotechnology** (Chair: O. Valentová, L. Burketová)
13:00-14:20 – **Microalgae Biotechnology I** (Chair: K. Bišová)
14:40-16:00 – **Microalgae Biotechnology II** (Chair: K. Bišová)
16:40-18:00 – **Microalgae Biotechnology III** (Chair: K. Bišová)

Thursday 17 June 2021
Hall 1

9:00-10:00 – **Plenary lecture:** Flavia Marinelli *Department of Biotechnology and Life Sciences, University of Insubria, Italy*
10:00-11:20 – **Medical and Pharmaceutical Biotechnology I** (Chair: O. Maťátková, F. Marinelli)
11:50-13:40 – **Medical and Pharmaceutical Biotechnology II** (Chair: O. Maťátková, F. Marinelli)
14:10-15:50 – **Medical and Pharmaceutical Biotechnology III** (Chair: O. Maťátková, F. Marinelli)
16:10-18:00 – **Poster Session**

Friday 18 June 2021
Hall 1

9:00-10:00 – **Plenary lecture:** Peter Dürre *Institute of Microbiology and Biotechnology, University of Ulm, Germany*
10:00-11:00 – **Food and Agriculture Biotechnology I** (Chair: P. Dostálek, F. Chen)
11:40-13:10 – **Food and Agriculture Biotechnology II** (Chair: P. Dostálek, F. Chen)
14:00-15:10 – **Food and Agriculture Biotechnology III** (Chair: P. Dostálek, F. Chen)
15:20-16:30 – **Industrial Biotechnology I** (Chair: P. Bojarová)
16:40-17:40 – **Industrial Biotechnology II** (Chair: P. Bojarová)
17:40-18:00 – Closing Ceremony

Program

Wednesday 16 June 2021
Hall 1

9:00-9:10 – Official opening

9:10-9:30 – Welcome by officials

9:30-10:30 – **Plenary lecture:** Type VI Secretion System: From the Discovery to the Mode of Action of a Dynamic Bacterial Nanomachine (PL1)
Marek Basler *Biozentrum, University of Basel, Switzerland*

10:30-10:50 – break

10:50-12:20 – **Environmental Biotechnology I** (Chair: K. Demnerová, P. Dürre)

10:50-11:40 – Butanol stress response mechanisms and butanol tolerance in *Clostridium beijerinckii* NRRL B-598 (L1)
Petra Patáková *Department of Biotechnology, UCT Prague, Czech Republic*

11:40-12:00 – *Clostridium beijerinckii* and diolis: high similarity of genomes versus different behavior (L2)
Karel Sedlář *Department of Biomedical Engineering, Brno University of Technology, Czech Republic*

12:00-12:20 – Engineering *Pseudomonas putida* whole-cell biocatalysts for biotechnological processing of lignocellulosic substrates (L3)
Pavel Dvořák *Masaryk University, Czech Republic*

12:20-13:00 – break

13:00-14:30 – **Environmental Biotechnology II** (Chair: K. Demnerová, P. Dürre)

13:00-13:50 – Challenges for bioleaching at mine sites (L4)
Maria Vila *Faculty of Engineering, University of Porto, Portugal*

13:50-14:10 – Surface properties and colonization of newly developed flexible polyurethane foam biofilm carriers (L5)
Martin Halecký *Department of Biotechnology, UCT Prague, Czech Republic*

14:10-14:30 – Estrogens in wastewaters and evaluation of different types of treatment processes for estrogens removal (L6)
Eliška Maršálková *Institute of Botany of the CAS, Czech Republic*

14:30-14:50 – break

14:50-16:10 – **Environmental Biotechnology III** (Chair: K. Demnerová, P. Dürre)

14:50-15:10 – Secondary plant metabolites and the ecology of plant-associated bacteria (L7)
Ondřej Uhlík *Department of Biochemistry and Microbiology, UCT Prague, Czech Republic*

15:10-15:30 – Macroalgae as feedstock for fuels and chemicals (L8)
Ana Maria López-Contreras *Wageningen Food and Biobased Products, Netherlands*

15:30-15:50 – Biological biomethane production by conversion of CO, CO_2 and H_2 by technical anaerobic culture (L9)
Dana Pokorná *Department of Water Technology and Environmental Engineering, UCT Prague, Czech Republic*

15:50-16:10 – Benefits and limitations of different agriculture soil amendments (L10)
Hana Stiborová *Department of Biochemistry and Microbiology, UCT Prague, Czech Republic*

Wednesday 16 June 2021
Hall 2

10:40-12:20 – **Plant Biotechnology** (Chair: O. Valentová, L. Burketová)

10:40-11:00 – Plant viruses in the genome editing era (L11)
Tomáš Moravec *Institute of Experimental Botany of the CAS, Czech Republic*

11:00-11:20 – Production of recombinant antimicrobial peptides in barley (L12)
Ivo Frébort *Czech Advanced Technology and Research Institute, Palacký University Olomouc, Czech Republic*

11:20-11:40 – How extracellular vesicles of bacteria interact with plants (L13)
Martin Janda *South Bohemia University in České Budějovice, Czech Republic*

11:40-12:00 – A novel insight into the effect of *Pythium oligandrum* isolates as biological control agents (L14)
Kateřina Bělonožníková *Department of Biochemistry, Charles University, Czech Republic*

12:00-12:20 – Impact of noble metal nanoparticles on plants and associated microorganisms (L15)
Lenka Burketová *Institute of Experimental Botany of the CAS, Czech Republic*

12:20-13:00 – break

13:00-14:20 – **Microalgae Biotechnology I** (Chair: K. Bišová)

13:00-13:20 – Mutagenesis of green algae to recover desired phenotypes (L16)
Kateřina Bišová *Institute of Microbiology of the CAS, Czech Republic*

13:20-13:40 – Green Bioprinting – a tool for creating functional 3D-cell structures (L17)
Felix Krujatz *Institute of Natural Materials Technology, TU Dresden, Germany*

13:40-14:00 – Luxury phosphorus uptake: pushing to the limits (L18)
Alexei Solovchenko *Lomonosov Moscow State University, Russia*

14:00-14:20 – Construction of bio-inspired algal-bacterial consortium capable of phosphorus biosequestration from waste waters (L19)
Petr Zaytsev *Lomonosov Moscow State University, Russia*

14:20-14:40 – break

14:40-16:00 – **Microalgae Biotechnology II** (Chair: K. Bišová)

14:40-15:00 – Cyanobacterial biofilms as light-driven biocatalysts – obstacles and achievements (L20)
Katja Bühler *Helmholtz Center for Environmental Research, Germany*

15:00-15:20 – Microorganism in the LED-spotlight: standardized illumination systems for biotechnological applications (L21)
Harald Schöbel *MCI – THE ENTREPRENEURIAL SCHOOL, Austria*

15:20-15:40 – Bio-inspired materials from microalgal cells on chitosan-based carriers for biocapture of nutrients (L22)
Svetlana Vasilieva *Lomonosov Moscow State University, Russia*

16:00-16:40 – break

16:40-18:00 – **Microalgae Biotechnology III** (Chair: K. Bišová)

16:40-17:00 – Engineering Recombinant Antimicrobial Efficacy and Yield from Algae (L24)
Nanakow Baiden *Rothamsted Research, United Kingdom*

17:00-17:20 – Electroflocculation – a way to minimize microalgae harvesting cost (L25)
Simona Lucáková *Department of Biotechnology, UCT Prague, Czech Republic*

17:20-17:40 – New production models of valuable bio-products from microalgal biomass using countercurrent chromatography (CCC) technology (L26)
José Cheel *Centre ALGATECH, Institute of Microbiology of the CAS, Czech Republic*

17:40-18:00 – A biorefinery approach to obtain high-value polyunsaturated fatty acids from the microalgae *Schizochytrium limacinum* using liquid-liquid chromatography (L27)
Daniela Bárcenas Pérez *Centre ALGATECH, Institute of Microbiology of the CAS, Czech Republic*

Thursday 17 June 2021
Hall 1

9:00-10:00 – **Plenary lecture:** Where Will Novel Antibiotics Come from in Nature? (PL2)
Flavia Marinelli *Department of Biotechnology and Life Sciences, University of Insubria, Italy*

10:00-11:20 – **Medical and Pharmaceutical Biotechnology I**
(Chair: O. Maťátková, F. Marinelli)

10:00-10:20 – Galectin-glycoconjugate interactions in biomedical research (L28)
Pavla Bojarová *Institute of Microbiology of the CAS, Czech Republic*

10:20-10:40 – Engineering patients' immune cells with chimeric antigen receptor to treat hematological cancers (L29)
Vera Luginbuehl *Novartis Pharma Schweiz AG, Switzerland*

10:40-11:00 – Cryo-electron tomography: structural biology in situ (L30)
Martin Obr *IST Austria, Austria*

11:00-11:20 – Engineered antibodies for prostate cancer imaging and therapy (L31)
Zora Nováková *Institute of Biotechnology of the CAS, BIOCEV, Czech Republic*

11:20-11:50 – break

11:50-13:40 – **Medical and Pharmaceutical Biotechnology II**
(Chair: O. Maťátková, F. Marinelli)

11:50-12:10 – Magnetic nanoconjugated glycopeptides as novel tools for fighting bacterial infections (L32)
Francesca Berini *Department of Biotechnology and Life Sciences, University of Insubria, Italy*

12:10-13:00 – Use of RLI-15, a clinical grade fusion protein with IL-15 superagonistic activity for the activation of anti-tumor immune response (L33)
Radek Špíšek *Sotio, Czech Republic*

13:00-13:20 – Interaction patterns of natural killer cell receptors and ligands with CTL fold (L34)
Jan Dohnálek *Institute of Biotechnology of the CAS, Czech Republic*

13:20-13:40 – Production of therapeutic plasmid DNA in single-use technology (L35)
Melanie Ottinger *Thermo Fisher Scientific, Switzerland*

13:40-14:10 – break

14:10-15:50 – **Medical and Pharmaceutical Biotechnology III**
(Chair: O. Mať átková, F. Marinelli)

14:10-14:30 – Antimicrobial properties of biosynthesized nanoparticles (L36)
Olga Mať átková *Department of Biotechnology, UCT Prague, Czech Republic*

14:30-14:50 – Spontaneous in situ endothelialization as an advanced approach for construction of vascular replacements (L37)
Lucie Bacakova *Institute of Physiology of the CAS, Czech Republic*

14:50-15:10 – Non-thermal plasma for management of clinically relevant pathogens (L38)
Vladimír Schöltz *Department of Physics and Measurements, UCT Prague, Czech Republic*

15:10-15:30 – The PIXL&PIER methodologies (photo-induced cross-linking & electron release): novel experimental tools for structural and functional biochemistry (L39)
Miroslav Šulc *Department of Biochemistry, Charles University, Czech Republic*

15:30-15:50 – The epidemiological situation of measles in Tirana, Albania, from 2017 to 2019 (L40)
Blerta Laze *Department of Biology, University "Ismail Qemali" of Vlora, Albania*

15:50-16:10 – break

16:10-18:00 – **Poster Session**

Friday 18 June 2021
Hall 1

9:00-10:00 – **Plenary lecture:** Metabolic Engineering of Anaerobic Acetogens for Converting Greenhouse Gasses into Sustainable Products (PL3)
Peter Dürre *Institute of Microbiology and Biotechnology, University of Ulm, Germany*

10:00-11:00 – **Food and Agriculture Biotechnology I** (Chair: P. Dostálek, F. Chen)

10:00-10:20 – Gas management in the cultivation of microalgae: Use of membrane contactors for bubble-free gas transfer in tubular photobioreactos (L41)
Wolfgang Riedl *FHNW, Switzerland*

10:20-10:40 – How to reduce extract losses in industrial beer fermentation? (L42)
Edyta Kordialik-Bogacka *Lodz University of Technology, Poland*

10:40-11:00 – From traditional culture collection to sustainable and healthy foods (L43)
Fabian Wahl *Agroscope, Switzerland*

11:00-11:40 – break

11:40-13:10 – **Food and Agriculture Biotechnology II** (Chair: P. Dostálek, F. Chen)

11:40-12:30 – Biotransformation in Food Processing (L44)
Pavel Dostálek *Department of Biotechnology, UCT Prague, Czech Republic*

12:30-12:50 – Non-Saccharomyces yeasts in brewing (L45)
Gabriella Kun-Farkas *Institute of Food Science and Technology, Hungarian University of Agriculture and Life Sciences, Hungary*

12:50-13:10 – The use of sensomics in controlling of brewing process (L46)
Jana Olšovská *Research Institute of Brewing and Malting, Czech Republic*

13:10-14:00 – break

14:00-15:10 – **Food and Agriculture Biotechnology III** (Chair: P. Dostálek, F. Chen)

14:00-14:50 – Advances in Acid Resistance Mechanisms of Acetic Acid Bacteria (L47)
Fusheng Chen *Huazhong Agricultural University, P.R.China*

14:50-15:10 – Metabolites and their applications of *Monascus* spp. and the underlying essentials of genetics (L48)
Yanchun Shao *Huazhong Agricultural University, P.R.China*

15:10-15:20 – break

15:20-16:30 – **Industrial Biotechnology I** (Chair: P. Bojarová)

15:20-16:10 – The Golgi Glycan Factory (GGF) – Enzyme Modules for Glycoconjugate Synthesis (L49)
Lothar Elling *Institute for Biotechnology and Helmholtz-Institute for Biomedical Engineering, RWTH Aachen University, Germany*

16:10-16:30 – Biotechnological production of polyhydroxyalkanoates employing stress-adapted microorganisms (L50)
Stanislav Obruča *Faculty of Chemistry, Brno University of Technology, Czech Republic*

16:30-16:40 – break

16:40-17:40 – **Industrial Biotechnology II** (Chair: P. Bojarová)

16:40-17:00 – Biotechnology potential of aldoxime- and nitrile-converting enzymes (L51)
Ludmila Martínková *Institute of Microbiology of the CAS, Czech Republic*

17:00-17:20 – Substrate oxidation and electron transfer mechanisms in the active site of bilirubin oxidase (L52)
Tomáš Kovaľ *Institute of Biotechnology of the CAS, BIOCEV, Czech Republic*

17:20-17:40 – Can Nanotextiles Enhance Filtration Capacity of HEPA Filters for Microorganisms? (L53)
Daniela Obitková *Department of Healthcare, CTU Prague, Czech Republic*

17:40-18:00 – Best Posters Awards and Closing Ceremony

Abstracts

Plenary lectures

PL1

Type VI secretion system: from the discovery to the mode of action of a dynamic bacterial nanomachine

Marek Basler[1]

[1] Biozentrum, University of Basel, Switzerland

Bacteria have evolved several nanomachines to deliver proteins from their cytosol to target cells. These nanomachines differ in their structure, secrete various substrates and are often essential for bacterial survival in diverse environments. In my talk, I will describe the journey from the discovery of the Type VI secretion system (T6SS) to the current understanding of its structure, dynamics and mode of action. I will explain how we used a combination of live-cell imaging, cryo-electron microscopy and genetics to show that T6SS is a powerful speargun that pushes proteins across membranes of both eukaryotic and bacterial cells. I will provide evidence that T6SS is evolutionarily related to contractile phage tails and show that subcellular localization of T6SS assembly is in many bacteria regulated with a remarkable precision with implications for its function. I will discuss how T6SS influences bacterial pathogenesis, competition and horizontal gene transfer.

PL2

Where will novel antibiotics come from in nature?

Flavia Marinelli[1]

[1] Department of Biotechnology and Life Sciences, University of Insubria

Discovery of novel antibiotics has dramatically slowed down. After the 'golden era' peaked around 1950s, the number of antibiotics marketed each decade has declined. Conversely, the rapid spread of antibiotic resistance among pathogenic bacteria makes the development of novel drugs compulsory. Notably, ESKAPE pathogens (six multidrug-resistant, nosocomial pathogens: *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* spp.), were recently included by the World Health Organization in the list of the life-threatening disease agents which need to be tackle using new drugs. Many are the factors, which exert a negative impact on the antibiotic discovery rate: from the unease to identify new essential susceptible targets and to discover novel active chemical entities, to the regulatory challenges and the limited economic returns that often discourage pharmaceutical companies from investing into the field. In this post-antibiotic era, the best hope for developing a new generation of antibiotics is to discover novel microbial natural products by combining the classical successful biological activity-guided screening (so called Waksman platform) with the perspectives opened by the new genomic tools for genome mining and for the rational engineering of homologous and heterologous hosts. In this presentation, we focus on the exploitation of these tools for discovering and developing novel and old glycopeptide antibiotics (GPAs) facing the spread of multi-drug resistant gram positives. Genome sequencing has in fact recently revealed the organization of GPA biosynthetic gene clusters in pharmaceutically valuable actinobacteria, including long known GPA-producers, as well as in novel producing strains and metagenomic DNA. In addition, although the global regulation of GPA biosynthesis is still largely unexplored, the pathway specific regulation controlling the expression of BGCs is being elucidated in model systems, paving the way to better understand how to effectively produce GPAs for clinical practice.

PL3

Metabolic engineering of anaerobic acetogens for converting greenhouse gases into sustainable products

Peter Dürre[1]

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Autotrophic acetogenic bacteria employ the so-called Wood-Ljungdahl pathway to produce naturally acetate, ethanol, and/or 2,3-butanediol from gaseous substrates such as CO_2 + H_2 or syngas (mostly a mixture of CO + H_2). To date, different acetogens are used in industrial applications in pilot and demonstration plants aiming at ethanol formation from different syngas sources. A full scale commercial facility for ethanol production from steel mill waste gases started operation in Chinain 2018. A major challenge is to metabolically reengineer these bacteria for formation of other interesting bulk or speciality chemicals, allowing fermentation with an abundant, cheap carbon source and, in parallel, even consumption of greenhouse gases. Two such examples will be presented.

A pathway for acetone production was introduced into *Acetobacterium woodii*, the model acetogen for growth on CO_2 + H_2. The pathway consisted of the genes encoding thiolase, coenzyme A transferase, and acetoacetate decarboxylase, originally stemming from *Clostridium acetobutylicum*. Further improvements were made by optimizing ribosome binding sites and using a thiolase gene from *C. kluyveri* and CoA transferase genes from *C. aceticum*.

Other low molecular platform chemicals are isobutanol and 3-hydroxybutyrate. A pathway for isobutanol formation based on ketoisovalerate: ferredoxin oxidoreductase (Kor) and aldehyde/alcohol dehydrogenase (AdhE2) was successfully established in *A. woodii.* A model organism for utilization of CO-containing gas mixtures (syngas) is *Clostridium ljungdahlii*. In this organism, a pathway consisting of ketoisovalerate decarboxylase (Kiv) and alcohol dehydrogenase proved to be suited for isobutanol formation.

In another acetogen, *C. coskatii*, 3-hydroxybutyrate could be successfully produced after heterologous expression of other clostridial genes encoding thiolase, CoA transferase, and secondary alcohol dehydrogenase.

Clostridial genomes were screened for genes leading to poly-3-hydroxybutyrate (PHB) formation. *C. acetireducens* was found to carry *phaC*, *phaE*, and *phaJ* genes. These were subcloned, together with genes leading to crotonyl-CoA formation, into *C. ljungdahlii* and *C. coskatii*. Both recombinant species were able to produce PHB under autotrophic conditions.

Lectures

L1

Butanol stress response mechanisms and butanol tolerance in *Clostridium beijerinckii* NRRL B-598

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1-butanol can be produced by acetone-butanol-ethanol (ABE) fermentation by different species of clostridia including *C. beijerinckii*. The ABE fermentation can be considered a great example of sustainable technology based on circular economy where different kinds of waste from agriculture and industry can be utilized. While industrial ABE process was utilized for butanol and/or acetone production throughout the world in past, nowadays it cannot compete with oil processing mainly because low attainable butanol titer. This bottleneck is mainly caused by low butanol tolerance which results in severe product inhibition. Deep understanding of butanol stress mechanisms is of key importance for increase of butanol tolerance. Transcriptomic profiling during standard [1, 2] and butanol shock [3] ABE fermentations was used to detect main mechanisms of butanol induced stress response. Butanol shock resulted in upregulation of heat shock protein genes and genes for cyclopropanation of membrane fatty acids and formation of plasmalogens. Although, it is questionable whether efflux pumps activation belong to butanol stress response too, upregulation of several putative butanol efflux pumps genes were found after butanol addition. Further evidences for efflux as a mechanism involved in butanol tolerance are obtaining random mutants with increased efflux and increased tolerance and profile of enhanced efflux activity during solventogenic phase under standard conditions in comparison to the conditions under which the solventogenesis was impaired. A new flow cytometric assay based on direct efflux of ethidium bromide was used for measurement of efflux capacity.

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References:

1. Sedlar K., Koscova P., Vasylkivska M., Branska B., Kolek J., Kupkova K., Patakova P., Provaznik I. Transcription profiling of butanol producer *Clostridium beijerinckii* NRRL B-598 using RNA-Seq. *BMC Genomics* 2018, 19(1): 415

2. Patakova P., Branska B., Sedlar K., Vasylkivska M., Jureckova K., Kolek J., Koscova P., Provaznik I. Acidogenesis, solventogenesis, metabolic stress response and life cycle changes in *Clostridium beijerinckii* NRRL B-598 at the transcriptomic level. *Sci. Rep.* 2019, 9:1371

3. Sedlar K., Kolek J., Gruber M., Jureckova M. Branska B., Csaba G., Vasylkivska M., Zimmer R., Patakova P., Provaznik I.. A transcriptional response of *Clostridium beijerinckii* NRRL B-598 to a butanol shock. *Biotechnology for Biofuels* 2019, 12: 243

L2

Clostridium beijerinckii and diolis: high similarity of genomes versus different behavior

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Solventogenic bacteria from the genus *Clostridium* are studied for their potential industrial use in production of biofuels. *Clostridium beijerinckii* belongs among widely studied species thanks to its ability of acetone-butanol-ethanol (ABE) fermentation utilizing wide range of waste materials, e.g. feathers, wheat straw, etc. During the reidentification of the strain *C. beijerinckii* NRRL B-598 (GenBank accession no. CP011966.3), formerly known as *C. pasteurianum* NRRL B-598, we found out its high genome similarity to the strain *C. diolis* DSM 15410. Last year, *C. beijerickii* and *C. diolis* were reclassified to be the same species based on digital DNA—DNA hybridization (dDDH) of incomplete genomes. We believe this should be studied in more detail. Therefore, we decided to sequence and assemble the first complete genome sequences of type strains *C. diolis* DSM 15410 and *C. beijerijckii* DSM 791 and to compare them with other complete *C. beijerinckii* genome sequences. Our results showed that genomes share very high similarity as their dDDH value were estimated to be close to 90%. Although values higher than 70% suggest that the genomes belong to the same species and values higher than 79% even the same subspecies, analyzed strains are characterized by substantial differences in phenotype. For example, while the strain *C. beijerijckii* NRRL B-598 is unable to utilize glycerol and produces mainly butanol during ABE fermentation, the strain *C. diolis* DSM 15410 utilizes glycerol and produces propanediol. In this presentation, we provide a genome comparison of several strains and we try to explain the differences in their phenotype on a genome-wide scale as their identification seems to be crucial for a potential industrial use.

L3

Engineering *Pseudomonas putida* whole-cell biocatalysts for biotechnological processing of lignocellulosic substrates

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Pseudomonas putida KT2440, the best-characterized and safe pseudomonad, be-

longs among the most promising bacterial hosts for synthetic biology and biotechnology endeavors [1,2]. Recently, it has attracted attention as a new microbial platform for valorization of aromatic compounds and sugars derived from lignocellulosic waste [3]. The strain has been employed for the production of value-added chemicals from glucose, but the lack of certain metabolic traits and functions hinders its application for the utilization of a wider spectrum of (hemi)cellulosic carbohydrates.

We sought to meet this challenge by empowering *P. putida* with novel catabolic pathways and a system for efficient display of cellulases on *P. putida* surface. For that purpose, metabolic engineering, and synthetic biology approaches were combined with in-house *P. putida* KT2440-derived strains with reduced genomes and altered physiological properties. Isomerase pathway from *Escherichia coli* and intracellular beta-glucosidase from *Thermobifida fusca* implanted in *P. putida* mutant enabled rapid growth of the recombinant on D-xylose (μ=0.17 h^{-1}) and D-cellobiose (μ=0.35 h^{-1}) and co-utilization of these biotechnologically relevant sugars [3]. β-Glucosidase was also efficiently anchored to the surface of *P. putida* with a designer display system mimicking the function of natural cellulosomes. The project provides a showcase of expanding the catalytic scope of environmental bacterium toward biotechnological applications.

1. Nikel P.I., de Lorenzo V. *Pseudomonas putida* as a functional chassis for industrial biocatalysis: From native biochemistry to trans-metabolism. *Metab. Eng.* 2018, 50:142-155.

2. Dvořák P., Nikel P.I., Damborský J., de Lorenzo V. Bioremediation 3.0: Engineering pollutant-removing bacteria in the times of systemic biology. *Biotechnol Adv.* 2017, 35:845-866.

3. Dvořák P., de Lorenzo V. Refactoring the upper sugar metabolism of *Pseudomonas putida* for co-utilization of cellobiose, xylose, and glucose. *Metab. Eng.* 2018, 48:94-108.

L4

Challenges for bioleaching at mine sites

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Biomining or bioleaching is based on certain microorganisms' ability to break down minerals in order to obtain a soluble form of metals [1], [2]. Usually most metallic ore deposits contain iron or sulfide, but these elements have no special commercial value. The activity of leaching bacteria that obtain energy from the oxidation of ferrous iron, elemental sulfur, or partially oxidized sulfur compounds, could release other valuable metals [3], [4]. Literature reports bioleaching laboratory works of different metals as Cd, Co, Cu, Mo, Ni, Sn, Ti, U3O8 and Zn with recoveries in the range of 25-100% and leaching time from 6 to 500 days. However, at industrial scale, the number of success cases are reduced to Cu, Au, Co, Ni and U_3O_8.

The presence of indigenous chemoautotrophic bacteria at mine sites is widely known, being particularly abundant in acid mine drainage strains of Acidithioba-

cillus ferrooxidans and Leptosirillum ferrooxidans. Cultures of these autochthonous microorganisms are the most widely used in bioleaching of base and high-tech metals occurring in polymetallic sulphides.

In recent years there has been an emergence of bio-hydrometallurgical methods applied to mineral processing, as well as site remediation. However, many unanswered questions remain concerning the microbial dynamics and its role in bioleaching, bioaccumulation or even biosorption of metals (some with important economic value, others with harmful consequences for the environment).

In this work, the challenges of bioleaching/biomining are discussed, based on the experience and knowledge acquired in the last years the research projects authors have been involved. In particular, batch flask and column tests using a microbial consortium from different mine sites allowed to evaluate the biorecovery of different metal(oid)s varying experimental and natural (geological and mineralogical origin of samples) conditions.

References

[1] OKIBE, N.; Gericke. "Enumeration and Characterization of Acidophilic Microorganisms Isolated from a Pilot Plant Stirred-Tank Bioleaching Operation". Applied and. 2003, vol 69, p. 1936-1943.

[2] OLSON, G. J.; Brierley. "Bioleaching review part B: progress in bioleaching: applications of microbial processes by the minerals industries". Applied Microbiology and Biotechnology. 2003, vol 63, p. 249-257.

[3] PETERSEN, J .; Dixon. "Competitive bioleaching of pyrite and chalcopyrite". Hydrometallurgy. 2006, vol 83, p. 40-49.

[4] RAWLINGS, D. E.. "Heavy metal mining using microbes". Annu Rev Microbiol. 2002, vol 56, p. 65-91.

L5

Surface properties and colonization of newly developed flexible polyurethane foam biofilm carriers

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Four water-blown fully aliphatic polyurethane foams based on hexamethylene diisocyanate and four different diols of ethylene glycols and adipic acid were prepared and tested as porous biomass carrier for biotechnological applications in both production and decontamination areas. Foams were designed as microporous polyurethanes with adjustable rate of biodegradation and different surface properties. Fungus *Fusarium solani* and bacterium *Pseudomonas* sp. isolated from combined bioreactor treating waste air polluted with styrene and acetone were used as biological agents for foam testing. Carrier biodegradability was determined using respirometric measuring system OxiTop IS 6 as biological oxygen demand (BOD). Surface colonization was predicted by measurement of Zeta potential and contact angle followed by use XDLVO theory and thermodynamic approach and quantified by image ana-

lyses. Different molecular structure of foams affected all tested parameters, e.g. biodegradability, surface properties and biofilm development. *Fusarium solani* shown higher degradation ability against polyurethane foams based on triethylene glycol while *Pseudomonas* sp. against foams based on tetraethylene glycol. Results obtained using the XDLVO theory corroborated the trend obtained using thermodynamic approach. Above this, predictions of interactions in cell-water-carrier system are in agreement with measurements of carrier surface colonization.

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L6

Estrogens in wastewaters and evaluation of different types of treatment processes for estrogens removal

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The levels of five estrogenic hormones in influents and effluents from two different types of wastewater treatment plants (WWTPs) – conventional, i.e. mechanical-biological WWTPs and constructed wetlands (CWs) – were investigated in this study. The synthetic hormone 17α-ethynylestradiol (EE2) and natural 17β-estradiol (β-E2) were shown to be the source of 99% of the overall estrogenic activity in surface waters and effluents from WWTP. Estrone (E1) and estriol (E3) have lower estrogenic potencies. Solid-phase extraction (Oasis HLB) was used for the preconcentration of estrogens from the samples and two clean-up steps (using Florisil and NH_2 sorbents) ensured maximal removal of the interfering matrix. An LC-MS/MS method with dansyl chloride derivatization was used for measuring environmentally relevant steroid concentrations. Estrogen removal from CWs ranged from 38.0 to 97.8% and from conventional WWTPs, from 23.4 to 99.4%. Levels of E3, E2 and E1 in CW effluents exceeded the lowest-observed-effect concentrations (LOEC) which were proposed for vitellogenin induction. Two of the three investigated CWs showed higher removal efficiencies of estrogens in warm season (83.0-97.8%) than in cold season (18.0-81.8%). The plant composition, abundancy and especially the metabolic activity of the plants and microbial assemblages play an important role in removal estrogenic compounds from the constructed wetlands.

L7

Secondary plant metabolites and the ecology of plant-associated bacteria

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Given their intertwined evolutionary history and close environmental proximity, plants and microorganisms have developed extensive interactions which are essential for the proper functioning of entire ecosystems. Plants, or more specifically rhizodeposition, are the major suppliers of carbon in soil, soil prokaryotes and fungi, in turn, are the primary decomposers of this photosynthetically-derived organic carbon. Among the plant-derived organic matter, secondary plant metabolites (SPMs) seem to be of outstanding importance in controlling soil ecology: not only can SPMs serve as carbon and/or energy sources for bacteria but they often have antimicrobial activity or ability to disrupt bacterial quorum sensing and some SPMs have been predicated of regulating the decomposition of organic matter. This all leads to the **hypothesis that SPMs are among key features controlling rhizosphere microbial community structure**. At the same time, rhizosphere is the primary entry point into the endosphere which then leads to the **hypothesis that community composition of the endophytic microorganisms is, at least partially, controlled by rhizodeposition.** Because insects and other herbivores are continually developing mechanisms of resistance to SPMs, plants are driven to modify and develop new mechanisms of protection, including modification of SPMs. These changes in plant SPM content and composition in turn affect microbial populations which have established detoxifying enzymes often with broad substrate; this then leads to the **hypothesis that enzymes originally evolutionarily developed for the detoxification and/or degradation of SPMs are also fortuitously involved in the degradation of anthropogenic pollutants**. To this end, I will present the most recent findings from the Laboratory of Microbial Ecology of the UCT Prague which center on the above hypotheses and deepen our insight into how SPMs shape composition of plant-associated microbiota and how they relate to the function of biodegradative enzymes.

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L8

Macroalgae as feedstock for fuels and chemicals

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Marine macroalgae (seaweeds) has attracted attention as the new feed stock for 3rd generation production of biofuels, as an alternative to present fossil transportation fuels and bulk chemicals[1]. In contrast to 1st and 2nd generation feedstock, seaweed neither competes for fresh water nor agricultural land. In addition, it is an abundant and fast-growing resource, it is renewable , takes up CO_2 from the environment and assimilates inorganic elements from sea water. As biomass for biorefinery, in general it has the advantage compared to lignocelluloses that it does not require harsh/extreme conditions of temperature or pH in their pretreatments for disruption of the seaweed cell wall and enzymatic hydrolysis prior to fermentation into bio-fuels[2].

Cultivation costs of seaweeds are high, but demand for seaweed derived products, especially for the production of food in Japan, China and the republic of Korea, is larger than supply from the wild, which stimulates the development of new and efficient cultivation methods. Brown seaweeds such as kelps (including *Saccharina* sp. and *Laminaria* sp.) can be cultivated in the sea in the Atlantic ocean, and show potential to be a good substrate for production of fuels and chemicals by fermentation since they can be rich in fermentable components. Kelp biomasses are made up of several different polysaccharides, mainly laminarin, fucoidan and alginate and the monosaccharide mannitol in season dependent ratios. In this presentation, results on the advances in the fractionation, desalting and fermentation to acetone, butanol and ethanol (ABE) of *Saccharina latissima* cultivated biomass will be shown. The organism used for fermentation was a strain of *Clostridium beijerinckii* that was adapted to grow on seaweed hydrolysate showing utilisation of all sugars for ABE production. Upscaling of the ABE fermentation from seaweed sugars for fuel production for use in engine tests will be shown.

Acknowledgement

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References

[1] van Hal, Jaap W., W. J. J. Huijgen, and A. M. López-Contreras. "Opportunities and challenges for seaweed in the biobased economy." Trends in biotechnology 32.5 (2014): 231-233.

[2] del Río, P. G., et al. (2019). "Recent trends on seaweed fractionation for liquid biofuels production." Bioresource Technology: 122613.

L9

Biological biomethane production by conversion of CO, CO_2 and H_2 by technical anaerobic culture

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Methanogenic Archae are part of technical cultures of anaerobic fermenters processing complex organic matter into biogas, the majority components of which are methane and carbon dioxide. The concentration of CH_4 in biogas depends on the reduced state of substrate and used to be 52 – 65 % [1]. For better distribution and utilization of methane, its concentration needs to be higher than 95 %. Amount of H_2 from organic substrates is not enough to convert all CO_2 to CH_4, therefore external H_2 or other sources of reducing equivalents e.g. CO are introduced to the process. Hydrogenotrophic methanogens in the technical culture are of particular importance because they are able to reduce CO_2 to methane using H_2. In anaerobic fermenters, microorganisms are also present to some extent, which can metabolize CO [2].

Syngas, a gaseous product of the pyrolysis of poorly degradable organic substances, contains mainly CO and H_2 in different proportions depending on the pyrolysis conditions. The syngas components fed to the anaerobic fermenter can be converted

to methane directly or through the linked metabolisms of several cooperating microbial groups, such as carboxydotrophic and hydrogenotrophic methanogens and homoacetogenic bacteria [3].

The direct supply of these gases into the anaerobic fermenter may inhibit other anaerobic microorganisms and it is important to find the optimum gas dosing and process conditions. The experiments were performed in a stirred thermophilic bioreactor processing the sewage sludge; the technological parameters corresponded to a full-scale bioreactor at the WWTP. Some inhibition problems can be avoided by using a separate external bioreactor, where only gases are fed and the active culture consists mainly of those species that are able to metabolize them; a trickle bed biofilm reactor was successfully used in our project.

Acknowledgement

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References

1. Enzmann, F., et al., *Methanogens: biochemical background and biotechnological applications. AMB Express*, 2018. **8**(1): p. 1.

2. Zabranska, J. and D. Pokorna, *Bioconversion of carbon dioxide to methane using hydrogen and hydrogenotrophic methanogens. Biotechnology Advances*, 2018. **36**(3): p. 707-720.

3. Phillips, J.R., R.L. Huhnke, and H.K. Atiyeh, *Syngas Fermentation: A Microbial Conversion Process of Gaseous Substrates to Various Products.* 2017. **3**(2): p. 28.

L10

Benefits and limitations of different agriculture soil amendments

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Nowadays, agriculture address multiple challenges to meet the criteria for higher crop production. The main purpose of fertilizer application is to improve soil physicochemical properties, water retention, nutrient utilization, alteration of pH and increase soil organic carbon. All of these changes influence the soil microbial communities which are crucial for overall soil health. Many studies have revealed that the application of agricultural amendments impacts both microbial diversity and microbial biomass. In our laboratory, apart from organic and inorganic fertilizers, we studied the influence of biochar which also belongs to the most extensively studied soil amendments. However, its effect on soil strongly depends on the type of material used for its production, temperature of py-

rolysis and application rate. Therefore, the addition of biochar prepared from two different feedstock (plant biomass and waste from poultry slaughterhouse) under two different temperatures (350 °C and 500 °C) applied at two ratios, 2% and 5% (w/w), in two different soils, Cambisol and Luvisol, was compared. The impact on soil physicochemical properties and microbiota in terms of soil enzyme activities, diversity and microbial community structures of treated soils were evaluated at different time intervals within 6 months. To obtain the more comprehensive picture about the soil amendments, the effect of organic (manure and sewage sludge) and inorganic fertilizers was also assessed. In addition to the effect on bacterial and fungal community structures and soil properties, attention was also focused on the presence of antibiotic resistance genes and occurrence of pathogenic microorganisms.

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L11

Plant viruses in the genome editing era

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Plant viruses played an important role since the emergence of plant biotechnology. Viruses were used as vectors for overexpression of proteins in plants as well as tools for targeted silencing of endogenous genes (VIGS). Virus regulatory sequences were instrumental in the generation of the first generation of transgenic crops (CaMV 35S promoter). How can plant viruses contribute to the ongoing genome-editing revolution? Here we will discuss the use of plant viruses or their components as versatile tools to deliver genome-editing reagents into the plant cells and use of viruses as vectors for amplification of DNA template to facilitate homologous recombination. Last but not least plant viruses have the potential to facilitate the gene editing in crops that are recalcitrant to classical Agrobacterium transformation and regeneration protocols.

L12

Production of recombinant antimicrobial peptides in barley

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Antimicrobial peptides are key components of innate immunity in mammals. Numerous studies indicated that those peptides have potential applications in therapeutic applications, sanitization or cosmetics. However, the high cost of their chemical synthesis is a major limiting factor for commercial use. We have previously reported the production of the human antimicrobial peptide cathelicidin LL-37 in barley seeds driven by an endosperm specific promoter [1,2]. Besides, N-terminal

signal peptide for translocation through the endoplasmic reticulum and C-terminal return signal both facilitate the accumulation of the peptide in barley seeds. The isolated product then exhibited antimicrobial activity. In 2019 and 2020, field trials were conducted to prove the scalability of the production. For effective and low-cost purification, the fusion domain of the biosurfactant protein DAMP4 separated from LL-37 by a linker with an acidic cleavage site was included in the next generation of the production constructs. Such fusion proteins were first produced in bacteria, then purified by thermal denaturation in combination with sodium sulfate precipitation that removed most of the ballast proteins, and finally cleaved with acid to liberate the LL-37 product. The final product was purified to high homogeneity by a semi-preparative UPLC/MS. Selected fusion constructs were then optimized to create new barley lines for field production.

References

1. Holásková E., Galuszka P., Mičúchová A., Šebela M., Öz M.T., Frébort I. Molecular farming in barley: development of a novel production platform to produce human antimicrobial peptide LL-37. Biotechnol. J. 218, 13(6) 1700628

2. Holásková E., Galuszka P., Frébort I., Milek J. Method of production of barley plants producing antimicrobial peptides. WO/2019/052588

L13

How extracellular vesicles of bacteria interact with plants

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Extracellular vesicles (EVs) are produced by all forms of life including Gram-negative bacteria. EVs are cytosol-containing membrane spheres that provide selection, storage and protection against degradation of enclosed cargoes in a highly dynamic and environmental cue-responsive manner. Additionally vesiculation enables bacteria to quickly adapt to changing environments. Being underexplored, we are focus on the role of EVs from bacterial pathogens in shaping the interactions with plants. We recently found that the tomato pathogen *Pseudomonas syringae* pv. *tomato* (*Pto*) DC3000 releases EVs, characterized by electron microscopy (SEM, TEM) and nanoparticles tracking analysis (NTA). We demonstrated the vesiculation *in vitro* and *in planta*. The application of purified *Pto* DC3000 EVs induced prototypic immune responses in plants, i.e. defence gene expression, and promote

anti-bacterial immunity but not repressed seedlings growth. Using proteomics, we determined the *Pto* DC3000 EV proteome. Interestingly, a large proportion of the EV proteins appears to be regulated in response to microbial pattern-triggered immunity (PTI). Highly interesting group of proteins found in the proteome were connected with siderophore transport indicating possible role of EVs in bacteria iron acquisition. In conclusion, we will provide an overview about the recent knowledge of the bacterial EVs role in plant-bacteria interactions and EVs cargoes to be explored for biotechnology solutions in plant protection.

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L14

A novel insight into the effect of *Pythium oligandrum* isolates as biological control agents

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Pythium oligandrum is a promising biological control agent. This soil oomycete acts as a mycoparasite and thus protects plants from fungal pathogens. *P. oligandrum* interacts also with plant roots, activates plant defense response to pathogens *via* specific elicitors, thus functions as priming. In addition, *P. oligandrum* can synthetize auxin precursors and thus stimulate plant growth and fitness. In order to find out a novel, more effective strain with unique features, secretome and biochemical properties of 11 soil sample isolates from around the world were analyzed. Even closely related *P. oligandrum* isolates differ significantly in the content of secreted compounds into the medium. All the strains secrete proteins, amino acids, tryptamine, phenolic compounds, and hydrolytic enzymes capable to degrade cell walls (endo-β-1,3-glucanase, chitinases, and cellulase), exoglycosidases (especially β-glucosidase), proteases, both alkaline and acid phosphatases.

We tested the effect of seed coating by *P. oligandrum* isolates on the rapeseed me-

tabolism. While basic parameters were not affected, significant differences were found in the free individual amino acids, glucosinolates content, activity of antioxidant enzymes and phytohormone content (auxin and its precursors, all the forms of cytokinins, salicylic acid, jasmonate, and ethylene). The role of priming effect on the course of fungal pathogen (*Alternaria brassicicola* and *Verticilium longisporum*) and viral (*Tobacco mosaic virus*) infection was also tested.

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L15

Impact of noble metal nanoparticles on plants and associated microorganisms

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Within past decades, nanoparticles (NPs) become common components of electronics, batteries, cosmetics, clothing, and even dietary supplements. Despite their undisputed advantages consisting in the possibility of engineering their novel physical, thermal, optical, and biological properties, safety questions arise around their wide exploitation. NPs interact with living organisms such as humans, animals, plants or microorganisms, in which can interfere with essential life processes. Besides this direct effect, NPs can also modify a natural microbiome present on the organism's surface. NPs can also enter the soil and adversely affect soil microbiome, which can in the upshot attenuate plant growth and health as a side-effect.

Our study focuses on the impact of engineered noble metal NPs of silver, gold, and palladium on plant growth, immune system and phytopathogens, which has been poorly studied so far. Experiments performed on the model plant *Arabidopsis thaliana* and economically important crop oilseed rape (*Brassica napus*) showed a distinct impact of different noble metal NPs both on a cellular level (cytoskeleton rearrangement, callose deposition) and plant growth parameters [1]. Study of a direct antimicrobial effect against bacterial (*Pseudomonas syringae* pv. *tomato*) and fungal (*Leptosphaeria maculans* and *Alternaria brassicicola*) phytopathogens demonstrated the efficiency of silver and palladium NPs. In addition, the effect of silver and gold NPs on soil microbiome was investigated, showing the significant impact of silver NPs on microbial communities and disturbances in their total numbers, enzymatic activities and catabolic diversity.

1. Siegel J., Záruba K., Švorčík V., Kroumanová K., Burketová L., Martinec J. Round-shape gold nanoparticles: effect of particle size and concentration on *Arabidopsis thaliana* root growth. *Nanoscale Res. Lett.* 2018, **13**(1):95. doi: 10.1186/s11671-018-2510-9.

L16

Mutagenesis of green algae to recover desired phenotypes

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Microalgal biotechnology traditionally relies on bioprospecting in order to isolate algal strain with desired properties. It has been becoming clear that although powerful, bioprospecting is not omnipotent. Thus, there is an increasing demand for further modification of the algal strains already in use. Mutagenesis can be performed on any organism without previous knowledge on its genetic information or establishing methods of crossing. Moreover, the resultant mutants are not considered GMO and their cultivation is therefore not limited by legislation. This way any algal strain can be manipulated to produce mutant cells with desired phenotype. Here, we used two types of mutagens, UV light and ethyl-methanesulphonate (EMS), to produce mutants with desired properties in three different green alga species, *Desmodesmus quadricauda*, *Haematococcus pluvialis* and *Chlorella vulgaris*. For each of the species we established specific screening conditions to recover the desired phenotypes, established the optimal doses of mutagen and screened the mutant population. I will discuss the limitations and bottlenecks of the process and will suggest guidelines for successful mutant screens of biotechnologically relevant species.

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L17

Green Bioprinting – a tool for creating functional 3D-cell structures

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3D-Bioprinting - additive manufacturing with integrated living cells - is a strong field of research mainly in tissue engineering and regenerative medicine. The technology of Green Bioprinting, developed by interdisciplinary research at the TU Dresden, stands for a new approach combining the fields of additive manufacturing technologies, biotechnology, optical sensors and material & medical science. The structural embedding of cells within a hydrogel environment protect the production hosts from shear stress, improves the separation of cells from the medium and allows the development of new process strategies. The talk will discuss recent research on creating structured 3D-immobilization matrices for microalgae and plant cells and their potential medical and biotechnological applications [1,2]. A special focus will be on the selection of appropriate printing

matrices for particular cell types and material properties, and on monitoring cellular health [3] and growth in the scaffolds using optical technologies [4], e.g. fluorescence microscopy, optical active sensor-nanoparticles and pulse-amplitude modulated fluorometry which can be applied to viszualize respiration and photosynthetic processes within the hydrogel microenvironment. Green Bioprinting offers a wide range of medical and biotechnological applications as well in basic research (e.g. research on symbiotic living organisms, e.g. quorum sensing, artificial construction of natural multi-specie environments, local- and time resolved analysis of cell properties in response to external stimuli) as applied research in medicine and biotechnology (e.g. multi-step metabolic bioreactions, microalgae as natural oxygen source for mammalian cells).

References:

[1] Lode, A.et al. Green bioprinting: Fabrication of photosynthetic algae-laden hydrogel scaffolds for biotechnological and medical applications. *Eng. Life Sci.* 2015, **15**(2) 177-183.

[2] Krujatz, F. et al. Additive Biotech—Chances, challenges, and recent applications of additive manufacturing technologies in biotechnology. *New Biotech.* 2017, **39**: 222-231.

[3] Krujatz, F. et al. Green bioprinting: Viability and growth analysis of microalgae immobilized in 3D-plotted hydrogels versus suspension cultures. *Eng. Life Sci. 2015,* **15**(7): 678-688.

[4] Trampe, E. et al. Functionalized Bioink with Optical Sensor Nanoparticles for O_2 Imaging in 3D-Bioprinted Constructs. *Adv. Func. Mater.* 2018, **28**(45): 1804411.

L18

Luxury phosphorus uptake: pushing to the limits

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It is believed that the overwhelming majority of microalgal species evolved under conditions of scarce and fluctuating availability of phosphorus (P). Adaptations to P shortage include rearrangements of lipid metabolism and polar metabolome, downregulation of photosynthetic activity, and luxury uptake of P. The latter is defined as taking up more P that necessary for the progression of current cell cycle. All these adaptations are exploited in microalgal biotechnology. Thus, nutrient starvation induces accumulation of valuable carotenoids and lipids in the microalgal cells whereas luxury uptake is the basis of conversion of the nutrients from waste streams to phosphorus biofertilizers from microalgal biomass.

The mechanisms of microalgae acclimation to P starvation are relatively well studied, but luxury uptake of P remains enigmatic in many regards. Phosphorus starvation induces smaller penalties for the culture growth e.g. in comparison with those of nitrogen starvation since nutrient-replete microalgal cells normally possesses a sizeable reserve of P, frequently in the

form of inorganic polyphosphates which are created during the periods of luxury P uptake. Nevertheless, high concentrations of exogenic P can be toxic resulting in the inhibition of microalgal cell division or even death which can be a concern for wastewater biotreatment with microalgal cultures.

We hypothesize that P toxicity arises when the rate of P uptake is much faster than its conversion into long-chain polyphosphate coupled with their transport into the cell vacuole(s). Instead of this, a lot of short-chain polyphosphate molecules chaotically distributed in the cytoplasm are formed disturbing with protein folding. This can be the case during re-feeding of P-starved cultures with large amounts of P.

We argue that understanding the relationships between P availability, cell viability and capacity for P uptake is essential for the development of efficient knowledge-based microalgal biotechnologies both for valuable substance production and protection of the environment.

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L19

Construction of bio-inspired algal-bacterial consortium capable of phosphorus biosequestration from waste waters

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Phosphorus is a key biogenic element for the maintenance of biochemical processes, information handling and energy storage in the cell. Its availability often limits the growth of crop plants. A viable alternative to the traditional chemical fertilizer is biofertilizer from biomass of the microalgae enriched with phosphorus they capture from waste waters. Algal-bacterial consortia are shown to be an efficient and robust tool for waste waters treatment [1]. A promising approach to bioprospecting of efficient consortia for biotechnology is their bio-inspired design. This approach implies the reconstruction of artificial consortia combining the most useful features and component of natural microbial communities.

The aim of this research is to construct the bio-inspired microbial consortium based on photosynthetic microorganisms with potential ability for luxury phosphorus uptake from waste waters [2]. The consortium was constructed around the core of *Micractinium sp.* microalgae obtained from the polluted area near apatite-nepheline ore mine from the Khibiny deposit phosphorus. The constructed consortium was studied in two types of photobioreactors mimicking two different cases of phosphorus pollution: ponds near apatite-nepheline rock phosphate mines and municipal waste waters. The biouptake time in both systems did not exceed two weeks, which was demonstrated by measuring the residual amounts of phosphate by spectrophotometry and chromatography methods. The intercellular accumulation of phosphorus was confir-

med with energy-dispersive X-ray (EDX) spectroscopy. The stability of the constructed consortium was estimated by observing microbial dynamics by combination of 16S rRNA metabarcoding and scanning electron microscopy (SEM). The results indicated the formation of algal-bacterial consortia capable of efficient phosphorus uptake from waste waters during the long period.

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1. Novoveská L., Zapata A.K., Zabolotney J.B., Atwood M.C. and Sundstrom E.R. Optimizing microalgae cultivation and wastewater treatment in large-scale offshore photobioreactors. *Algal research*. 2016, **18** 86-94.
2. Solovchenko A.E., Ismagulova T.T., Lukyanov A.A., Vasilieva S.G., Konyukhov I.V., Pogosyan S.I., Lobakova E.S. and Gorelova O.A. Luxury phosphorus uptake in microalgae. *Journal of Applied Phycology.* 2019, **31**(5) 2755-2770.

L20

Cyanobacterial biofilms as light-driven biocatalysts – obstacles and achievements

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Despite photo-biocatalysis developing remarkably and the huge potential of photoautotrophic microorganisms for eco-efficient production scenarios, photobiotechnology is still in its infancy. The lack of scalable photo-bioreactors that provide efficient light transmission, CO_2 supply, and O_2 degassing and thus enable high cell densities (HCD), constitutes a key bottleneck, especially if cost-sensitive bulk chemicals are the product of choice. Commercialized tubular photo-bioreactors with 100 to 600 mm inner diameter offer a surface area to volume ratio (SA/V) of over 100 m^2 m^{-3} enabling the efficient capturing of incident solar radiation.[1] Here we introduce a new generation of photo-bioreactors based on capillary biofilm reactors. The biofilm is composed of two strains, namely the photoautotrophic strain *Synechocystis* sp. PCC 6803 and the chemoheterotrophic strain *Pseudomonas taiwanensis* VLB120, which serves as a biofilm supporter strain. *Pseudomonas* sp. is lowering the pO_2 in the system, which otherwise would toxify the Cyanobacteria. Furthermore, it produces extrapolymeric substances (EPS) and produces a kind of seeding layer promoting the attachment of *Synechocystis* sp.. *Synechocystis* sp. on the other hand produces organic compounds and oxygen consumed by *Pseudomonas* sp. The system is run completely without any organic carbon source.

The flow reactor concept for phototrophic biofilm cultivation was coupled to the challenging C-H oxyfunctionalization of cyclohexane to cyclohexanol with a remarkable conversion of $> 98\%$ and selectivity of 100 % (KA oil). High photoautotrophic biocatalyst concentrations were established and resulted in a productivity of 3.76 $g_{cyclohexanol}$ m^{-2} day^{-1}, which was

maintained for at least one month.[2]

This work demonstrates prototrophy as a biological strategy for the cultivation of photobiocatalysts in a stable and high cell density format up to 51.8 g_{BDW} L^{-1}, thereby overcoming a key-bottleneck in photo-biotechnology.

References

[1] Posten, C. Eng. In Life Science. (2009) 9:165-177.

[2] Hoschek, A. et al Bioresource Technology. (2019) 282:171-178.

L21

Microorganism in the LED-spotlight: standardized illumination systems for biotechnological applications

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Light plays a crucial role for all life on earth - from being a fundamental energy source to triggering biological functions such as metabolism or phototaxis. When phototrophic organisms like plants or algae are applied for biotechnology purposes, it is rather obvious that the parameter light is important and has to be defined and controlled. Besides photosynthesis, light can influence metabolic pathways and induce the production of secondary metabolites. Like any other bioprocess parameter, light should be applied in a standardized way to ensure constant and reproducible conditions. Due to their intrinsic properties, light emitting diodes are well-suited to provide controlled illumination conditions and to enable the design of versatile and standardized lighting systems for research of photo-biological processes.

In this work we address the development of a modular lighting system based on LED-technology to realize standardized illumination conditions for research applications. The system consists of individual controlled LED units to ensure well defined light spectra. Intensities and duration of light exposure are tunable variables, and integrated light sensors guarantee constant conditions. In order to ensure comparability and to provide standardization, the modules are characterized by radiometric standards and are aligned with a calibrated spectrometer. These modules can be custom-made in such a way that they fulfill the desired experimental requirements.

Furthermore, we present two applications of such LED systems for biotechnological purposes. With modules equipped with UV-A LEDs we performed irradiation experiments on different terrestrial algal strains to induce the production of secondary metabolites. First results with *Chromochloris zofingiensis* show a significant enhancement of carotenoids such as canthaxanthin and astaxanthin. In a second application, our modules were adapted to commercially available bioreactors. Hereby, the influence of different illumination settings was studied in experiments with fungi. First tests with *Penicillium ochrochloron* CBS 123.823 show that the production of xanthoepocin, polyketide

and fatty acids strongly depends on the illumination conditions, among other factors.

L22

Bio-inspired materials from microalgal cells on chitosan-based carriers for biocapture of nutrients

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The use of immobilized microalgae (MA) in bioremediation of wastewaters, in biocapture of nutrients and heavy metals shows an upward trend during recent years. Main advantages of immobilized cells are facilitation of biomass harvesting, increase the stress-tolerance of the cultures and prolongation of cultivation period. The immobilized MA are especially suitable for removal of nutrients from wastewater and attached microalgae biomass enriched with N and P can be considered as a slow-releasing environment-friendly biofertilizer.

New chitosan-based polymeric materials offer a range of advantages for the immobilization of MA. Chitosan as the positively charged natural polysaccharide has high affinity to the negatively charged cell surface of MA and can be easily derived from the partial deacetylation of chitin which is the second most abundant biopolymer after cellulose. The cross-linking of chitosan with dialdehydes at low temperatures brings polymers with high porosity and high surface area, enabling the efficient diffusion of nutrients and gases, allowing high microalgae concentrations in matrix. When compared to the widespread bio-based materials (alginate, agar, carrageenan) chitosan polymers have higher mechanical strength and higher durability in aqueous environments.

The tested microalgae (*Lobosphaera CALU 925, Nanochloropsis oculata, Haematococcus pluvialis)* became rapidly attached to the chitosan and the efficiency of immobilization (at initial chlorophyll 10-15 mg/L) comprised 50% after 5-6 h of incubation. Growth rate of the suspended and immobilized culture monitored via accumulation of chlorophyll was similar. Judging from the high retention of the photosynthetic activity (Fv/Fm > 0.62 throughout the experiment), attachment to the carrier had no deteriorative effect on the cells. The attached cells of *Lobosphaera CALU 925* demonstrated a reasonably good capability of nutrients bioremoval. Collectively, the bioinspired materials constructed from MA attached to the non-expensive, renewable, biocompatible and biodegradable chitosan-based carriers can be used in the wastewater treatment and other fields of microalgae biotechnology.

L23

Multifunctionalizing microalgae for sustainable production of industrially useful proteins and fatty acids

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There is an urgent requirement for sustainable sources of food and feed due to continuing world population growth. The importance of a sustainable supply of omega-3 long chain polyunsaturated fatty acids (LC-PUFAs), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) has long been established. These biologically active fatty acids are essential constituents of human nutrition and have key roles in maintaining health through their effects on immune system. Marine microorganisms are the primary producers of LC-PUFAs in the aquatic food chain, and EPA and DHA enter our diet through the consumption of marine fish. Aquaculture now supplies the largest proportion of the fish for human consumption, relying heavily on the fishmeal and fish oils derived from capture fisheries, challenging sustainability of the production system. Substitution of fish oil with vegetable oil and fish meal with plant seed meals in aquaculture feeds reduces the levels of valuable omega-3 LC-PUFAs and lowers the nutritional value due to the presence of phytate. Addition of exogenous phytase to fish feed is beneficial for enhancing animal health and reducing phosphorus pollution.

Microalgae represent an attractive and robust platform for the production of recombinant proteins and high value lipids. We have engineered the marine diatom *Phaeodactylum tricornutum* to accumulate high levels of EPA and DHA and demonstrated the potential of this transgenic strain for industrial production of omega-3 LC-PUFAs. In addition, we evaluated the possibility of producing these important nutrients together with recombinant proteins, the fungal *Aspergillus niger* PhyA or the bacterial *Escherichia coli* AppA phytases. The best engineered strain achieved up to 40,000 phytase activity units (FTU) per gram of soluble protein, thus demonstrating the feasibility of developing multifunctionalized microalgae for the production of industrially useful proteins and fatty acids to meet the demand of modern intensive fish farming.

L24

Engineering Recombinant Antimicrobial Efficacy and Yield from Algae

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With a pressing need for new antibacterial agents globally, and significant interest in the usage of antibacterials in livestock management, a clear need for new, affordable antibiotics exists. If intestinal infla-

mmation in livestock is not addressed, suboptimal absorption of nutrients lowers feed conversion efficiency and increases costs for farmers. Microalgae may prove to be an ideal vector to deliver recombinant antimicrobial proteins to both terrestrial and aquatic farm animals. Addressing both inflammation and infection with transgenic microalgae is an opportunity to employ a wide array of peptide and protein antimicrobials to mitigate farmer costs and possibly alleviate dependence on a fairly limited set of veterinary petrochemical antibiotics whilst the prevalence of antimicrobial resistance remains a global health concern.

The marine diatom *Phaeodactylum tricornutum* is a genome-sequenced, genetically tractable microalgae with a relatively efficient generation time and routine methodology for introducing transgenes to its nuclear genome. Independent of native antibacterial activity, we have established that nuclear genome transformation of *P. tricornutum* is as a feasible and adaptable platform to express an expanding suite of active antimicrobial peptides. We also share recent advances in developing chloroplast genome transformation for *P. tricornutum*, a methodology that would avoid nuclear transformant positional effects and could increase protein yield. Beyond antimicrobials, developing the *P. tricornutum* expression platform provides a versatile conduit for administering a wider array of single and combination therapeutic proteins to livestock.

L25

Electroflocculation – a way to minimize microalgae harvesting cost

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Due to low biomass concentration in the culture medium during autotrophic production of microalgae in a large scale, separation of cells from the culture medium is one of the most challenging parts of the process. Depending on the type of cultivation device, the biomass concentrations are usually in a range 0.5-3 g of dry weight pet litre. Centrifugation is a commonly used method for microalgae harvesting although it is economic inefficient. A way of decreasing the volume which has to be centrifuged is a pre-concentration of microalgal suspension by flocculation. During electroflocculation, the sacrificial anode is releasing metal cations, which are bound to the negatively charged microalgal cells and form easily separable aggregates called flocks.

During laboratory-scale experiments, the influence of following parameters on the separation efficacy was studied: voltage, time of current application, interelectrode distance, agitation, biomass concentration, culture medium composition, temperature and pH value. Optimization of these parameters was performed in order to achieve high biomass separation efficacy and the lowest possible content of iron in the algal biomass. Subsequently,

three types of continuous bench-scale harvesting devices (pneumatically aerated reactor, fluidised bed reactor, channel flow reactor) with working volumes in range 3-20 L were constructed and tested. Based on gained results, a pilot-scale continuous electroflocculation + flotation or sedimentation device (channel flow reactor with perforated baffles) with working volume of 150 L was designed, constructed and tested for *Chlorella vulgaris* harvesting. Harvesting costs were decreased from 2 kWh/kg of dry weight (centrifugation) to 0.19 kWh/kg of dry weight (electroflocculation + sedimentation + subsequent centrifugation of 30 times lower volume). The efficacy of separation higher than 95 % was achieved while iron content in the biomass met the legal food requirements (lower than 3 mg Fe/g of dry weight of *Chlorella* biomass).

L26

New production models of valuable bio-products from microalgal biomass using countercurrent chromatography (CCC) technology

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The identification of valuable bio-products from microalgae is a fact of wide dissemination in light of the advances in the development of increasingly efficient and sensitive analytical techniques. However, the methods developed so far to obtain these compounds from microalgae have limitations when scaled up in size and have implied time- and solvent-consuming operations. In this context, countercurrent chromatography (CCC) has emerged as a valuable alternative due to its high efficiency and proved scalability. The group of algal bio-refinery of the centre ALGATECH has recently developed liquid-liquid separation platforms using CCC technology to obtain high-value bioproducts from microalgae biomass including lutein, astaxanthin and docosahexaenoic acid (DHA). Lutein is a yellow carotenoid with beneficial effects in age-related eye diseases [1]. A novel approach for obtaining lutein from microalga biomass by integrating the extraction and CCC isolation steps was developed. A *Chlorella vulgaris* extract was processed applying multiple injections using CCC, yielding lutein at 97% of purity. Astaxanthin is one of the most powerful natural antioxidants found in nature [2] and possesses several health-promoting properties; however, its ester forms have yet to be extensively valorized. Five astaxanthin derivatives esterified with α-linolenic acid, linoleic acid, palmitic acid, oleic acid and stearic acid with purity over 98% were produced from *Haematococcus pluvialis* using a multi-injection CCC method combining two elution modes (reversed-phase and co-current). DHA, an omega-3 fatty acid, is fundamental for the formation and function of the human brain and retina [3]. DHA with purity of 99 % was obtained from micro-

algae *Schizochytrium* sp. using a multi-injection CCC method combining two elution modes (reversed-phase and elution-extrusion). Overall, the developed CCC methods may serve as references for promoting the development of new production models of microalgae-based bioproducts.

References

1. Bernstein P.S., Li B., Vachali P.P., Gorusupudi A., Shyam R., Henriksen B.S., J.M. Nolan. Lutein, zeaxanthin, and meso-zeaxanthin: the basic and clinical science underlying carotenoid-based nutritional interventions against ocular disease. *Prog. Retin. Eye Res.* 2016, 50, 34–66.

2. Miki W. Biological functions and activities of animal carotenoids. *Pure Appl. Chem.* 1991, 63, 141–146.

3. Echeverría F., Valenzuela R., Catalina Hernandez-Rodas M., Valenzuela A. Docosahexaenoic acid (DHA), a fundamental fatty acid for the brain: New dietary sources. *Prostaglandins Leukot. Essent. Fatty Acids.* 2017, 124, 1–10.

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L27

A biorefinery approach to obtain high-value polyunsaturated fatty acids from the microalgae *Schizochytrium limacinum* using liquid-liquid chromatography

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Docosahexaenoic acid (DHA n-3) and docosapentaenoic acid (DPA n-6) are polyunsaturated fatty acids that are present in the human brain and breast milk [1,2]. The microalgae *Schizochytrium* has arisen as a valuable alternative to fish as a commercial source of polyunsaturated fatty acids [3]. The present study reports an isolation process for obtaining DHA n-3 and DPA n-6 ethyl esters from *Schizochytrium limacinum* using countercurrent chromatography (CCC).

The dried biomass of *S. limacinum* was processed by direct transesterification affording a transesterified algal oil, from which docosahexaenoic acid (DHA) and docosapentaenoic acid n-6 (DPA n-6) ethyl esters were isolated using CCC. A multiple sequential injection CCC separation method was developed combining two elution modes (reverse phase and extrusion). During the initial reverse phase elution, the two target compounds were obtained, which was followed by the extrusion of the stationary phase. Once the column was

refilled with new stationary phase, a new hydrodynamic equilibrium condition was again reached for a new separation cycle. Ten consecutive sample injections (1000 mg of algal oil, each) were performed in this way leading to the separation of DHA n-3 ethyl ester (797 mg, 99% purity) and DPA n-6 ethyl ester (164 mg, 97% purity) with recoveries of 99% and 92%; respectively. The process throughput (amount of algal oil processed by CCC per time unit) was 1.149 g/h, while the efficiency (amount of target compounds obtained by CCC per time unit) was 0.110 g/h. Environmental risk and process evaluation factors were used for evaluation of the separation process. Overall, this separation strategy may represent an efficient model for the co-production of DHA and DPA n-6 from microalgae under a bio-refinery perspective.

References

1. Echeverría F., Valenzuela R., Catalina Hernandez-Rodas M., Valenzuela A. Docosahexaenoic acid (DHA), a fundamental fatty acid for the brain: New dietary sources. *Prostaglandins Leukot. Essent. Fatty Acids* 2017, 124 1-10, (DOI: 10.1016/j.plefa.2017.08.001).

2. Yuhas R., Pramuk K., Lien E.L. Human milk fatty acid composition from nine countries varies most in DHA. *Lipids* 2006, 41(9) 851-858, (DOI: 10.1007/s11745-006-5040-7).

3. Ratledge C. Microbial production of polyunsaturated fatty acids as nutraceuticals, in: McNeil B., Archer D., Giavasis I., Harvey L. (Eds.). Microbial Production of Food Ingredients, *Enzymes and Nutraceuticals.* Woodhead Publishing 2013, 531-558, (DOI: 10.1533/9780857093547.2.531).

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L28

Galectin-glycoconjugate interactions in biomedical research

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Galectins are soluble human lectins participating in pathological processes such as cancerogenesis, metastatic formation, inflammation, fibrotization, cardiopathologies and related disorders [1]. Galectins generally bind beta-galactoside-terminated glycans; however, structural preferences, and, consequently, a varying degree of selectivity exist between individual galectin representatives, depending on the differences in their carbohydrate-binding domains (CRD).

Since *in vivo* regulations and signaling pathways involving galectins are extremely complex and largely unexplored, it is cru-

cial to decode and understand the galectin-carbohydrate interaction at the molecular level. For this aim, a variety of *in vitro* assays [2], both for a simple affinity determination, and for analyzing particular biological processes, are very useful. A reliable assessment of affinity of synthetic ligands of galectins enables to reveal structure-affinity relationships and identify the most promising candidates for further study [3,4]. The present contribution will describe the design of novel glycoconjugates targeting galectin-3 and the determination of their anti-tumor activity in a variety of in vitro assays with cancer cells overexpressing galectin-3. These compounds represent an alternative research direction prospective for combined immunotherapy of cancer.

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[1] D. Laaf, P. Bojarová, L. Elling, V. Křen, *Trends Biotechnol*. **2019**, *37*, 402-415.

[2] L. Bumba, D. Laaf, V. Spiwok, L. Elling, V. Křen, P. Bojarová, *Int. J. Mol. Sci*. **2018**, *19*, 372.

[3] M. R. Tavares, M. Bláhová, L. Sedláková, L. Elling, H. Pelantová, R. Konefał, T. Etrych, V. Křen, P. Bojarová, P. Chytil, *Biomacromolecules* **2020,** 21, 641-652.

[4] M. Filipová, P. Bojarová, M. R. Tavares, L. Bumba, L. Elling, P. Chytil, K. Gunár, V. Křen, T. Etrych a O. Janoušková *Biomacromolecules* **2020**, *21*, 3122-3133.

L29

Engineering patients' immune cells with chimeric antigen receptor to treat hematological cancers

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Chimeric antigen receptor (CAR) T cell therapy combines the concept of using the patients' immune system and genetic engineering to make immune cells more effective and powerful to fight against cancer. Therefore T cells are collected from the blood, genetically modified with a CAR-expressing vector, expanded in vitro and given back to the patient [1]. CD19-specific CAR T cell therapies have achieved high remission rates in patients with various hematological cancers leading to the approval of the first CAR T cell therapy products [2, 3]. Infused CAR T cells can cause a systemic inflammatory response by the release of cytokines resulting in a widespread reversible organ dysfunction and neurotoxicity [4]. Delivering CAR T therapy requires an individualized approach and multidisciplinary medical care as well as a strong collaboration between hospitals, manufactures and the health care systems. While there is growing clinical experience and real-world evidence with CAR T cell therapy, future developments are on its way to optimize the CAR design, to target different antigens, to explore new indications in clinical trials and to automate and accelerate manufacturing.

References

1. June C.H., O'Connor R.S., Kawale-

kar O.U. et al. CAR T cell immunotherapy for human cancer. *Science* 2018, 23;359(6382):1361-1365.
2. Maude S.L., Laetsch T.W., Buechner J. et al. Tisagenlecleucel in Children and Young Adults with B-Cell Lymphoblastic Leukemia. *N Engl J Med.* 2018, 1;378(5):439-448.
3. Maloney D.G. Anti-CD19 CAR T cell therapy for lymphoma — off to the races! *Nat Rev Clin Oncol* 2019, 16:279–280.
4. Lee D.W., Santomasso B.D., Locke F.L., Ghobadi A. et al. ASTCT Consensus Grading for Cytokine Release Syndrome and Neurologic Toxicity Associated with Immune Effector Cells. *Biol Blood Marrow Transplant* 2019, 25(4):625-638.

L30

Cryo-electron tomography: structural biology in situ

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Over the past two decades, cryo-electron microscopy (cryo-EM) has become a method of choice for biomolecule structure determination at high resolution. Recent breakthroughs in hardware and software development enabled reaching near-atomic resolution for a wide range of protein and nucleic acid samples, also those inaccessible by X-ray crystallography. Because of its potential to study protein-protein interactions, conformational changes, small molecule binding, etc. cryo-EM is now becoming a frequently used technique in pharmaceutical research.

The so-called single particle analysis cryo-EM relies on acquisition of large amount of 2D images spanning different orientations of the molecule of interest in space. On the other hand, cryo-electron tomography (cryo-ET) is used to capture and visualize 3D information, allowing exploration of complex biological environments. When combined with subtomogram averaging, this technique can also be used for biomolecule structure determination. This is especially relevant for samples, where the objects of interest are overlapping with each other, or samples that are non-uniform in terms of shape and geometry, and therefore cannot be analyzed by the single-particle approach.

Cryo-ET and subtomogram averaging has been a vital tool for structural characterization of retroviruses and understanding the mechanisms of their assembly. Retrovirus particles are formed by hexagonal arrays of capsid protein, constituting a lattice of local symmetry, but pleomorphic and heterogeneous in global shape. Recent advancements in structural biology of retroviruses demonstrate the power and potential of cryo-ET and subtomogram averaging for biomolecule structure determination within challenging samples.

L31

Engineered antibodies for prostate cancer imaging and therapy

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Prostate cancer (PCa) stands out as one of leading death-causing malignancies. High incidence of the disease in countries with high standards of living has triggered an intensive search for efficient diagnostic and therapeutic modalities. Prostate-specific membrane antigen (PSMA) is an established biomarker of PCa as it is up-regulated and strongly expressed on PCa cells associated with high grade primary, androgen independent, and metastatic tumors.

We have recently developed a PSMA-specific antibody, termed 5D3, with subnanomolar affinity and high specificity for native PSMA that is particularly suitable for *in vivo* applications. Here we describe cloning and heterologous expression and purification of engineered single-chain Fv and Fab fragments of 5D3. These fragments were then characterized in detail using and arrays of biochemical, biophysical and *in vivo* experiments and these characteristics compared to the intact parent mAb. To enhance translational potential of 5D3, we determined an X-ray structure of isolated scFv and Fab as well as their complexes with PSMA. Structural data facilitated the rational design of humanized 5D3 molecules that were heterologously expressed in HEK293T cells in milligram quantities. Ongoing studies are aimed at elucidating the clinical applicability of humanized mAbs for PCa imaging and therapy.

L32

Magnetic nanoconjugated glycopeptides as novel tools for fighting bacterial infections

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One of the most promising approaches in the field of antimicrobial therapy is the use of nanotechnology-tailored agents for preventing and treating infections caused by resistant bacteria. Iron oxide nanoparticles (IONPs) are particularly attractive as nanocarriers for antibiotics thanks to their biocompatibility and magnetic properties, which allow to specifically direct conjugated antibiotics to infection sites.

Our purpose is preparing nanoformulations of the 'last-resort' glycopeptides teicoplanin and vancomycin -clinically used for fighting severe infections caused by multiresistant Gram-positive pathogens [1]- by conjugating them to IONPs via surface functionalization with (3-aminopropyl)triethoxysilane (APTES) [2,3]. Antimicrobial activity and surface interaction of these NP-conjugated antibiotics against differently resistant bacteria (*Staphylococcus* spp., *Enterococcus faecalis,* and *Bacillus subtilis*) were analyzed by a combination of

classical microbiological methods and fluorescence and electron microscopy, in comparison to non-conjugated teicoplanin and vancomycin. Achieved results suggest that bacteria sensitivity to NPs was species-specific, being variable according to bacterial surface. NP-conjugated antibiotics presented the same spectrum of action as free glycopeptides, with high and prolonged antimicrobial activity towards Gram-positives. Moreover, NP-TEICO was effective in inhibiting the formation of *S. aureus* biofilm, with an improved activity towards adherent cells if compared to non-conjugated teicoplanin. Finally, glycopeptide conjugation to IONPs increased their cytocompatibility towards two human cell lines [2,3].

[1] Marcone G.L. et al. Old and new glycopeptide antibiotics: From product to gene and back in the post-genomic era. Biotech. Adv. 2018, 36 (2) 534-554.

[2] Armenia I. et al. Magnetic nanoconjugated teicoplanin: A novel tool for bacterial infection site targeting. Front. Microbiol. 2018, 9 2270.

[3] Berini F. et al. Antimicrobial activity of nanoconjugated glycopeptide antibiotics and their effect on *Staphylococcus aureus* biofilm. Submitted to Front. Microbiol.

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L33

Use of RLI-15, a clinical grade fusion protein with IL-15 superagonistic activity for the activation of anti-tumor immune response

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Interleukin 15 (IL-15) represents one of the most promising cytokine in cancer immunotherapy. IL-15 activates cytotoxic functions of NK and CD8+ T cells similarly to IL-2. Unlike IL-2, IL-15 does not mediate activation-induced cell death and does not expand T regulatory cells. Both cytokines share the intermediate affinity IL-2/IL-15βγ receptors, but they differ in binding to their respective high affinity α receptor subunits. IL-15 bound to IL-15Rα on the surface of antigen pre-

senting cells transmits signals to IL-2/IL-15Rβγ expressed on target immune cells by a process called transpresentation. RLI-15 is a superagonist fusion protein of interleukin (IL)-15 and the IL-15 receptor α (IL-15Rα) sushi+ domain designed to bypass the need of endogenous IL-15Rα, thereby leveraging the activity of IL-15 in vivo on target immune cells. RLI-15 stimulates the proliferation and the cytotoxic activity of NK cells and memory CD8+ T cells with no significant expansion and activation of the regulatory T cell compartment. RLI-15 as a monotherapy exhibits a potent anti-metastatic activity and significant delay in tumor growth in various mouse tumor models. RLI-15 also significantly delayed tumor growth and prolonged survival when combined with anti-PD-1 therapy. RLI-15 was also explored to create novel immunocytokines targeted against CD20 and GD2 to leverage the activity of therapeutic monoclonal antibodies combined with its immune-stimulatory function. Currently, RLI-15 (SO-C101) is being tested in Phase I/Ib to evaluate the safety and preliminary efficacy of SO-C101 as monotherapy and in combination with pembrolizumab in patients with selected advanced/metastatic solid tumors.

L34

Interaction patterns of natural killer cell receptors and ligands with CTL fold

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Natural killer cells of the innate immune system recognize and degrade virally infected or tumour cells [1]. They utilize a range of receptors and corresponding ligands on the cell surface. Interactions between receptors and ligands determine the cascade of events leading to either inhibition of defense events or to activation of processes leading to elimination of the encountered cell.

C-type lectin like (CTL) receptors and ligands form one group of these molecules. The mammalian receptors of the NKR-P1 family possess the extracellular part formed by a CTL domain, a neck region, a transmembrane region formed by a helix and an intracellular part responsible for signaling. The CTL fold of the extracellular domain is utilized also by some of the protein ligands of these receptors found on the surface of the encountered and recognized cell. The protein-protein contacts are of interest following some recent results. In the cases studied, interaction patterns were conserved in terms of molecular topology, however, not so much on the level of sequence and particular interaction types. The CTL fold is a basic building block of many other proteins, such as the macrophage mannose receptor, selectins, collectins and others [2], where intermolecular interactions define their function.

Interpretation of our structures of receptors and ligands [e.g. 3-6] in the context of other known CTL receptor structures, complemented by computational analysis of other types of CTL fold-utilizing proteins allows for generalization of CTL-based interaction principles, role of ions in some cases and glycosylation in other. The presented data are a prerequisite for protein

engineering and potential biotechnological and biomedical application of the fold.
1. Vivier E., Tomasello E., Baratin M., Waltzer T., Ugolini S. Functions of natural killer cells. Nat. Immunol. 2008, 9, 503-510.
2. Zelensky A.N., Gready J.E. The C-type lectin-like domain superfamily. FEBS J. 2005, 272, 6179-6217.
3. Kolenko P., Rozbesky D., Vanek O., Kopecky V., Hofbauerova K., Novak P., Pompach P., Hasek J., Skalova T., Bezouska K., Dohnalek J. Molecular architecture of mouse activating NKR-P1 receptors. J. Struct. Biol. 2011, 175, 434.
4. Skalova T., Kotynkova K., Duskova J., Hasek J., Koval T., Kolenko P., Novak P., Man P., Hanc P., Vanek O., Bezouska K., Dohnalek J. Mouse Clr-g, a Ligand for NK Cell Activation Receptor NKR-P1F: Crystal Structure and Biophysical Properties. J. Immunol. 2012, 189, 4881-4889.
5. Skalova T., Blaha J., Harlos K., Duskova J., Koval' T., Stransky J., Hasek J., Vanek O., Dohnalek J. Four crystal structures of human LLT1, a ligand of human NKR-P1, in varied glycosylation and oligomerization states. Acta Crystallogr. D-Biol. Crystallogr. 2015, 71, 578-591.
6. Blaha, J., Skalova, T., Vanek, O., Stransky, J., Koval, T., Hasek, J., Yuguang, Z., Harlos, K., Dohnalek, J. Complex of human NKR-P1 and LLT1 in deglycosylated forms. Protein data bank, access code 5MGT.
This work was supported by the Czech Science Foundation (18-10687S), and by the ERDF fund (CZ.02.1.01/0.0/0.0/16_013/0001776).

L35

Production of therapeutic plasmid DNA in single-use technology

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Recent progress in the field of gene therapies has primed the creation of a completely novel and potentially highly efficacious therapeutic approaches for currently unmet medical needs such as cancer or diseases caused by distinct mutations. Due to rapidly growing demand for various gene-targeting biotherapeutics the manufacturing of plasmid DNA is becoming a supply bottleneck. Plasmid DNA production is almost exclusively based on *Escherichia coli*, because of its relative simplicity, with rapid and inexpensive high-density cultivation, well-known genetics, and many compatible molecular tools available.

Many producers are moving towards expanding or implementing single-use production facilities to efficiently meet the demand. Single-use technologies enable development of platform processes that rapidly produce a large range of plasmids for research and clinical application. The usage of disposables eliminates the risk for cross-contaminations, the need for cleaning, cleaning validation, and preparation of glass and steel containers and devices. Furthermore, single-use systems have fewer utility requirements, and much lower investment costs.

While Single-Use Bioreactors for mam-

malian and insect cells have relatively few engineering challenges to achieve the necessary mixing, temperature, and dissolved oxygen control, Single-Use Fermentors present significant engineering challenges.

However, the modern Thermo Scientific HyPerforma SingleUse Fermentor addresses these unique needs of microbial applications especially regarding high-performance mixing and mass transfer and enables production facilities to achieve equivalent yields in the same time frame as traditional stainless steel fermentor vessels. In this presentation, three procedures for production of therapeutic plasmid DNA at 6—30 L working volumes will be reviewed.

L36

Antimicrobial properties of biosynthesized nanoparticles

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Metal nanoparticles are extensively studied due to their various potential applications in biotechnological, environmental and medicinal fields. Several antimicrobial nanoparticles and nanosized carriers for antibiotic delivery have proven their effectiveness against microbial pathogens, both in vitro and in animal models. Silver and gold nanoparticles are among the most widely studied among the metal nanoparticles, due to their broad potential of antimicrobial effects and other medicinally exploitable properties. A great effort has been dedicated to finding new, environmentally friendly and economical methods for nanoparticles production, among which biosynthesis, or green synthesis, plays a prominent role.

In our work, we have studied the biosynthesis and subsequent antimicrobial properties of metal nanoparticles. The extracts for biosynthesis are obtained from *Vitis vinifera* canes, a waste product from cultivation and processing of wine. The synthetized nanoparticles were studied for their antimicrobial activity against planktonic cells and biofilms of opportunistic pathogen *Pseudomonas aeruginosa*. The antimicrobial properties determination focused both on overall growth suppression and the estimation of cell viability, as well as the impact of nanoparticles on quorum sensing, a communication system which governs the biofilm formation and is essential for microbial virulence expression.

L37

Spontaneous in situ endothelialization as an advanced approach for construction of vascular replacements

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Small-caliber vascular grafts (inner diameter < 6 mm) are increasingly needed in cardiovascular surgery, namely for aortocoronary bypasses and for replacements of irreversibly damaged peripheral blood vessels. However, these grafts often fail (0-30% patency after 5 years) due to thrombosis, inflammation or hyperplastic neointima formation [1]. It has been proved that the best prevention of these undesirable phenomena is endothelialization of the grafts. However, the endothelialization with patient's autologous endothelial cells (ECs) *in vitro* is time consuming and associated with a risk of microbial contamination. A more advanced strategy lies in implantation of cell-free vascular replacements functionalized with various agents, which capture endothelial progenitor cells (EPCs) from the blood and promote the ingrowth of ECs from the sites of anastomosis between the graft and the original blood vessel. These agents include oligopeptidic ligands for adhesion receptors on EPCs and ECs, such as REDV, YIGSR or CAG, antibodies against CD34, CD133 or against receptors for vascular endothelial growth factor (VEGF), DNA, RNA or peptide aptamers, growth factors (mainly VEGF), neuropeptide substance P, stromal cell-derived factor 1α or plant-derived molecules, such as polyphenols, gallic acid, ferulic acid or icariin. All these agents are usually attached to the grafts pre-coated with anti-fouling substances, such as heparin, poly(ethylene glycol) or dextran. This system is applicable on synthetic polymeric vascular grafts, including clinically used grafts made of expanded polytetrafluoroethylene, polyethylene terephthalate and polyurethane, and also on biological tissue-engineered blood vessels, based on decellularized vascular matrices or collagen tubes. In our experiments, we are developing a unique system based on oligosaccharides, capturing EPCs or ECs through galectin-3 molecules on these cells. Our recent results suggest a dual role of galectin-3 in this system: it can either act as a cell adhesion receptor for extracellularly-bound oligosaccharides, or it can act as an adhesion ligand attaching cells to a biomaterial surface.

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[1] Liebler S, Grunert F, Thompson J, Wedel M, Schlosshauer B. Towards a biofunctionalized vascular prosthesis: immune cell trapping via a growth factor receptor. *J Tissue Eng Regen Med.* 2017;**11**(10):2699-2709. doi: 10.1002/term.2165.

L38

Non-thermal plasma against viruses including SARS-CoV-2

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The recent epidemiology situation has accented the call for efficient decontamination of surfaces and objects to prevent virus infections. The decontamination tech-

niques have several limitations and are not suitable to use in universally, so the call to find some alternatives are actual. One possibility for object decontamination from microorganisms is the non-thermal plasma – NTP. The plasma technology is inexpensive, gentle to the exposed material and applicable on biomaterials, live tissues including human skin. The efficacy of plasma mediated disinfection is well established for bacteria and fungi, but recent studies and our preliminary data also indicated its suitability for efficient virus inactivation.

L39

The PIXL&PIER methodologies (photo-induced crosslinking & electron release): novel experimental tools for structural and functional biochemistry

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The photo-induced crosslinking (PIXL) technique is a promising experimental approach that in combination with mass spectrometry (MS) is suitable for determination of the many structural/functional aspects of structural biochemistry of protein in their native states at reasonable time-scales using their relatively small quantities. This approach is an alternative to the chemical crosslinking, but without limitations in reaction specificity and without restrictions on reaction conditions that are inherent in chemical crosslinkers (e.g. interaction between protein transmembrane parts buried in membrane environment [1]).

The PIXL workflow employs an amino acid structural analogue (e.g. in our work we utilize two structural analogues of methionine: photolabile diazirine or azide) that is introduced into protein sequence during its recombinant expression. This incorporation of structural analogues is allowed by the miscoupling activity of aminoacyl-tRNA-synthase during amino acid activation in protein biosynthesis. The studied recombinant protein with photolabile structural analogue of methionine is purified and incubated in a designed system with following activation by UV irradiation forming reactive species that is able to attack any molecule in its close vicinity bellow 5Å). Finally the high resolution or tandem mass spectrometry analysis are employed after sample processing (covalent crosslinks enrichment, proteolysis, reverse phase liquid chromatography, etc.) to identify crosslinks (intermolecular or intramolecular crosslinks formed between the interacting protein parts) and to determine their constrain-distances that can serve for subsequent 3D model building or model evaluation.

The introduced PIXL technique with MS detection was successfully applied to several biochemically interesting subjects/questions comprising: (i) the hetero-

dimer interface mapping of calmodulin and adenylate cyclase toxin of *Bordetella pertussis* involved in its bacterial pathogenesis, (ii) the protein fold description and protein multimerization of the metal-labeled blue copper protein azurin of *Pseudomonas aeruginosa*. Moreover, the PIER methodology was described in azurin case (central Cu^{II} ion oxidation following by UV-VIS changes) for the first time.

We have established and validated the methodological approaches such as PIXL&PIER that are able to revealed unknown processes or to contribute to the elucidation of many biologically important mechanisms (e.g. the identification of target protein partners, the composition and stoichiometry of the supramolecular complexes, the identity of the crucial parts of protein responsible for interaction or for electron transfer).

Acknowledgement: This work has been supported by Charles University (project GAUK n. 1538119) and Grant Agency of Czech Republic (GAČR 20-28126S).

[1] Ječmen T, Ptáčková R, Černá V, Dračínská H, Hodek P, Stiborová M, Hudeček J, Šulc M: *Methods* (2015) 89:128-37.

L40

The epidemiological situation of measles in Tirana, Albania, from 2017 to 2019

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Background: Measles is a highly contagious disease and as long as the measles virus is circulating anywhere in the world, the risk of cross-border transmission and importation to countries that have achieved elimination remains high. Albania is one of the countries that have successfully interrupted endemic transmission of measles in 2015 due to the national immunization programme. From 2017, the country has reported sporadic imported cases of measles, that were followed by a few separate outbreaks with a small number of cases and limited period of transmission. The aim of this study is to monitor the epidemiological situation of measles in Tirana, Albania, from 2017 to 2019.

Materials/methods: A total of 433 samples from patients, who were examined for the measles immunization status were involved in this study. The specimens were tested for anti-Measles-IgG and anti-Measles-IgM antibodies with ELISA technique, at "Intermedica"Medical Clinic, in Tirana, Albania.

Results: The evaluation of the results showed that 18 % of samples (78 patients) resulted negative for anti-Measles-IgG antibodies. 10.8% of patients (47 samples), who resulted negative for anti-Measles-IgG antibodies, were over 13 years old, while 7.2 % of patients (31 samples), younger than 13 years, are under the immunization process. Only 0.46 % of patients (2 samples), in adulthood, resulted positive for anti-Measles-IgM antibodies.

Conclusions: In conclusion, a considerable percentage of cases tested in this study are unimmunized, or do not have information available about immunization

status. A part of them are related to the cohorts of children born in 1989–1992, who remained either partially vaccinated or not vaccinated due to lack of vaccine. We hope the measures implemented by the Albanian country, especially raising the public awareness, will help to reduce the number of unimmunized people and the spread of disease.

L41

Gas management in the cultivation of microalgae: Use of membrane contactors for bubble-free gas transfer in tubular photobioreactors

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Tubular photobioreactors (TPB) are more advantageous than open ponds or raceways in reducing contamination (at ambient pH and low salinity) and being independent of climate, season, and natural light source. However, the management of gas transfer in TPB-systems and, thus, the economics of microalgal production are challenging. To support growth of microalgae under conditions of non-limiting carbon dioxide concentrations, and simultaneously protecting these cultures from excess oxygen, gas exchangers need to be installed; at each 70-90 meters of reactor length. In addition, the usable reactor volume is limited by gas bubbles, i.e., an excess of gas, suboptimal distribution of carbon dioxide, or released oxygen that inhibits photosynthesis and thus, microalgal growth.

Membrane contactors are an established technology for bubble-free gassing and degassing, for instance, carbonization of soft drinks or a reduction in oxygen concentrations in water for breweries. Membrane contactors typically used for these purposes are, however, not routinely used for gas management in microalgal cultures since these membranes tend to suffer blockages in non-filtered aqueous liquids containing suspended solids and cannot be easily cleaned [1].

Using tubular polytetrafluoroethylene (PTFE) membranes of inner diameters of up to 8 mm, gas transfer to and from the microalgal culture can be managed optimally. These membranes offer excellent cleanability and can be easily adapted to almost any tubular reactor system by a 3D-printed holder. The first results from bubble-free bioprocesses using membrane contactors underline their potential to increase the space-time-yields (productivities in g per liter per hour) of microalgal cultures in tubular photobioreactors.

[1] Riedl,W.: Membrane-Supported Liquid-Liquid Extraction – Where Do We Stand Today? *ChemBioEng. Rev.* 2021, 8, No. 1, 1–10.

L42

How to reduce extract losses in industrial beer fermentation?

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Breweries strive to achieve higher productivity using the same resources, while maintaining high quality beer. Greater efficiency in the brewing process can be attained by reducing an excessive yeast production during fermentation since higher yeast quantity involves the extract losses and entails extra cost in yeast separation from beer. However, an adequate yeast growth is necessary to obtain a proper fermentation speed and attenuation. Many factors, including yeast strain, inoculum size, temperature, aeration, original gravity and composition of wort affect the quantity of yeast produced.

The aim of the study was to optimize industrial fermentation performance for the production of high gravity beer. Industrial-scale trials were conducted to improve fermentation efficiency and cost-savings without compromising yeast and beer quality. In our study different yeast inoculation rates and quantities of dissolved oxygen were applied to ferment wort with 17.1°P extract.

Initially, five of the seven brews per CKT were aerated with 8 mg/l air, increasing the yeast dose from 0.6 to 1.1 kg/hl. The tests resulted in a shorter fermentation time as well as an increase in extract losses. Therefore, the amount of supplied oxygen was reduced to 4 brews per CKT and the yeast dose was increased from 1.1 to 1.2 kg/hl.

The extract losses in the fermentation department decreased with increasing yeast inoculum size from 0,6 to 1,2 kg/hl. Doubling the yeast inoculation rate allowed to reduce the losses of the fermentation department by approx. 0.7% without an adverse influence on beer quality and the viability of cropped yeast. Nonetheless, the use of higher amounts of pitching yeast requires a more flexible yeast management.

L43

From traditional culture collection to sustainable and healthy foods

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The «Liebefeld Kultursammlung» is more than a traditional culture collection. It preserves the unique character of high-quality Swiss cheese and is the foundation of original Swiss secret recipes.

Resulting from more than 100 years of public research, more than 12,000 natural (non-GMO) single species or their mixed cultures were collected and characterized. They include distinctive cultures for making cheese (with its specific aroma, texture, wholes, and rind) that are less prone to quality defects (including those by bacteriophages) as well as cultures to make other fermented dairy products. The following species are the signatures for traditional, high quality Swiss dairy products: Lacto-

coccus spp., Lactobacillus spp., Lacticaseibacillus sp., Leuconostoc sp., Propionibacterium freudenreichii, Streptococcus thermophilus as well as Corynebacterium spp., yeasts, Geotrichum, and Fusarium).

However, the potential of this culture collection goes far beyond a repository of species, their reproduction and redistribution. Our continuing research, using advanced scientific approaches, focusses on the following fields and applications:

- combined phenotypic and genotypic characterization using bioinformatic tools
- authentication of foods by detecting the presence of intrinsic signature organisms
- cultures for protecting fermented foods and plants
- fermentation of innovative plant-based foods
- cultures for various food components or to promote a healthy human microbiome
- innovation in the production of traditional dairy products

These activities, aimed at protecting and developing the Swiss cheese brand and its cultural heritage, are based on a Public Private Partnership (PPP) between the federal government and the dairy industry, including associations of Swiss cheesemakers and milk producers as well as 14 cheese variety organizations and 6 milk processing companies.

References:

https://www.liebefeld-kulturen.ch/
https://www.agroscope.admin.ch/agroscope/en/home/about-us/organization/competence-divisions-strategic-research-divisions/food-microbial-systems.html

L44

Biotransformation in Food Processing

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In the food industry, microorganisms or functional enzymes are very often involved in food production. These processes are often referred to as food biotechnology. These biotechnologies include historically very old biotechnologies such as beer and wine production. The basic process applied in their production is fermentation. The essential difference between fermentation and biotransformation is that there are several catalytic steps between the substrate and the product in fermentation while is only one or two in biotransformation. The distinction is also in the fact that the chemical structure of the substrate and the product resemble one another in biotransformation, but not necessarily in fermentation. Biotransformation is defined as the transformation of a substrate to a product using biocatalyst. The aim of our work is not only targeted application of biocatalyst in the food industry – production of invert sugar, isomerization of glucose to the production of high fructose syrups, production gluconic acid, ascorbic acid, and lactose hyd-

rolysis, but also natural biotransformation reactions which occur during food production and storage. An example of these reactions is the transformation of ferulic acid to 4-vinylguaiacol by brewing yeast *Saccharomyces cerevisiae* during the production of wheat beer. This substance is giving fine aroma to wheat beer like a clove. Study of these type of reactions is very important because in the near future can be used in the food and biotechnology industry.

L45

Non-Saccharomyces yeasts in brewing

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Brewing is one of the most traditional food industries. Although the application of pure brewer's yeast cultures started only in the late 19th century, brewers had decades to select the suitable yeast for their own beer. In the meantime *Saccharomyces cerevisiae* became one of the most studied microorganism, and brewers had the opportunity to use this common knowledge to adopt technology. Nowadays brewers produce wort with composition that suits best the yeast, and provide circumstances (in propagation and fermentation) that is just right for it – given that it is a *S. cerevisiae* or a *S. pastorianus* strain.

In the past decades due to curiosity and innovation pressure non-*Saccharomyces* yeasts appeared in brewing. The strains of different *Brettanomyces*, *Torulaspora* and *Saccharomycodes* species have been studied, even from the brewer's point of view, but much less than traditional brewer's yeast. Today's brewers do not have decades to get to know strains, and some targeted examination would provide useful information.

Several strains of *Brettanomyces*, *Torulaspora* and *Saccharomycodes* yeast was included in fermentation trials and other analyses relevant to brewing. Observations and results confirmed that many of the strains have positive features like low diacetyl productions, good flocculation abilities, or smaller needs for N source like in case of *Brettanomyces* strains. In basic characteristics variousness was found even among strains of a given species. Two out of four tested *Torulaspora delbrueckii* strains produced alcohol in very low concentration (below 1.7 ABV).

Eventually some of the non-*Saccharomyces* yeast strains can become accepted and reliable ingredients in brewing, but brewers have to find the right strain, and find out the right circumstances for them. There is already one good example to follow: *Saccharomyces ludwigii* used in non-alcoholic beer production!

L46

The use of sensomics in controlling of brewing process

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Beer, from the chemical point of view is a very complex sample and a compre-

hensive view is necessary for understanding the intertwined aspects of sensory properties of this worldwide popular beverage. The sensomics, a member of a wider family of -omics technologies applied to food, e.g., foodomics, food metabolomics, and flavoromics, aims to describe the sensory properties of foodstuffs at a molecular level. Last few years our research group has been focused on the expansion of this field into brewing science [1]. As it has been shown in many scientific papers, not only volatiles and/or compounds above their flavor threshold, but also non-volatiles and compounds at sub-threshold concentrations can influence the final sensory impression of a given product. The sensory impact of the latter group has been usually neglected in sensory studies. The sensomics in our conception operates with both groups of compounds. Besides, our motivation is to identify novel beer compounds with not yet known sensory effects.

The aim of this lecture will be to explain the basic principles, steps, and tools in the sensomics, such as the design of the experiment, the performance of an experiment and analysis, data treatment and evaluation. An introduction will be given to the application of sensomics in the brewing process, for example during mashing, hopping, and fermentation. Successful applications of sensomics will be demonstrated by the study of sensory properties of European and Czech lager beers.

[1] Vrzal, T., & Olšovská, J. (2019). Sensomics-basic principles and practice. KVASNÝ PRŮMYSL, 65(5), 166-173.

L47

Advances in Acid Resistance Mechanisms of Acetic Acid Bacteria

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Acetic acid bacteria (AAB) are gram-negative aerobic bacteria that can oxidize sugar, sugar alcohol, and alcohol compounds to produce corresponding alcohols, ketones, and organic acids. In the 90 years from 1898, when Acetobacter, the first genus of AAB, was proposed, to 1989, AAB contained only three genera and less than 10 species. Nowadays, AAB have been reported nearly 100 species in 19 genera. Besides being used in vinegar production, AAB also have the functions of producing cellulose, pigments, indoleacetic acid, ascorbic acid and nitrogen fixation, etc. AAB are also important parts of insect intestinal microorganisms, some of which are also human pathogens. Some AAB have very high acid production (resistance) capacity, to produce and tolerate up to 20% acetic acid, while most other bacteria usually can only tolerate less than 0.5% acetic acid, therefore the acid resistance mechanism has been the focal and difficult points of AAB research. The known mechanisms of AAB acid resistance mainly included acetic acid assimilation and transport, cell morphology, membrane remodeling and cell stress response. Recently, we have used transcriptomics to compare the acid production (resistance) capacities of different AAB to find out that AAB can mobilize the whole genome to respond

in high-acid environments, and discovered some previously unreported acid production (resistance) mechanisms.

L48

Metabolites and their applications of *Monascus* spp. and the underlying essentials of genetics

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The genus of *Monascus*, nominated by van Tieghem in 1884, is used to produce red yeast rice (RYR) which has been used as folk medicines, food colorants, and fermentation starters for approximate 2000 years in oriental countries. Accumulation of published documents demonstrated diverse metabolites produced by Monascus spp. possessed numerous biological properties with hypolipidemic, anti-atherosclerotic, anti-cancer, neurocyto-protective, hepato-protective, anti-osteoporotic, anti-diabetic, anti-obesity, immunomodulatory, antiinflammatory, anti-hypertensive, and anti-microbial activities. Genomic DNA sequence of Monascus strains revealed that it had great potential to produce unknown secondary metabolites. So this is always a hot issue to improve the yields of beneficial compounds. The introduction of molecular biology techniques facilitate us to elucidate biosynthetic pathways of these metabolites and their modulation mechanisms at the genetic level. Here, progress will be emphasized on the underlying essentials of genetics of secondary metabolites and their hierarchical modulation.

L49

The Golgi Glycan Factory (GGF) – Enzyme Modules for Glycoconjugate Synthesis

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The translation of multi-enzyme glycoconjugate synthesis into larger scale is hampered by multi-parameter optimization of enzyme-modules. In this respect, nucleotide sugars are considered as bottleneck and expensive substrates for glycan synthesis with glycosyltransferases. We have set up modular multi-enzyme cascades for the synthesis of sixteen different nucleotide sugars starting from monosaccharides and sucrose as substrates. Multiplexed CE (MP-CE) as fast analytical tool was established for optimization of reaction parameters [1]. The repetitive use of enzyme cascades in batch synthesis significantly increased productivity up to a multi-gram product scale [2]. A high mass based total turnover number (TTN_{mass}) of up to 500 g product/g enzyme and space-time-yield (STY) of up to 19 g/L*h were obtained. With these basic technologies, nucleotide sugars are readily available for *in vitro* Leloir-glycosyltransferase based glycoconjugate [3] biopolymer synthesis [4] and automated enzymatic glycan synthesis [5].

1. Wahl, C., Hirtz, D., and Elling, L. Mul-

tiplexed Capillary Electrophoresis as Analytical Tool for Fast Optimization of Multi-Enzyme Cascade Reactions – Synthesis of Nucleotide Sugars, *Biotechnol. J.* 2016, **11**, 1298-1308.

2. Fischöder, T., Wahl, C., Zerhusen, C., and Elling, L. (2019) Repetitive Batch Mode Facilitates Enzymatic Synthesis of the Nucleotide Sugars UDP-Gal, UDP-GlcNAc, and UDP-GalNAc on a Multi-Gram Scale, *Biotechnol. J.* 2019, **14**, doi:10.1002/biot.201800386.

3. Fischöder, T., Cajic, S., Grote, V., Heinzler, R., Reichl, U., Franzreb, M., Rapp, E., and Elling, L. Enzymatic Cascades for Tailored $^{13}C_6$ and ^{15}N Enriched Human Milk Oligosaccharides, *Molecules* 2019, **24**, 3482.

4. Gottschalk, J., Zaun, H., Eisele, A., Kuballa, J., and Elling, L. Key Factors for A One-Pot Enzyme Cascade Synthesis of High Molecular Weight Hyaluronic Acid, *Internat. J. Mol. Sci.* 2019, **20**, 5664.

5. Heinzler, R., Fischöder, T., Elling, L., and Franzreb, M. Toward Automated Enzymatic Glycan Synthesis in a Compartmented Flow Microreactor System, *Adv. Synth. Catal.* **361** 2019, 4506-4516.

L50

Biotechnological production of polyhydroxyalkanoates employing stress-adapted microorganisms

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Polyhydroxyalkanoates (PHA) are polyesters accumulated by numerous prokaryotes. These materials are generally considered being biodegradable, biocompatible and bio-based alternatives to synthetic petrochemical polymers. Nevertheless, due to high production cost of PHA, these environmental-friendly polymers can hardly compete with synthetic polymers, which prevents their massive industrial production. There are several strategies which can possibly decrease cost of PHA production, one of the most promising one is so called concept of "Next Generation Industrial Biotechnology" (NGIB) relying on utilization of extremophilic microbial producers. Extremophiles provides numerous benefits, most of all, processes based on extremophiles are naturally robust against contamination by ubiquitous microflora and; therefore, they can be operated in semi-sterile or non-sterile conditions even under continuous or semi-continuous *modus operandi* which substantially reduces cost of the process.

Therefore; we performed evolutionary engineering experiments aimed at adaption of selected heterotrophic but also photoautotrophic bacteria capable of PHA synthesis to various stressors. Furthermore, we also focused on screening and isolation of extremophilic bacteria and archaea capable of PHA biosynthesis. We have identified that numerous halophiles such as *Halomonas halophila*, *Halomonas organivorans* or *Halomonas hydrothermalis* are very potent PHA producers from various low-cost substrates such as lignocellulose hydroly-

sates or waste frying oils. Nevertheless; apart from many benefits, utilization of halophiles brings also many drawbacks related to high concentration of salt in cultivation media. Therefore; we focused on screening of thermophiles capable of PHA biosynthesis. It should be stated that, as compare to halophiles, PHA biosynthesis is far more unexplored so far since PHA production capacity was described only for few thermophiles. Therefore, we systematically screened selected thermophilic strains available in public collections of microorganisms to identify novel potent PHA producers. Moreover; we also developed unique isolation protocol which enabled us to isolate PHA accumulating thermophiles from various microbial consortia such as compost or activated sludge. Therefore; we identified several very promising thermophiles which can be considered being auspicious candidates for industrial production of PHA. For instance, our isolate *Aneurinibacillus* sp. H1 is capable of efficient biosynthesis of PHA copolymer consisting of 3-hydroxybutyrate, 3-hydroxyvalerate and 4-hydroxybutyrate with very high 4-hydroxybutyrate fraction. Such a material reveals unique mechanical, technological and biological properties which open gates to numerous high-value applications in cosmetics or medicine.

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L51

Biotechnology potential of aldoxime- and nitrile-converting enzymes

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The transformation of aldoximes to carboxylic acid (via nitriles) proceeds in bacteria and fungi. Though widespread, this process is not ubiquitous. Its probable role is the detoxification of aldoximes and nitriles, which are plant defence compounds. The enzymes catalyzing this cascade are aldoxime dehydratases (AODHs) and nitrilases (NLases), the latter also occurring in plants. In some bacteria, NLases are replaced or supplemented with nitrile hydratases (NHases) and amidases. The activity of these enzymes for non-natural substrates makes them attractive for biotechnology. Moreover, they surpass the common che-

mical tools in many aspects. AODHs catalyze a unique reaction – dehydration of aldoxime in water, and thus they enable to synthesize nitriles without using the toxic cyanide. In addition, they are *E*/*Z*-selective and enantioselective for certain substrates. NLases catalyze the energy-demanding hydrolysis of nitriles at ambient temperature and mild pH, and enable to produce optically pure carboxylic acids/amides and cyano acids/cyano amides. The selection of a suitable gene is the key to the success of the enzyme production. Sequence alignments were used to classify AODHs and NLases into clusters (clades). Selected members of the clades were studied using a homology modeling/docking approach. The role of specific residues in enzyme affinity to various substrates was analyzed and the predicted affinities were verified experimentally. Thus the substrate specificities of the clades were elucidated and the biotechnology potential of the enzymes was assessed. AODHs and NLases are promising for the fine chemical production and for some environmental applications such as the detoxification of free cyanide using cyanide hydratases, which form a subclass within NLases.

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L52

Substrate oxidation and electron transfer mechanisms in the active site of bilirubin oxidase

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Bilirubin oxidase from *Myrothecium verrucaria* (EC: 1.3.3.5) as well as other members of the multicopper oxidase family (e.g. laccases) are widely studied and used for their ability to oxidize a great variety of substrates in different conditions and transfer electrons from them to oxygen. Because of these properties, multicopper oxidases are used for many applications in medicine and industry. Their usage comprehends applications in medical diagnostics (e.g. level of bilirubin in blood), processing of various organic compounds (e.g. dyes or lignin), development of biosensors and biofuel cells or treatment of dangerous chemicals in waste processing [1, 2].

Our recent structural, biophysical and enzymological studies of bilirubin oxidase revealed structural features responsible for its high substrate promiscuity. We were able to explain the role of the recently discovered unique posttranslational modification present in the active site: the

tryptophan – histidine covalent adduct [3]. The newly obtained knowledge regarding the active site of this enzyme can lead to engineering of modified oxidoreductases with broader substrate range with many possible applications in biotechnology.

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References

1. Mate D.M. & Alcalde M. Laccase: a multi-purpose biocatalyst at the forefront of biotechnology. *Microb Biotechnol*. 2017; **10**:1457–1467.

2. Guan Z., Luo Q., Wang H. *et al*. Bacterial laccases: promising biological green tools for industrial applications. *Cell. Mol. Life Sci*. 2018, **75**:3569–3592.

3. Kovaľ T., Švecová L., Østergaard L.H. *et al*. Trp–His covalent adduct in bilirubin oxidase is crucial for effective bilirubin binding but has a minor role in electron transfer. *Sci Rep* 2019, **9**:13700.

L53

Can Nanotextiles Enhance Filtration Capacity of HEPA Filters for Microorganisms?

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To evaluate the contribution of the air conditioning system to the air borne microorganism transmission, the microbial contamination of selected air filters was investigated. High Efficiency Particulate Air (HEPA) filters removed aseptically from commercial aircraft cabin air conditioning system and HEPA filter from household air conditioner were tested. On both HEPA filters, the pathogenic microorganisms were searched for. The present study is aimed to compare the microbial contamination of inlet and outlet surface of the filters. For this aim, cultivation of bacteria and real time RT PCR techniques were used. The aircraft air filter was highly contaminated with pathogens and potential pathogens on both sides of the filter, namely *Escherichia coli, Staphyloccoccus aureus, Streptococcus pyogenes, Citrobacter spp,* and *Yersinia pseudotuberculosis.* Quantification revealed ten times higher values of colony forming unit per milliliter (CFU/ml) on the outlet surface of the filter [1].

The HEPA filter removed from a household air conditioner underwent the tests using the FilmArray® multiplex PCR detection system with Respiratory panel and Pneumonia panel [2.;3]. On the inlet sur-

face of the filter there we detected no pathogens included in the portfolio of Respiratory and Pneumonia panel respectively. The outlet side of the filter contained rhinovirus, enterovirus, and coronavirus 229E. Nanotextiles may considerably enhance the filtration capacity of filters used in any applications. When a single layer of nanotextile (producer Nanovia Ltd, nanotextile developed for air filtration, diameter of pores 70 nm) was placed behind the HEPA filter of household air conditioner, the inner side of the nanotextile was able to capture *Acinetobacter calcoaceticus-baumannii complex, Escherichia coli, Serratia marcescens, Staphylococcus aureus,* and coronavirus. This study gives valuable information on the capacity of used nanotextile to capture bacteria and viruses of lower sizes. Therefore, these nanotextiles could be useful for air conditioning systems.

The study was partially funded under the projest SGS20/205/OHK4/3T/17

[1] Obitková, D.; Pavlík, E.; Mráz, M.; Gvozdeva, V. **Microbial Contamination of Different Types of Air Filters.** In: Ochrana obyvatelstva v případě krizových situací a mimořádných událostí nevojenského charakteru VII. Kladno: ČVUT, Fakulta biomedicínského inženýrství, 2019. p. 179-201. ISBN 978-80-01-06651-5.

[2] BIOFIRE DIAGNOSTICS LLC. *FilmArray Respiratory Panel (RP): Instruction Booklet*. 2017.

[3] BIOFIRE DIAGNOSTICS LLC. *FilmArray Pneumonia Panel Plus: Instruction manual*. 2018.

Posters

P01

New specialized metabolites with a 4-alkyl-L-proline moiety derived from L-tyrosine

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4-alkyl-L-proline derivatives (APDs) are a group of microbial specialized metabolites with a common APD moiety, but diverse biological functions and final structures. APDs are biosynthesized from L-tyrosine (L-tyrosine-derived APDs) or L-leucine (L-leucine-derived APDs). The L-tyrosine-derived APD is incorporated for instance into clinically used lincosamide antibiotics (e.g. lincomycin A), pyrrolobenzodiazepines with antitumor activity (e.g. tomaymycin), while the L-leucine-derived APDs constitute a part of antituberculotic compound griselimycin and other compounds. The above mentioned specialized metabolites are used in medicine or have a great potential for such applications. Therefore, this study focuses on searching and characterizing new metabolites with an APD moiety; APDs derived from L-tyrosine in particular (referred to as APDs only in the ongoing text).

Our laboratory has developed a new method for the detection of new metabolites with APD moieties produced by soil and marine Actinobacteria, which bear a cryptic gene cluster (BGC) for the biosynthesis of such metabolites. This method is based on feeding the production medium with isotopically labelled 3,4-dihydroxy-L-phenylalanine (L-DOPA), which is an intermediate of the APD biosynthetic pathway. Then, the MS spectra obtained for cultures, which were supplemented with labelled L-DOPA are compared with MS spectra of those without the labelled L-DOPA. When the $^{13}C2$, ^{18}O-L-DOPA incorporates into an APD compound, the respective pseudomolecular ion in the MS spectra obtained from cultures with labelled L-DOPA increases. This method has facilitated the discovery of a new metabolite with an APD moiety. In addition, we have analysed the BGC of this new APD metabolite and this observation has shown us that the new APD metabolite is distinct from known compounds with L-tyrosine-derived APDs. Currently, this new APD metabolite is under investigation in terms of structure elucidation and biological function characterization.

P02

Bacterial aryl sulfotransferase: Substrate specificity and enzyme structure

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Sulfation is an important reaction in human metabolism. The biological activity of many molecules *in vivo* appears to be primarily linked to their sulfated form. Sulfation is performed by specific **PAPS-independent aryl sulfotransferases** (**ASTs**) of bacterial origin in a one-step mechanism using *p*-nitrophenyl sulfate, which is more suitable for laboratory application, as a donor.

In this project we used the His-tagged construct of recombinant AST from ***Desulfitobacterium hafniense*** (***Dh*AST**). Compared to the original construct used in the literature [1], the new construct makes the purification simple and effective using IMAC [12 % yield]. The enzyme activity was measured using *p*-nitrophenyl sulfate as a donor and phenol as an acceptor of the sulfate group. The approximate molecular weight of the *Dh*AST was ca **70 kDa** as estimated from the SDS-PAGE. The **pure recombinant enzyme** was biochemically characterized (pH optimum, temperature optimum and enzyme stability). The achieved results correspond to the published data measured with the original construct. This project is primarily aimed to analyze the relation between the substrate specificity of the enzyme and the structure of the respective sulfate donor. Several **sulfated phenols** were tested as donors, e.g., *m*-nitrophenyl sulfate, 4-methylumbellifenyl sulfate, 4-nitrocatechol sulfate etc. The acquired kinetic parameters give us important information on the structure of the enzyme active site and the interaction between the enzyme and the substrate. The optimized method of high-yield production and easy purification of *Dh*AST will enable to crystallize the enzyme and examine its structure by **X-ray analysis**.

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1. van der Horst M. A., van Lieshout J. F. T., Bury A., Hargot A. F., Wever R. Sulfation of various alcoholic groups by an arylsulfate sulfotransferase from *Desulfitobacterium hafniense* and synthesis of estradiol sulfate. Adv. Synth. Catal. 2012, **354**, 3501-3508.

P03

Pseudomonas putida consortium for utilisation of oligosaccharides from plant biomass

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Lignocellulosic biomass is gaining increased attention as a cheap and renewable carbon source for sustainable biotechnologies. However, lignocellulose hydrolysis is an expensive and imperfect process resulting in a mix of monosaccharides (mostly glucose, xylose, and arabinose),

oligosaccharides, and toxic aromatic compounds derived from lignin. As the field of microbial bioengineering advances, lignocellulose depolymerisation could be partially handed over to a suitably robust bacterial host, such as *Pseudomonas putida*. This organism has native pathways for the degradation of aromatics and can be engineered to use disaccharides instead of sugar monomers.

This study aims to prepare a *Pseudomonas putida* consortium metabolically engineered to consume non-native substrates – xylobiose and cellobiose. We tested the expression of β-xylosidase *xyl43A* and β-glucosidase *bglC* (both from *Thermobifida fusca*) under various conditions in *P. putida* and confirmed their functionality in degrading xylobiose and cellobiose to xylose and glucose, respectively. In addition, our data suggest that there are native transporters for oligosaccharides in *P. putida*. Based on these findings, a synthetic mutualistic consortium was constructed. The consortium consists of two strains: a glucose consuming strain expressing *xyl43A* and a xylose consuming strain expressing *bglC*. This setup leads to a mutual dependence of the two consortium strains *via* carbohydrate cross-feeding.

This study lays a foundation for the construction of a new bacterial platform for the production of value-added chemicals from lignocellulosic biomass. The employed oligosaccharide catabolism could not only enable more complete utilisation of lignocellulose-derived carbohydrates but also bypass carbon catabolite repression – a big challenge for microbial biotechnology.

P04

Engineering *Pseudomonas putida* for co-utilisation and valorization of cellobiose with glucose

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Lignocellulose is an abundant, cheap, and renewable source of carbon and can therefore serve as a substitute for fossil fuels that cause an increasing environmental burden. Among the reasons, why lignocellulose is still not massively used in the bioindustry, are its complex monomeric composition, and the limitations that stem from the microbial conversion of monomers and oligomers into products.

In our previous work, we successfully established cellobiose metabolism in the biotechnologically relevant bacterium *Pseudomonas putida*. However, the recombinant strain with β-glucosidase (BglC) from *Thermobifida fusca* showed diauxic behavior when grown on a mixture of glucose and cellobiose [1]. In this study, we tackled this issue.

First, we proved that D-glucono-δ-lactone produced in the periplasm from glucose by glucose dehydrogenase (Gcd) inhibits BglC and is responsible for the observed diauxia. The deletion of *gcd* re-

moved the diauxic growth but also significantly reduced the growth rate of the mutant on glucose. The correction strategy included expression of glucose facilitator gene *glf* from *Zymomonas mobilis* and lactose transporter gene *lacY* from *Escherichia coli* in *P. putida* Δ*gcd*. These modifications increased the growth rate on glucose almost to the level of the wild type strain and also improved utilization of cellobiose. We now plan to apply computational approaches to further improve the resulting *P. putida* mutant and redirect carbon toward the production of bioplastics – polyhydroxyalkanoates.

1. Dvořák P., de Lorenzo V., Refactoring the upper sugar metabolism of *Pseudomonas putida* for co-utilization of cellobiose, xylose, and glucose. Metab. Eng. 2018, 48, 94–108.

P05

The reticuline epimerase – an important enzyme for the identification of poppy varieties with high content of alkaloids

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Opium poppy (*Papaver somniferum L.*) is an important oilseed for a food and pharmaceutical industry. Poppy varieties cultivated for food have a good nutritional and sensory quality and low levels of opiates. Its seeds are an important food item in many countries of Central-Eastern Europe. Others varieties, containing high levels of alkaloids, are the only commercially viable source of narcotic raw materials used by the alkaloid pharmaceutical industry. Seeds of these varieties have worse sensory and nutritional properties and, therefore, are not suitable for food. Nevertheless, there have been several cases of poppy seed falsification when the seeds of pharmaceutical varieties were branded as food. In this work, we tested the possibility to differentiate *P. somniferum L.* species and varieties by DNA analysis of gene coding reticuline epimerase (REPI), an important enzyme for the biosynthetic pathway of opium alkaloids. We sequenced REPI gene of different *P. somniferum* varieties and compared the differences among them. Finally, we used PCR-RFLP for DNA analysis. REPI gene seems to be suitable for the differentiation of poppy varieties with low- and high alkaloid content.

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P06

Antimicrobial activity of extracts of arrayan (*Luma apiculata*) and peumo (*Cryptocarya alba*)

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Arrayan (*Luma apiculata*) and peumo (*Cryptocarya alba*) fruits are commonly used in the traditional medicine of Chile. In this study, we characterized the antimicrobial activity of methanol fruit extracts. The half-maximal inhibitory concentration of extracts inhibiting both drug-sensitive and drug-resistant strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa*, inhibition of biofilms formation and disruption of mature biofilms (IC_{50}, mg/mL) were determined. Also, the chemical composition of extracts was analyzed by high-resolution liquid chromatography coupled with mass spectrometry (U-HPLC/MS).

Overall, the arrayan extract (IC_{50}: 0.35-0.01 mg/mL) was more effective than peumo extract (IC_{50}: 0.53-0.02 mg/mL) in the inhibition of drug-sensitive and drug-resistant *S. aureus* planktonic cells. Concerning the inhibition of biofilm formation, arrayan extracts were more effective against *S. aureus* (IC_{50}: 0.23 mg/mL) and *P. aeruginosa* (IC_{50}: 0.29 mg/mL) than peumo extracts (against *S. aureus* IC_{50}: 0.47 mg/mL, *P. aeruginosa* IC_{50}:0.35 mg/mL). Arrayan and peumo showed no activity on the disruption of *S. aureus* mature biofilm, contrary to disruption of *P. aeruginosa* mature biofilm observed for both extracts (arrayan: IC_{50}:0.56 mg/mL; peumo: IC_{50}:0.59 mg/mL). U-HPLC/MS results showed that arrayán fruit extracts mainly possess quercetin compounds and the peumo fruit extract showed quercetins, phenolic acids and phenylpropanoids compounds as potential bioactive compounds.

Our results suggest that extracts of native fruits of Chile can be used as natural antimicrobials to control bacteria responsible for some skin and nosocomial infections. This antimicrobial activity is likely due to the complex bioactive profile.

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P07

A novel approach to the assessment of current state quality of natural reservoirs

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Outdoor swimming has always been popular among citizens living in larger cities, including Prague. The assessment of bathing water quality in the Czech Republic is regulated by Act No. 258/2000 Coll. and Decree No. 238/2011 Coll., which introduce five quality categories for natural bathing waters. According to these regu-

lations, the evaluation of microbiological indicators should be based on results obtained over four bathing seasons, while for other indicators (e.g. cyanobacteria) on the latest analysis. Since 2018, we have analyzed the current state of 57 potential bathing sites in Prague, Czech Republic. We evaluated several microbial parameters (enterococci, *Escherichia coli* and fecal coliform bacteria) by standard culture methods, the occurrence of cyanobacteria was determined visually, if necessary confirmed by microscopy. These data were supported by measurement of water temperature and Secchi plate transparency and visual evaluation of water pollution. To assess the water quality of these localities, as an alternative to Decree No. 238/2011 Coll., a simplified procedure for the approximate assessment of natural reservoirs was proposed, which can be used to assess a single sampling. Based on the novel approach, the natural reservoirs were classified into 3 categories ("satisfactory", "satisfactory with reservation" and "unsatisfactory") and this approach will be also designed for the purposes of the indicative assessment of the current water quality condition.

P08

Relationship of (1,3;1,4)-β-D-glucan content and transription profile of CslF6 gene in oat (*Avena sativa* L.)

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(1,3;1,4)-β-D-glucans are unsubstituted, unbranched polysaccharides containing β-D-glucopyranosyl monomers polymerized by both (1,3)- and (1,4)-bonds found in family Poaceae. They are found in cell walls of type II, where, along with glucoarabinoxylans, were bound on cellulosic microfibers, thereby providing support and elasticity of the cell wall. This may also be related to the plant defense barrier. The aim of experiment was relationship of (1,3;1,4)-β-D-glucan content in five genotypes of oats comparing to abiotic (growing on Cd^{2+}) and biotic stress (artificial infection *Blumeria graminis*) and analysis of transcription of CslF6 gene, which is dominant in biosynthesis of (1,3;1,4)-β-D-glucans in cereals. Level of (1,3;1,4)-β-D-glucan decrease during abiotic and biotic stress, however transcript levels of CslF6 were not significant higher during biotic stress. Growth on cadmium did not affect the level of CslF6 transcription.

Key words: *Avena sativa*, (1,3;1,4)-β-D-glucans, ClsF6 gene

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P09

Applications of capillary electrophoresis for the determination of honey composition

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Evaluation of quality and safety as well as nutritional and functional properties of foods require the use of reliable, accurate, flexible and cost-effective analytical methods. Nowadays, capillary electrophoresis (CE) is a versatile method in the field of food analysis, with advantages related to its high separation efficiency, rapid method development, small sample and reagent requirement and relatively low operational costs; offering opportunities to obtain valuable information which can be correlated with food quality and safety, food interaction and processing and authenticity evaluation.

Honey is a very complex matrix; consisting mainly of sugars and other elements like amino acids, organic acids, carotenoids, minerals and aromatic substances. The composition of honey varies according to botanical sources, and is also determined by climate conditions, processing and storage. Also, honey is a product that during storage undergoes several changes in its composition, due to different chemical reactions.

Different CE techniques were applied for the determination of sugars (fructose, glucose, sucrose), hydroxymethylfurfural, organic acids, polyphenols and mineral content in honey.

Analysis of carbohydrates in honey by CE requires indirect UV detection using appropriate co-ions added to the electrolyte solution.

Micellar electrokinetic chromatography (MEKC) can be used to characterize honey flavonoids, capillary zone electrophoresis (CZE) with UV detection to determine the most important nonaromatic organic acids, while capillary zone electrophoresis – mass spectrometry (CZE-ESI-MS) method has been used to identify and characterize phenolic compounds after solid-phase extraction (SPE).

The determination of mineral content of honey by CE does not require previous steps like sample mineralisation and digestion, and generally an indirect method of separation is applied, using a complexation agent. Mineral content of honey samples can be used for the classification of honey by geographical origin.

The current presentation summarizes the principles, advantages and disadvantages of CE methods applied for the determination of honey components.

References:

1. Pascual-Mate A., Oses S., Fernandez-Muino M., Sancho T. Methods of analysis of honey. J Apic Res. 2018, 57(1) 38-74.

2. Lu Y., Hu Y., Wang T., Yang X., Zhao Y. Rapid determination and quantitation of compositional carbohydrates to identify honey by capillary zone electrophoresis. CyTA - Journal of Food. 2017, 15(4) 531-537.

3. da Silva PM., Gauche C., Gonzaga LV., Oliveira Costa AC, Fett R. Honey: chemi-

cal composition, stability and authenticity. Food Chem. 2016, 196 309-323.

4. Rizelio VM., Gonzanga LV, da Silva Campelo Borges G., Maltez HF., Oliveira Costa AC, Fett R. Talanta. 2012, 99, 450-456.

P10

Selective β4-galactosidase from *B. circulans*: heterologous production and site-directed mutagenesis

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Glycosidases, which naturally cleave glycosyl residues from carbohydrates or glycoproteins, may also be used for synthetic applications in the laboratory. By site-directed mutagenesis of the catalytic nucleophile it is possible to abolish their hydrolytic activity, providing synthetically potent glycosynthases that use glycosyl fluorides of the opposite anomeric configuration as glycosyl donors [1]. In this work, the synthetic potential of new recombinant β-galactosidase from *Bacillus circulans* isoform A (BgaD-A; EC 3.2.1.23) was studied through site-directed mutagenesis. The expression of this large protein (189 kDa) cloned in the expression vector pCOLD II was realized at 15 °C to ensure its selective induction and correct folding [2]. Three tentative glycosynthases were prepared by mutating the active-site catalytic nucleophile. However, none of the mutants processed α-galactosyl fluoride as a galactosyl donor, which was caused by sterical conditions in the enzyme active site as explained by molecular modelling. Thanks to the remaining hydrolytic activity, two of the mutants were able to selectively synthesize azido-functionalized *N*-acetyllactosamine using the standard *p*-nitrophenyl β-galactoside as a galactosyl donor. Thus, they behaved as transglycosidases. This study demonstrates for the first time that the substitution at the catalytic nucleophile is not a versatile tool for glycosynthase construction in retaining glycosidases and that the overall mutagenesis effects depend on the particular relations in the enzyme active site.

Keywords: galactosidase, site-directed mutagenesis, glycosynthase, transgalactosidase

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[1] Q. Wang, S. G. Withers, R. W. Graham, D. Trimbur, R. A. J. Warren, "Changing Enzymic Reaction Mechanisms by Mutagenesis: Conversion of a Retaining Glucosidase to an Inverting Enzyme," *J. Am. Chem. Soc.*, vol. 116, no. 25, pp. 11594–11595, Dec. 1994, doi: 10.1021/ja00104a060.

[2] J. Song, K. Abe, H. Imanaka, K. Imamura, M. Minoda, S. Yamaguchi, K. Nakanishi, "Cloning and expression of a

β-Galactosidase Gene of Bacillus circulans," *Biosci. Biotechnol. Biochem.*, vol. 75, no. 6, pp. 1194–1197, 2011, doi: 10.1271/bbb.110014.

P11

Effect of red yeast rice extract on germination of bacterial spores

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Red yeast rice is the rice prepared by fermentation with the *Monascus* fungi. *Monascus* fungi are ascomycetes known and used in Asia for centuries for food dyeing and conservation, or as a part of traditional Chinese medicine. The most important and most used feature is pigment production. The pigments exhibit antiproliferative, anti-inflammatory, or antimicrobial activity. Effects of red yeast rice extract on germination of *Bacillus subtilis* and *Clostridium beijerinckii* spores were studied. *Bacillus spp*. and *Clostridium spp*. are important food contaminants, especially *Clostridium botulinum* in meat products. For the suppression of clostridial spores germination, nitrite solutions are used, their side effect is the pleasant colour of processed meat products. To study the effect of nitrite solution on germination, sodium nitrate, sodium nitrite (300 mg/l), and butcher's nitrite pickling mixture (1.3 and 2 g/L) were used. The independent effect of NaCl (1.3 and 2 g/l) was tested too. Besides polyketide pigments, some species of fungus *Monascus* also produce monacolins and mycotoxin citrinin. The citrinin effect on spores germination was not observed. As our results indicate, *Monascus* pigments have a potential for safe use as a dye and meat products preservative with desired inhibition effect on clostridial spores germination.

P12

Pterostilbene – a potent antibiofilm drug for fight against representatives of genus *Staphylococcus*

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Antibiotic resistance among microorganisms represents an urgent problem of today clinical practice. With common antibiotics failing to cure infectious diseases caused by resistant pathogens, an alternative strategy for their treatment emerged acting via inhibition of infectious properties of the pathogens rather than their killing [1]. Virulence enables pathogen to cause host infection in the first place, to further promote it and colonize other host tissues and effectively evade host immune system. The virulence factors comprise of various toxins, extracellular enzymes or adhesins. Biofilm formation and morphology switching act also as a manifestation of virulence in pathogen [2]. Therapeutics capable of suppression of virulence could enhance modern antibiotic therapy and effectively treat infections caused by these otherwise persistent untreatable microbes [3].

Pterostilbene, a natural antioxidant found in various plants (*Vaccinium* berries, bark of *Pterocarpus* trees or in wine grapes) has a wide spectrum of beneficial pharmacological effects, among which the most outstanding is the antimicrobial combinatory activity with antibiotics [4,5]. In this study, its effect was observed on *Staphylococcus aureus* and *Staphylocccus epidermidis*. The effect of pterostilbene alone and in combination with erythromycin was studied on suspension growth and biofilm formation of mentioned species. Its antivirulence effect on production of extracellular virulence factors (proteases, phospholipases, haemolysins etc.) was further examined by agar plate and colorimetric methods. The results were supported by scanning electron microscopy.

Pterostilbene was found to have permeabilization activity on cell membranes in *Staphylococcus* spp., which is likely crucial for potent synergistic activity with antibiotics. The biofilm formation was supressed as proved also by fluorescent microscopy and decreased haemolytic activity of *S. epidermidis*. Pterostilbene was proved to have antivirulence activity on genus *Staphylococcus* and the enhancement of antibiotics activity was shown to be very beneficial.

References:

1. Ventola C. L. The Antibiotic Resistance Crisis. Pharm. Chemother. 2015, *40(4)*, 277-283.
2. Barczak A. K., Hung D. T. Productive steps toward an antimicrobial targeting virulence. Curr. Op. Microbiol. 2009, *12(5)*, 490-496.
3. Tong S. Y. C., Davis J. S., Eichenberger E., Holland T. L., Fowler V. G. Staphylococcus aureus Infections: Epidemiology, Pathophysiology, Clinical Manifestations, and Management. Clin. Microbiol. Rev. 2015, *28(3)*, 603-661.
4. Lee W. X., Basri D. F., Ghazali A. R. Bactericidal Effect of Pterostilbene Alone and in Combination with Gentamicin against Human Pathogenic Bacteria. Molecules 2017, 22(3), 463-475.
5. Rimando A. M., Kalt W., Magee J. B., Dewey J., Ballington J. R. Resveratrol, pterostilbene, and piceatannol in vaccinium berries. J. Agr. Food Chem. 2004, 52(15), 4713-4719.

P13

Overexpression of Trp-related genes leads to increased ergot alkaloid production in *Claviceps purpurea*

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A biotrophic fungus *Claviceps purpurea* that infects more than 400 monocotyledonous plant species including barley, rye and wheat is widely used in the industry for the ability of ergot alkaloid (EA) production. Although EAs are normally produced by the wild-type fungus only *in planta* when a purple dark sclerotium is formed, various strains of *C. purpurea* producing EAs also

in axenic culture have been prepared. EAs production by these strains is usually induced by tryptophan (Trp) and inhibited by high levels of inorganic phosphate.

To increase EAs production, we prepared *C. purpurea* strain P1 overexpressing a mutated *TrpE* gene encoding anthranilate synthase (TrpE), a key enzyme in Trp biosynthesis, and *dmaW* gene encoding dimethylallyltryphophan synthase (DMATS), a key enzyme in EA biosynthetic pathway. As the physiological function of *TrpE* gene has not yet been fully confirmed in *C. purpurea*, a knock-out mutant of the native gene was also created. Growth of this mutant was examined on a minimal medium supplemented with anthranilate and Trp and its ability to infect rye plants was tested. The content of anthranilate and Trp was determined in mycelia as well as in the culture media of *C. purpurea* overexpressing mutated version of TrpE at both low and high level of phosphate. *C. purpurea* overexpressing DMATS was examined under the same conditions. Moreover, DMATS was fused to green fluorescent protein to follow the overproduction by fluorescence microscopy and western blot analysis. All samples were also used for EAs determination and subsequent qRT-PCR analysis.

Altogether, the overexpression of mutated anthranilate synthase insensitive to feedback inhibition by Trp and dimethylallyltryptophan synthase represents a promising way how to increase EAs production in *C. purpurea* strain P1.

P14

Basidiomycetes and agricultural waste – Promising agents for a new type of renewable mycelium based biomaterials

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Agricultural wastes are considered to be one of the serious pollutants, which because of their high nutritional value, represent a resource with considerable potential for the production of new value-added materials. The use of metabolite activity of *Agaricomycetes'* basidiomycetes in the process of utilization of agricultural lignocellulose waste is an attractive alternative for the production of innovative mycelium-based bio-composites. These composites could be applied in the construction industry and various industrial sectors together with recycling the lignocellulose substrate and conversion into value-added products using the natural capacity of the fungi.

The study is focused on the controlled solid state cultivation of three species of basidiomycetes - *Pleurotus ostreatus, Ganoderma resinaceum and Trametes versicolor* on different lignocellulose substrates. The assessment of the type and the chemical composition of different lignocellulose substrates on the morphological characteristics and metabolic profile as well as the ability of formation of new bio-composites

by the fungal species is studied. During the cultivation fungal mycelium secretes enzymes that break down lignocellulose polymers and synthesizes metabolites that bind the residual structures resulting in bio-composite material. The formation of mycelium based bio-composites and the mycelium growth of the fungi are monitored by determining the density and by different microscopic and spectral methods.

P15

Vitis vinifera endophytes and production of their beneficial metabolites

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Vitis vinifera is one of the most economically important crops. It has been used since the Middle Ages mainly for wine production, which consumes the highest part of harvested grapes. As with other plants, *Vitis vinifera* tissues are inhabited by various types of microorganisms. These organisms can be epiphytic, i.e. superficial, or colonizing internal tissues, i.e. endophytic. Endophytes are beneficial to their host in many ways, especially as a defence against foreign phytopathogens through production of variety of metabolites. These natural metabolites could be useful in agriculture, industry and medicine. Endophytic population is able to produce antibacterial, antifungal, antiviral, cytotoxic and immunosuppressive metabolites. Endophytes are also capable of producing antioxidants and siderophores. Nowadays, when new diseases caused by microorganisms are emerging and resistance to known drugs is expanding, it is siderophores from a relatively poorly studied endophytic population that could be used to develop active ingredients in the pharmaceutical industry. Screening of the endophytic micromycete population was the initial task of our study. The production of biotechnologically interesting metabolites (ability to form siderophores and antioxidant activity) was subsequently determined for the isolates.

P16

Polyamide nanofibers functionalized with natural substances as a promising active food packaging

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Nowadays, great emphasis is placed on the quality and safety of food. Indisputably, food packaging plays the main role in food protection and therefore new packaging technologies are sought. Active food packaging, based on active functions providing extended food shelf life, has become one of the most innovative approaches. Our long-term aim is to develop functional nanomaterials which can be used as an active food packaging providing a protection of the final consumer from foodborne microbial infections and intoxications. In this study, we analyzed overall interactions between electrospun polyamide (PA) nanofibrous materials functionalized with natural substances (natamycin, green

tea extract, rosemary extract) and bacteria *Escherichia coli*, *Listeria monocytogenes*, *Salmonella enterica*, and *Staphylococcus aureus*. We tested PA's permeability for bacterial cells, antimicrobial and antibiofilm properties. The results showed i) excellent retention of all the tested PAs reaching up to 6.4 $\log_{10}$removal, and ii) a significant ($p \leq 0.05$) inhibition of both microbial growth and biofilm formation (up to 3 $\log_{10}$suppression) by the functionalized PAs. Further, the PAs were tested as a packaging of chicken breast both non-inoculated and inoculated with *L. monocytogenes*; the functionalized PAs prolonged microbial, pH, and sensory quality of the chicken samples. Obtained results confirmed the potential of PA nanofibers in packaging applications and suggest that they could be a functional and ecological alternative for traditional materials.

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P17

In Vitro Pharmacokinetics of Therapeutic Nanoparticles

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A significant obstacle, which all intravenously administered nanoparticulate systems for drug delivery are facing is an extensive uptake by immune cells, which leads to the nanoparticle removal from the bloodstream and accumulation in organs such as the liver and spleen. In our previous research, we found out that by modulating the nanoparticle dosage the active tumor targeting efficiency can be improved. The presented work focuses on *in vitro* testing of antibody-modified nanoparticles in interaction with tumor and immune cells. The two basic processes which are studied are (i) the antibody-antigen interaction by which nanoparticles bind to tumor cells and (ii) non-specific phagocytosis of immune cells, a process responsible for the nanoparticle accumulation in the liver and spleen. These two processes differ not only in mechanism and extent, but also lead to different rates of saturation of specific cells (tumor cells are saturated faster). The kinetics of the processes was studied by flow cytometry and the targeting selectivity was confirmed in mixed cell culture via confocal microscopy. Future work will be focusing on designing a dose-dependent mathematical model for the targeting specificity prediction.

P18

Antifungal activity of pterostilbene and amphotericin B in combination against *Candida* biofilm

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Microbial biofilms can be defined as a community of microorganisms encased in a protective matrix of exopolymeric substances, adhering to various biotic and abiotic surfaces. The most common yeast species isolated from nosocomial infections are for example *Candida krusei, Candida parapsilosis*, *Candida glabrata* and *Candida albicans.* The treatment of biofilm-associated diseases is very difficult because biofilm cells often show resistance to a wide range of antimicrobial agents. Therefore, new antimicrobial or anti-biofilm substances are currently being developed. This study is focused on testing a combination of biologically active substances with an anti-biofilm effect. For this purpose, a natural substance pterostilben and antibiotic amphotericin B were selected. We studied their potential to inhibit the biofilm formation of opportunistic yeast strains *Candida albicans* ATCC 2091, *Candida parapsilosis* CCM 8260 and *Candida krusei* CCM 8271. The metabolic activity of the cells in biofilm was determined using MTT assay and the total biofilm biomass was quantified by crystal violet staining. The biofilm was subsequently visualized by a fluorescence microscope. The highest inhibitory effect was confirmed for 40 mg/l pterostilbene and 0.7 mg/l amphotericin B in combination in the case of *C. albicans* ATCC 2091. From these results, we concluded that the combination of these substances has potential for the treatment of *Candida* species infections.

P19

The effect of new psychoactive compounds on monoamine oxidase activity

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Monoamine oxidases are intracellular enzymes bound to the outer mitochondrial membrane. They occur in almost all tissues of the human organism, where catalyse oxidative deamination of neurotransmitters and biogenic amines. This deamination is affected by therapeutic inhibitors in the form of antidepressants and antiparkinsonian drugs. Once monoamine oxidases are inoperative, amines cannot be effectively degraded in the body. The consequences are very dangerous with reckless use of monoamine oxidase inhibitors or in their combination with other xenobiotics, especially if the cause of the intoxication is not identified in time. In the same way, very readily available new psychoactive substances can potentially act. They might be acquired online as preparations indirectly intended for internal use or food supplements. However, structurally are these substances close to psychoactive compounds already regulated by law. Due to the structural diversity, availability, and lack of *in vivo* effects information, it po-

ses a high risk to society. Therefore, the impact of new psychoactive substances from the groups of amphetamines, cocaine, ketamine, tryptamine derivatives, and several synthetic cannabinoids on human monoamine oxidases A and B activity were monitored in this project. The work also deals with the recombinant preparation of both isoforms of human monoamine oxidase in *Pichia pastoris*.

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P20

Preparation of oligosaccharide ligands for galectins

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Galectins are involved in many pathophysiological processes associated with cancer, inflammation, fibrosis, heart disease and some metabolic disorders. Their level is significantly elevated in patients suffering from these disorders and therefore, early detection of galectins is required to significantly improve patient's prognosis, allow better prediction of treatment response and reduce the risk of disease recurrence. Here, we focus on the methodology of the preparation of new oligosaccharide ligands derived from *poly*-LacNAc ([Galb4GlcNAcb3Galb4GlcNAc]$_n$) with high affinity to three selected galectins (Gal-1, -3 and -4) using a combination of chemical and enzymatic synthesis.

The preparation of these tetrasaccharide ligands required a regiospecific β-*N*-acetylhexosaminidase capable of transferring GlcNAc unit to non-reducing galactose residue with β1-3 selectivity. For this aim, we used β-*N*-acetylhexosaminidase from *Bifidobacterium bifidum* (*Bb*hI), which shows the desired 3-OH specificity. The gene of this enzyme (GenBank ID: AB504521) was prepared synthetically on a commercial basis, cloned into the pET21b expression vector and expressed in a suitable strain of *Escherichia coli* in a high yield. We prepared and characterized two new mutant variants of *Bb*Hex to increase the enzyme synthetic activity for the introduction of the β3-linked GlcNAc unit. The β4-galactosylation was performed under the action of recombinant β4-galactosidase BgaD-A from *Bacillus circulans*.

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P21

Antimicrobial activity of metal nanoparticles made using plant waste extract

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Nanotechnology has been an exponentially growing industry during the last half a century; metal nanoparticles in particular have been garnering attention in both scientific and industrial circles. Traditional methods of their synthesis, however, come with indisputable inconveniences: high cost due to energy consumption of the synthesis and environmental impact due to toxic solvent use. Therefore, in the last decades, biological methods of nanoparticle synthesis have been a subject of research. In this study, we explored the route of agricultural waste valorization. Scraps from industrial hemp processing were used for the production of metal nanoparticles. Their presence was later confirmed using UV-Vis. In the end, antimicrobial activity of the nanoparticles was investigated on a model microorganism – *Pseudomonas aeruginosa*. Our results showed, *Cannabis sativa* waste is capable of mediating metal nanoparticle synthesis and the nanoparticles produced exhibit considerable antimicrobial activity against the gram-negative bacterium.

P22

Green synthesis of silver nanoparticles using *Vitis vinifera* extract

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Green approach for metal nanoparticle synthesis is a current topic of modern nanobiotechnology. Especially phytosynthesis using plant extracts gains increasing attention due to its advantages such as environmental friendliness, cost-effectiveness, simplicity of the process, and easy scale-up. In this case, the metal ions are reduced and stabilized by plant constituents such as enzymes, polyphenols, or carbohydrates. In principle, plant extracts can be prepared from any part of the plant including leaves, flowers, or seeds. However, as very promising is considered the use of agrowastes – parts of the plants without further significant use. In our study, we decided to use viticultural waste for nanoparticle synthesis. *Vitis vinifera* dried canes from the wine region of Bohemia were used. This part of grape vine is usually used only for composting, but it has been found to contain many bioactive compounds such as polyphenols [1]. Ethanol extract (40 % v/v) from the canes was mixed with 1 mM aqueous solution of $AgNO_3$ and the pH of this mixture was altered to obtain nanoparticles. The presence of nanoparticles was observed by monitoring the UV-Vis spectrum of the prepared solution (concurrent with the color change of the solution from light brown to dark yellow-brown). Moreover, transmission electron

microscopy (TEM) was used to determine the size and shape of the nanoparticles and atomic absorption spectrometry (AAS) to determine the mass concentration of silver in the nanoparticles.

References

1. Cviner, P., K. Pádrová, and I. Kolouchová, *Moderní možnosti využití odpadních surovin ze zpracování vinných hroznů.* Chemické listy, 2017. 111(2): p. 103-108.

P23

Potential functional capacity of a non-model organism *Aneurinibacillus* species H1

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Polyhydroxyalkanoates (PHA) are polyesters similar to industrially processed synthetic polymers. *Aneurinibacillus* species H1 belongs to thermophilic gram-positive bacteria capable of accumulating the PHA in a high amount under certain conditions. Whereas the bacterium´s genetic information has not been studied for its ability to accumulate PHA yet, we bring the first genome assembly along with insight into its functional properties.

The genome was assembled using sequencing data obtained from Oxford Nanopore MinION and Illumina MiSeq platforms. In the first step, the initial assembly from Nanopore reads was created. Subsequently, trimmed Illumina reads were mapped to the initial assembly with the result of final assembly. The final assembly was rearranged according to the replication origin (oriC) in a way that the *DnaA* gene is the first gene. To reveal potential functional capacity of the genome, we analysed particular protein coding genes and divide them according to clusters of orthologous genes (COG) using the eggNOG-mapper tool. Finally, we have predicted operons using the Operon-mapper tool.

The presented insight into the genome is an ideal beginning for exploring the possibilities of polyhydroxyalkanoates production. The acquired knowledge will help with the future engineering of the strain *Aneurinibacillus* species H1. Moreover, the results can be generalized also for the accumulation of polyesters in related species.

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P24

Modulation of β-*N*-acetylhexosaminidase activity by a semi-rational approach

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β-*N*-Acetylhexosaminidases (GH20, EC 3.2.1.52) are *exo*-glycosidases with dual activity towards both *N*-acetylglucosamine (GlcNAc) and *N*-acetylgalactosamine (GalNAc) carbohydrates. They naturally catalyze *in vivo* cleavage of these moieties from glycostructures [1]. Under suitable reaction conditions, they can also synthesize the glycosidic bond in good yields but the dual substrate specificity complicates the synthesis of complex *N*-acetylhexosamine oligosaccharides containing both GlcNAc and GalNAc units. Substitution of target amino acid(s) in the enzyme active site by site-direct mutagenesis may change the enzyme's substrate specificity [2] or suppress its hydrolytic activity in favor of synthesis [3].

We present genetic engineering of β-*N*-acetylhexosaminidase from *Talaromyces flavus* (*Tf*Hex), a promiscuous enzyme with a high synthetic potential and a broad substrate specificity. *Tf*Hex WT has a GalNAcase/GlcNAcase ratio of 1.2. Crucial amino acids residues responsible for the enzyme affinity to both substrates were identified by molecular modeling. For this study, we selected the Glu332 active-site residue located in the proximity of the C-4 hydroxyl of the substrate, and subjected it to site-saturation mutagenesis. Mutant variants were produced at a small scale in the *Pichia pastoris* expression system in an optimized microtiter-plate set-up, and screened for their GalNAcase and GlcNAcase activity. From 1600 screened colonies, we identified those which produced enzyme with a significantly changed GalNAcase/GlcNAcase ratio for further characterization. The screening was followed by chromosomal DNA isolation, amplification of TfHex gene and identification of the inserted mutation. This study sheds further light on the relationship between the active site structure and the donor substrate specificity of a b-N-acetylhexosaminidase.

Support from the mobility project LTC19038 by the Ministry of Education, Youth and Sports of the Czech Republic and from the project 20-00215S by the Czech Science Foundation is acknowledged.

[1] Slámová K.; Bojarová P. *Biochim. Biophys. Acta - Gen. Subj*. **2017**, *1861*, 2070-2087.

[2] Nekvasilová P.; Kulik N.; Rychlá N.; Pelantová H.; Petrásková L.; Bosáková Z.; Cvačka J.; Slámová K.; Křen V.; Bojarová P. *Adv. Synth. Catal.* **2020**, 362, 4138-4150

[3] Bojarová P.; Kulik N.; Hovorková M.; Slámová K.; Pelantová H.; Křen V. *Molecules* **2019**, *24*, 599.

P25

Effect of high-fat diet on the composition of the intestinal metabolome in a mouse model of tau pathology

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Gut bacteria, commensal and pathogenic microorganisms, can have a major impact on the immune system, brain development, and behavior, as they can produce certain neurotransmitters, neuromodulators, or amyloids. The intestinal microflora participates in bidirectional communication between the gut and the brain, may even act as a "second brain" and can be responsible for neurodegenerative disorders such as Alzheimer's disease (AD). This neurodegenerative disorder is characterized by the presence of senile plaques (amyloid beta – Aβ aggregates); and neurofibrillary tangles (aggregated Tau protein). Obesity or type 2 diabetes contributes to the development of tau pathology; among other things, it is also involved in the dysregulation of lipid metabolism, increased oxidative stress, and the development of inflammation both in the periphery and in the brain.

Currently, the inflammatory-infectious AD hypothesis with the role of the intestinal microbiome is coming to the fore, while the hypothesis of the amyloid cascade, which has dominated for decades, is gradually being pushed into the shadow. The human microbiome is generally stable, but the change from natural intestinal microflora to pathological (dysbiosis), the development of local and systemic inflammation or dysregulation of the intestinal-brain axis can lead to dementia and even to the development of AD. Increased gut barrier permeability results in the invasion of various bacteria, viruses, and their neuroactive products, which contribute to neuroinflammatory reactions in the brain.

There are several well-studied mouse models of AD, especially with mutations that stimulate only the formation of Aβ plaques. This project contributes to the completion of the complex characterization of THY-Tau 22 – a unique mouse model in which neurodegeneration develops exclusively through the emergence of tau pathology. Changes in urine and feces' composition induced by obesity and tau pathology were monitored using nontargeted metabolomics based on liquid chromatography-mass spectrometry. According to the statistical analysis, significantly altered groups of metabolites were identified. For example, an increase in carnitines and a histamine metabolite in urine was found in mice groups on a high-fat diet, which may indicate mitochondrial dysfunction and inflammation.

P26

Proteomic analysis of phospholipid-binding proteins in the regulation of plant cell polarity

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Specific protein-lipid interactions are fundamental for all organisms. They regulate reproduction, growth, morphology, and responses to pathogens and more. In plants, lipid-protein interactions have not been satisfyingly described yet. Our laboratory has already proved that distribution and relative amount of anionic phospholipids, such as phosphatidylinositol phosphates, phosphatidylserine, and phosphatidic acid, on plasma membrane are affecting plant cell polarity. Various proteins are specifically bound to anionic lipids in membranes and these interactions then start various signaling processes, also involving exocytosis and endocytosis. We hypothesize that distinct combinations of anionic lipids are responsible for regulation of vesicular transport processes like exocytosis and endocytosis. Aim of this project is to identify peripheral membrane proteins interacting with anionic phospholipids, to analyse comparatively the specificity of these interactions towards distinct anionic lipids and to analyze the effect of membrane curvature on protein-phospholipid interactions. We will reach this aim by binding the proteins to prepared lipid vesicles with various composition and size. After the binding, we will isolate and analyze the proteins on MALDI-TOF MS and LC-MS/MS. This research will improve our understatement of cell polarization mechanisms and signalling protein targeting in cell.

Julkowska M M, et al. (2013). In Munnik T., Heilmann I. (eds) Plant Lipid Signaling Protocols. Methods in Molecular Biology, vol 1009.

McLoughlin F, et al. (2013). Biochemistry Journal 450:573–581

Potocký M, Pleskot R , Pejchar P, Vitale N, Kost B a Žárský V (2014), Live-cell imaging of phosphatidic acid dynamics in pollen tubes visualized by Spo20p-derived biosensor. New Phytology, 203: 483-494. doi:10.1111/nph.12814

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P27

Renaissance of *Arabidopsis* mutants with modulated salicylic acid pathway: "SA mutant collection" and its usage for study of salicylic acid effects on plant growth under stress

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Salicylic acid (SA) is a very important phytohormone. SA is, as a signal molecule, involved in plant response to bio-

tic (e.g. pathogen attack) and abiotic stresses (e.g. high salinity). If the SA content is abnormally high, plant activates immune system, which generally causes stunted growth phenotype.

We aim to study a role of SA in crosstalk of plant growth and response to stress. For that purpose, we are creating a collection of mutants with altered SA pathway. These mutants are already available based on the published literature.

Our "SA collection" includes seventeen *Arabidopsis thaliana* mutants with modulated SA pathway: three SA-downregulating mutants, seven canonical SA-over accumulators, three weak SA-over accumulating mutants and four SA-over accumulating mutants with introduced mutation for SA downregulation ("reverting" mutants).

We characterized their growth (size, weight, root length), SA content and gene transcription under very distinct growth conditions: i) growing in soil until age of four week under long and short day conditions; ii) growing *in vitro* on agar plates under distinct light intensity until the age of fourteen days.

Our data show that the length of the roots under *in vitro* growth does not correlate with size of the rosettes of plants cultivated in soil. It is strongly dependent on the mutation triggering SA pathway.

Additionally we show that distinct light conditions (intensity, day length), in connection with the type of mutation, affects strongly also rosette and root growth provide the evidence that it is not only activated SA pathway which is affecting Arabidopsis thaliana growth in mutants.

In summary, our mutant collection provides "new" tool for the study of SA pathway especially for investigating the role of SA under distinct stress conditions.

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P28

Research and developmental activites of the Biotechnological Pilot Plant (IMIC)

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The Core Facility Biotechnological Pilot-Plant of the Institute of Microbiology has a long history, but not as long as the Institute itself. Since mid eighties the main goal of the facility has been met: To facilitate the transfer of the research into practice.

The strategy of the pilot project of a typical submerged fermentation process followed by appropriate down-stream procedures will be demonstrated. Also a survey of the facility equipment will be displayed accompanied by a short explanation of the function.

Finally, a list of the current project would be shown completed with a more or less detailed description.

Currently there are many biotechnological projects of public and contract research dealt with. Since the equipment is mostly designed for aerobic submerged cultivation

technologies and the subsequent downstream processing, we are correspondingly engaged in cultivation of microorganisms, manufacturing of microbial products, optimization of bio-processes and, first of all, in scale-up of the microbial productions.

The Biotechnological Pilot-Plant follows up the areas of

- human and veterinary medicinals,
- antibiotics, vitamins, carriers, vaccines, biologically active compounds, proteins
- nutrition and feeds
- proteins, polysaccharides, lipids, enzymes
- enzyme technology
- detergents, pharmacy, food and feed industry
- decontamination
- environmental biotechnology
- soil amendments, fertilizers

P29

Microwave-assisted extraction of bioactives from *Satureja hortensis*

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Summer savory (*Satureja hortensis*) has a significant role in phytopharmacy because of its numerous pharmacological activities and presence in traditional medicine for the treatment of different diseases and conditions. Some of the activities that are scientifically proven are antioxidant, antimicrobial, analgetic, and antirpoliferative effects [1].

In order to obtain high quality extracts, microwave-assisted extraction (MAE) was employed for the extraction of polyphenolics from *S. hortensis*. Additionally, to achieve maximal exploitation of the material and investigate the possibility of increasing the efficacy of the extraction of polyphenols, supercritical carbon dioxide ($ScCO_2$) was applied before MAE to separate lipophilic components from the material. $ScCO_2$ was applied with three different pressures (100, 200, and 300 bar) at temperature of 40°C. For comparison, MAE (50% ethanol as solvent) was conducted on both the exploited and unexploited raw materials with two different microwave irradiation powers (470 and 800 W). Next, the content of total phenolics (TP) and total flavonoids (TF) in the obtained extracts was determined.

The highest exhaustion of material and $ScCO_2$ extraction yield were achieved at 200 bar pressure. It was determined that the content of phenols in the extract obtained without the $ScCO_2$ pretreatment was higher compared to the pretreated extracts at a lower irradiation power (3.63 mg GAE/mL), while the highest content of phenols was measured in the extract obtained by using a higher irradiation power and after the extraction with 100 bar. A similar trend was noticed with the content of flavo-

noids, hence the higher efficacy of the extraction of flavonoid was achieved from the untreated raw material by applying $ScCO_2$.

Taking the process and equipment of the $ScCO_2$ system costs into consideration, it can be concluded that the application of $ScCO_2$ is not justified following the procedure of improving the yield of polyphenolic compounds. Nonetheless, considering the importance of lipophilic components of *S. hortensis* extracted by applying this green extraction method, the justification for its application exists.

References

1. Momtaz, S., & Abdollahi, M. (2008). A systematic review of the biological activities of *Satureja* L. species. Pharmacology-online, 2, 34-54.

P30

Effect of grapevine extracts and their biologically active substances on selected gut-associated microbiota

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The human intestine contains a multitude of bacterial species, which are an important part of many vital processes. Dietary polyphenols have shown to modulate the gut-associated microflora composition and function. The biological activity of polyphenol substances contained in food supplements prepared from *Vitis vinifera* can affect the microorganisms present in the digestive tract in terms of their representation and activity of the individual species. This study deals with resveratrol and two polyphenol-rich extracts (extract from *V. vinifera* canes and commercially available dietary supplement, which contains the *Vitis vinifera* grape extract and extract of *Polygonum cuspidatum* root) and their effect on selected gut microbiota, both beneficial and opportunistic pathogens. The effect of the studied agents on planktonic and biofilm growth of the microorganisms was determined as minimum inhibitory concentration (MIC80) and minimum biofilm inhibitory concentration (MBIC80), respectively. The *V. vinifera* cane extract prepared from plant waste obtainable from viticulture was proved to have similar antimicrobial properties against opportunistic bacterial pathogens (*E. coli* DBM 3125, *C. freundii* DBM 3127) as the commercially available supplement, which was fortified to contain a certified amount of the resveratrol complex. Both extracts were able to positively influence the growth of probiotic strain *L. acidophilus* LA-5.

P31

Production of sourdoughs from special gluten-free flours

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Fermentation processes in cereal technology has been one of the oldest biotechnologies applied in food processing. While in the past the sourdoughs was obtai-

ned by spontaneous fermentation of a mixture of flour and water, currently is possible to use controlled fermentation processes using defined starter cultures. Sourdoughs are most often produced from wheat or rye flour, but may also be prepared from flours from other sources. In the present work gluten-free flours from sorghum, buckwheat and rice were used for the preparation of special sourdoughs. Basic analytical parameters - moisture, ash content, protein content and retention capacity were determined by these flours. In addition, spontaneous fermentation and fermentation using starter cultures were tested under pilot plant conditions. Prepared sourdoughs were evaluated in terms of pH and titratable acidity, content and ratio of organic acids and profile of volatile compounds. Sorghum and buckwheat sourdoughs are suitable for fermentation using starter cultures as well as for spontaneous fermentation. A starter culture was required to obtain rice sourdough with appropriate parameters. The selected sourdoughs were tested for their addition to the recipe of gluten-free bakery products by means of laboratory experimental baking.

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P32

Exploring cyanide-degrading enzymes in fungi

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The removal of cyanide from industrial effluents is a timely problem addressed by researchers and industries. The free cyanide (fCN) consisting of hydrogen cyanide (HCN) and cyanide ions (CN^-) is the most toxic form of cyanide. The fCN is the cause of acute or chronic poisoning in humans, animals and aquatic organisms. It occurs in the effluents from various industrial manufactures (metallurgy, organic synthesis, petrochemistry, coal coking etc.). These effluents often contain significant fCN concentrations at normal operation of the plant. In addition, the fCN levels in the effluents can be increased by accidental spills. The current methods of effluent treatment are largely based on oxidation requiring large amounts of chemicals but more eco-friendly biological processes emerge. Some of them are based on the use of enzymes. Many fungi contain cyanide hydratases (CynHs), which transform fCN into formamide. Within our studies we designed and overproduced six CynHs and examined their catalytic properties. Recently, we prepared a new CynH from *Exidia glandulosa*, which exhibited a good stability and activity over a wide pH range - desired properties for industrial applications. The activity of this enzyme at

up to pH 10 is important for the safety of handling fCN (prone to volatilize at pH below 8.5). This enzyme is resistant to typical contaminants occurring in the industrial effluents (phenol, sulfide, thiocyanate, ammonia, silver). It is also functional at high fCN concentration (up to 100 mM) mimicking cyanide spills.

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P33

Micromycetes proteolytic enzymes for biotechnology

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Proteases have a broad application potential, for example, in biodegradation and feed additives production. Micromycetes from the genus Aspergillus, in particular, are known as efficient producers of extracellular proteases with a different substrate specificity, which makes the investigation of proteolytic enzymes secreted by microscopic fungi an urgent task of modern biotechnology.

The screening was conducted to explore the ability of selected five Aspergillus species to perform a proteolytic activity. 0.2% suspensions of azocasein, azocollagen, elastin congo red (all prepared on 0.05M Tris-HCl buffer, pH 8.2), and fibrin blue (prepared on 0.05M acetate buffer, pH=5.5) were taken. Complexes of extracellular proteins were obtained by salting out from culture liquid, dialysis, and lyophilization. Proteolysis was carried out with diluted enzyme preparations (2 mg/ml) and was measured spectrophotometrically. One unit (U) of activity was defined as an increase of absorbance by 0.01 under the assay conditions (37 °C, 600 rpm).

The following results were observed: A. raperi and A. alliaceus showed the highest proteolytic activity with azocollagen (25.4 U and 20.5 units, respectively). Fibrin blue was considerably hydrolyzed by A. sydowii and A. flavipes (16.2 U and 21.0 U, respectively). A. flavipes had an insignificant general proteolytic activity (2.5 U) with azocasein when the highest values were provided by A.flavus and A.sydowii (100.2 U and 101.9 U). All micromycetes have shown insignificant activity with elastin congo red (<1.2 U), which indicates that these species are doubtful to be pathogenic.

Due to the shown enzymatic activity of micromycetes, Aspergillus may be considered as promising producers of proteases for application in biotechnology, e.g., biodegradation of animal waste, such as cartilage and bones containing a high percentage of hard-to-degrade proteins.

P34

Viola odorata: Influence of supercritical fluid extraction on the efficiency of microwave-assisted extraction of bioactive compounds

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Viola odorata (sweet violet) is an evergreen perennial herb and a member of the family *Violaceae*. Sweet violet shows anti-inflammatory, antipyretic, antibacterial activity, and hepatoprotective activity. While the majority of research is focused on essential oils or lipophilic compounds of the sweet violet, the potential of polar bioactive components in sweet violet remains largely unexplored.

In order to obtain high quality extracts of polar components, the raw material was firstly submitted to supercritical carbondioxide ($ScCO_2$) extraction which extracted the lipophilic ingredients. Then the exploited raw material was subjected to ultrasound-assisted extraction (UAE) in order to extract polyphenolic components.

The applied supercritical extraction was performed with pressure of 300 bar and at temperature of 40 °C during 4 hours. In order to see the benefits of $ScCO_2$, the ultrasound-assisted extraction (50% ethanol as solvent) was conducted on both exploited and unexploited raw materials. Also, the impact of the various UAE conditions was tested. The UAE was conducted on two different temperatures (40 °C and 50 °C) and two different times of extraction (40 min and 20 min). The research was focused on the content of total phenols, total flavonoids, and antioxidant activity of the extracts.

The yield of supercritical extraction was 1.43% (w/w). Next, it was determined that the extracts obtained with UAE after the $ScCO_2$ were noticeably richer in phenols (specifically on the temperature of 40°C and 20 min time of UAE; 70.38 mg GAE/g). This was most likely the consequence of exposure to high pressure CO_2 which weakened the cellular wall of the sweet violet raw material. The exposure to supercritical extraction, lower temperature (40 °C), and the lower time (20 min) of UAE resulted in the most efficient extraction of phenols (1.12 mg GAE/mL). The same trend was followed for the flavonoids as well. Antioxidant activity correlated with the concentration of polyphenols. Moreover,the highest antioxidant activity was recorded for the $ScCO_2$ exploited material and UAE conditions of 40 °C and 20 min.

It can be concluded that the application of $ScCO_2$ (apart from obtaining the lipophilic compounds) noticeably improves the UAE of polyphenolic compounds and also increases the antioxidant activity of the *V. odorata* extracts.

P35

Proteomic-based method for pancreatic cancer screening

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Pancreatic cancer is one of the most common lethal tumours of the gastrointestinal tract. Only 6-9% of patients will survive five years after diagnosis, but most of patients is revealed at the last stage due to long-discrete symptoms and will not survive for more than one year. Pancreatic cancer is a chemo- and radioresistant disease and therefore the treatment of patients with developed pancreatic cancer remains a major challenge and the prognosis is generally negative. Unfortunately, there are no specific biomarkers for pancreatic cancer yet.

In this pilot study, we focused to find panel protein biomarkers with increased sensitivity and specificity that would help to detect pancreatic cancer early on. We use plasma samples of three groups of patients: with pancreatic cancer (T3 and T4 disease stage), with fresh developed type II diabetes (risk group for developing of pancreatic cancer) and control group of healthy patients. The differences between the analysed groups were searched using combination of proteomic techniques as MALDI-TOF MS (Matrix-Assisted Laser Desorption/Ionization – Time of Flight Mass Spectrometry) with spectra analysis by Principal Component Analysis, and ESI-Q-TOF MS (Electrospray-Ionisation Quadrupole Time-of-Flight Mass Spectrometry) with statistical analysis by Principal Component Analysis/ Linear Discriminant Analysis. The promising results will be shown.

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P36

Resistance genes in *Listeria monocytogenes*, *Escherichia coli* and *Staphylococcus aureus*

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Resistance to antibiotics is steadily rising and it is considered to be one of the major global issues. Currently, 700 000 people die annually due to drug-resistant diseases and prognosis by 2050, is up to 10 million deaths each year. Research on genes encoding resistance seems to be one of the most promising strategies for solving this problem. In our work, we focused on two pathogenic bacteria, *Staphylococcus aureus* and *Escherichia coli*, and opportunistic pathogen *Listeria monocytogenes*. In 2018, the European population-weighted mean resistance of *E. coli* for aminopenicillins was 57.4%. and methicillin-resistant of *S. aureus* was 16.4%. For *L. monocytogenes* the incidence of resistance in such a wide extent has not been reported yet, but mortality due to listeriosis is up to 30% in the EU. Resistance genes are encoded in both geno-

mic (gDNA) and plasmid DNA (pDNA). However, extracellular DNA (eDNA) located in biofilm also contributes to the horizontal gene transfer. Therefore, we have studied all the above-mentioned types of nucleic acids. The bacteria were firstly characterized by the disc diffusion method, where 81.25% of the strains were antibiotic resistant, mainly to beta-lactams. Furthermore, the presence of *blaZ*, *ermB*, *lde* and *mcr-1* genes encoding resistance to beta-lactams, macrolides, lincosamides, streptogramins B, ciprofloxacin and colistin were tested by PCR. The analysis detected the *blaZ* gene in three strains of *S. aureus* and the *ermB* gene in the control strain verifying the correctness of the process. The *lde* gene was detected in both gDNA and eDNA of all six tested *L. monocytogenes* strains. The mcr-1 gene was confirmed in two *E. coli* strains.

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P37

Microbiological risk associated with the occurrence of *Asaia lannensis* in non-alcoholic beverages

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Bacteria of the *Asaia* genus represent health and technological undesirable bacteria often contaminating non-alcoholic flavoured non-carbonated beverages. They are found in the natural environment of tropical regions of Asia and Africa, mostly on fruits, flowers and insects. Due to globalization, they have spread across the world through raw materials and products of plant origin. Decreased quality of non-alcoholic beverages results in sensory defects such as intensive acid smell, turbidity, sediment or biofilm. *Asaia bogorensis* and *Asaia lannensis* are opportunistic pathogens causing serious nosocomial infection in immunocompromised individuals. Production of biogenic amines in *Asaia* spp. is interesting from the toxicological point of view.

The aim of this work is to identify 11 strains of *Asaia* spp., isolated from industrially produced contaminated non-alcoholic beverages, followed by their growth characteristics, tolerance to external cultivation factors, resistance to selected antibiotics, biofilm formation and biogenic amines production.

All industrial isolates of *Asaia* spp.: a) have been identified at the reliable species level as *Asaia lannensis* using the MALDI-TOF MS method, b) have been identified as *Asaia lannensis* species with at least 99.0 % compliance using the method of sequencing the gene encoding 16S rDNA, c) tolerated growth temperatures in the range of 25-30 °C, d) tolerated pH of Sabouraud broth with 4.0 wt. % glucose in the range of 3.0-6.0, e) were resistant to 11 antibiotics using the disk diffusion method, f) formed surface biofilms, e) produced 8 biogenic amines in the range of 68-890 mg l^{-1} (the total biogenic amine production) using the HPLC method after derivatization with

dansyl chloride.

The results of the work can be used to eliminate *Asaia* spp. in industrial production plants of non-alcoholic beverages to increase health microbiological safety and quality of final products using modern methods providing conclusive and reliable results.

P38

Enzymatic hydrolysis of guar gum galactomannan for mannose production

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Natural galactomannans such as guar gum (*Cyamopsis tetragonoloba* plant) can serve as very good sources of mannose, galactose and various oligosaccharides. In terms of chemical composition, these are heteropolysaccharides whose structural units are galactose and mannose. Guar gum consists of a linear chain of (1→4) -β-D-mannopyranosyl units and side chains of (1→6) -α-D-galactopyranosyl units (Miyazawa and Funazukuri, 2006; Hussain et al., 2015).

Enzymatic hydrolyses of a guar gum solution in excess of 100 mM phosphate buffer, which maintained a constant pH of 7 during the reaction, were carried out. The hydrolysis substrate solution had a total weight of 1500 ± 5 g. Guar gum concentration was 0.5% and D-arabinose as an internal standard was added in concentration of 0.5 mmol·L^{-1}.

Two types of reations were studied. The first reaction was catalysed by selected enzyme endo-(1,4)-β-mannanase (Megazyme Inc., Ireland) (EC 3.2.1.78) dissolved in 3.2M $(NH4)_2SO_4$ obtained by recombinant methods from the Gram-negative soil bacterium *Cellvibrio japonicus*. It is an enzyme from the genetic family GH26. Enzyme activity was determined by the manufacturer on carob galactomannan at pH 7 and 40 °C as 400 U·mg^{-1} and the concentration in the enzyme solution was 5000 U·mL^{-1}. Second reaction was catalysed by endo-(1,4)-β-mannanase and β-galactosidase for synergic effect. β-Galactosidase (Megazyme Inc., Ireland) (EC 3.2.1.23) obtained from *Aspergillus niger* is from genetic family GH35 and manufacturer declares activity of 170 U·mg^{-1} (40 °C, pH 4.5).

The reactions conditions were comparable, for example, to Tapie et al. (2008). The temperature was maintained at 40 °C (optimum for β-mannanase), the reactor was closed, sampling for the first two hours 10 min then for 60 min. Samples were precipitated in 60% ethanol for analysis, centrifuged at 14,000 rpm for 7 min and the supernatants directly analyzed by HPAEC/PAD.

Visually, during the first 15 minutes, there was decrease of viscosity. In addition to galactose and mannose, the hydrolysed samples contained a large number of lower galactomannans and mannans of different chain lengths, their content was not quantified. The composition of these unhydrolysed oligosaccharides and their quantification will be study in further research.

The single β-mannanase reaction gave maximal concentration of mannose **48.3 mg·L^{-1}** after 11 h and galactose achieved

only **3.5 mg·L^{-1}** maximal after 1.5 h. The synergic reaction of β-mannanase and β-galactosidase gave maximal concentration of mannose **43.8 mg·L^{-1}** after 11 h and **51.2 mg·L^{-1}** of galactose after 14 h. Although, enzymatic reactions are usable alternative to common acidic hydrolysis, it most probably will not be used in the mannose production technology development due to a lot of ballast compounds which need to be separated such as buffer, inactive enzymes, oligosaccharides and other.

References

Hussain M., Bakalis S., Gouseti O., Zahoor T., Anjum F. M., Shahid M. (2015): Dynamic and shear stress rheological properties of guar galactomannans and its hydrolyzed derivatives. *International Journal of Biological Macromolecules*, 72, 687-691.

Miyazawa T., Funazukuri T. (2006): Non-catalytic hydrolysis of guar gum under hydrothermal conditions. *Carbohydrate Research*, 341, 870-877.

Tapie N., Malhiac C., Hucher N., Grisel M. (2008): Determination of galactose and mannose residues in natural galactomannans using a fast and efficient high-performance liquid chromatography/UV detection. *Journal of Chromatography A*, 1181, 45-50.

P39

Biological properties of novel photoactive BODIPY-labelled colchicine derivatives

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Cancer is one of the greatest challenges of the modern medicine. Although a big effort has been made in development of novel therapeutics, it still remains one of the most common causes of human death in the developed world. Modern cancer research focuses on deeply targeted therapy seeking for minimization of side effects, which are often caused by commonly used chemotherapeutics. Photodynamic therapy (PDT) belongs to such targeted therapies. It uses three separately non-toxic components – a photosensitizer (PS), light and molecular oxygen. PS is able to generate radicals after light irradiation. Then, these radicals react with the molecular oxygen resulting in accumulation of reactive oxygen species, which cause cellular damage. Cytotoxicity occurs only on the illuminated site of a tissue eliminating undesired side effects. The aim of this work was to investigate biological properties of four newly synthesised colchicine derivatives labelled with fluorescent BODIPY molecules. Colchicine is a natural microtubule-binding agent with anticancer properties. Two derivatives were iodinated, which makes these compounds photoactive and, therefore, well applicable in PDT. In addition, fluorescent properties can be used for diagnostic imaging of tumours. Biological properties were tested using primary lung fibroblasts (MRC-5) and three cancer cell lines derived from breast (MCF-7) and lung (A549) carcinoma, and osteosarcoma (U2-OS). Using fluorescent markers of cellular compartments, we confir-

med localization of the colchicine derivatives in the endoplasmic reticulum. Cyto- and phototoxicity were tested by WST-1 assay. The most toxic compound was colchicine conjugated to iodo-BODIPY on the cycloheptane ring, while the other iodine derivative exhibited strong selectivity for cancer cells. Additionally, effects of derivatives on the cell cycle and mechanism of cell death have been evaluated. Further investigation of the iodine-derivatives is desirable, according to their great toxicity and selectivity for cancer cells, which, together with their photoactive properties, makes them potential cancer therapeutic candidates.

P40

Application of nanopore sequencing methods for the detection of antibiotic resistance genes in food

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Antibiotic resistance is a global public health problem, currently. The emergence and spread of antibiotic-resistant bacteria threaten most therapeutic and preventive measures to control bacterial infections. Most of the used antibiotics are no longer effective as the careless application in human medicine or even agriculture has put bacteria under the considerable evolutionary pressure.

Antibiotic resistance is the ability of a bacterium to withstand antibiotic action beyond the sensitivity common to its taxonomic group. It is caused by genes encoding antibiotic resistance (ARG), some of them, bacteria can successfully transmit among themselves through the horizontal gene transfer. Then ARG detection is one of the key steps to prevent their spread from the environmental bacteriome e.g. in soil, animal or human feces and water via food to natural human bacteriome.

The nanopore sequencing provides an excellently quick and a powerful tool for screening ARG in food to a deep and broad level.

Our goal was to establish efficient and appropriate protocols for nanopore sequencing of ARG in food to detect their individual alleles, abundance and species distribution. DNA was isolated by using ChargeSwitch gDNA Mini Bacteria Kit and nanopore sequencing was accomplished by the MinION system (Oxford Nanopore Technologies) using Ligation Sequencing kit and Epi2me software. The analysis of samples (mungo sprouts, dairy products) immediately and after 24h cultivation in buffered peptone water allowed to detect the present ARG, their distribution in different species and their ability to be multiplied. To confirm and specify these results, the most common bacterial groups were isolated by the appropriate ISO cultivation methods. The bacterial isolates were identified by MALDI-TOF MS and tested for their antibiotic resistance profile by phenotypic and genotypic methods (e.g. disc diffusion method, resp. by PCR). *This work was supported by the grant of Specific university research-grant No. A2_FPBT_2020_040".*

P41

Screening of microalgae mutants obtained by the heavy ion beam irradiation and their application to outdoor mass cultures

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Heavy ion beams have high linear energy transfer (LET) and can produce variants that cannot be obtained with low LET radiation such as gamma and x-rays [1]. In this study, we improved a method to effectively select mutants from microalgae irradiated with the heavy ion beams. *Parachlorella kessleri* was used, C and Ar were used as nuclides, and the dose was set to 50 Gy or 25 Gy with relatively low mortality. A single colony obtained by seeding the irradiated sample on an agar plate was picked up using a 384-well plate and subjected to screening on a 10^4 scale. The strains excellent in starch or oil production by Lugol staining or Nile Red staining were selected by an absorbance or a fluorescent plate reader [2]. The selected strains were picked up in a 96-well plate, the absorbance was measured according to culturing time, and a high growth strain was selected from its rate. However, a high-proliferation strain selected by a high-throughput plate reader does not mean that it can be cultured outdoors. In order to obtain a superior strain with high oil productivity that can be cultured outdoors, scale-up selection is performed in the order from100 mL x 12 rotary culture devices to 8 L x 4 photobioreactors. In the laboratory, the 8L-class cultures can be easily made, and 150L outdoor mass cultures using them as “seed” will be possible for the first time.

References

1. Takeshita, T., *et al.*: Comparison of lipid productivity of Parachlorella kessleri heavy-ion beam irradiation mutant PK4 in laboratory and 150-L mass bioreactor, identification and characterization of its genetic variation. Algal Research 35, 416–426 (2018).

2. Takeshita, T., *et al.*: A simple method for measuring the starch and lipid contents in the cellof microalgae. Cytologia, 80, 475-481, (2015) .

P42

Extremophile microorganisms from radon waters

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Bacteria and archaea are ubiquitous organisms whose occurrence is limited only by extremely inhospitable conditions such as temperature (above 121 ° C), extremely acidic or alkaline pH (below 0.5 and above 12.5), and limited availability of water [1]. Radon springs are an example of an extreme environment, but little is known about their microbiome. Micro-

organisms in radon springs are expected to withstand the influence of several abiotic factors at the same time, i.e. exposure to ionizing radiation and heavy metals, in some cases even high temperatures. Radon itself is a source of alpha radiation, which can cause the formation of reactive oxygen species and damage biomacromolecules [2, 3]. Survival in the radon spring is ensured by bacterial stress adaptation mechanisms. The main adaptation strategies include production of enzymatic and non-enzymatic antioxidants and efficient repair of damaged DNA [4]. Radon springs in Jáchymov, Czechia, are unique with their extremely high radon content [5]. In this work, dozens of bacterial isolates were obtained from radon springs in Jáchymov. Taxonomic identification was performed using MALDI-TOF methods and 16S rRNA sequencing. The conducted research was focused on the study of bacterial strains resistant to ionizing UVC radiation and oxidative stress (presence of free radicals in the environment).

1. Hendry, P., *Extremophiles: There's more to life.* Environmental Chemistry, 2006. **3**(2): p. 75-76.

2. Luig, H., A. Kellerer, and J. Griebel, *Radionuclides, 1. Introduction. In Ullmann's Encyclopedia of Industrial Chemistry*. 2011.

3. Close, D., W. Nelson, and B. W., *DNA Damage by the Direct Effect of Ionizing Radiation: Products Produced by Two Sequential One-Electron Oxidations.* The Journal of Physical Chemistry A, 2013. **117**: p. 12608-12615.

4. Pavlopoulou, A., et al., *Unraveling the mechanisms of extreme radioresistance in prokaryotes: Lessons from nature.* Mutation Research-Reviews in Mutation Research, 2016. **767**: p. 92-107.

5. Krejbichova, Z., *Radioactivity of mineral waters in Bohemia.* Czechoslovak Journal of Physics, 1999. **49**: p. 127-132.

P43

Submerged and solid-state fermentation of micromycetes to obtain enzymes active against fibrillar proteins

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Cost effective and sustainable utilization of agricultural waste containing a high percentage of difficult-to-degrade fibrillar proteins is an important goal of modern biotechnology, which can be achieved through the waste biodegradation by enzymatic hydrolysis. Micromycetes can secrete a wide range of proteases, but their possibility to degrade fibrillar proteins remains poorly understood. That is why nowadays the investigation of filamentous fungi proteases useful in biodegradation is an urgent direction in the science development.

Keratinolytic and fibrinolytic activity of extracellular proteases produced by *Aspergillus giganteus* and *Aspergillus sydowii*, respectively, was examined. Submerged fermentation (SF) and solid-state fermentation (SSF) on vermiculite were applied to micromycetes culturing. Enzymatic activity was measured spectrophotometrically

using suspensions of native proteins (keratin and fibrin). One unit (U) of activity was defined as an increase of A_{280} by 0.01 per 1 ml of culturing medium for keratinolysis and as the amount of released tyrosine (μmol) per minute in 1 ml of the sample for fibrinolysis under the assay conditions (37 °C, pH 8.2, 600 rpm).

Studied cultures showed high enzymatic activity against fibrillar proteins during cultivation under submerged conditions: keratinolytic activity of *A. giganteus* proteases – 0.56 U; fibrinolytic activity of *A. sydowii* proteases – 65.97 U. The proteolytic activity of both producers increased under solid-state conditions: 2.39 U and 78.16 U, respectively.

These results confirm the promising nature of microscopic fungi as producers of enzymes for agricultural waste biodegradation, as well as the possibility of using SFF to obtain the target product, which will make it possible to abandon the less profitable and sustainable SF common in industry. The application of such approaches will allow not only reduce the negative impact on the environment, but also ensure the production amino acids and oligopeptides that are in demand in many sectors of the bioeconomy.

P44

Butanol stress response in bacteria: role of efflux

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Butanol, a solvent and a potential biofuel, can be produced via ABE (acetone, butanol and ethanol) fermentation by solventogenic species of *Clostridium*. One of the biggest drawbacks of the process is the high toxicity of butanol towards cells, which results in low culture viability and decreased solvent production. However, butanol toxicity affects not only producers but also other bacteria that interact with butanol from the environment. Therefore, multiple species have developed different stress response mechanisms to survive in the presence of butanol and knowledge of such mechanisms can be used to engineer clostridial butanol producers. One of such mechanisms, butanol efflux, is still one of the least studied with few butanol efflux pumps reported for *Escherichia coli* or *Pseudomonas* species. However, recent publications report that clostridia might also possess and use butanol efflux systems and that their use can significantly improve butanol tolerance.

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P45

Valorisation of microalgae biomass obtained from poultry wastewater treatment

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According to the European Commission, the EU is one of the world's largest poultry meat producers and a net exporter of poultry products with an annual production of approximately 12.6 million tons. During the production process, huge amounts of wastewater are generated mainly due to the slaughtering of animals and cleaning of the slaughterhouse facilities and meat processing plants [1]. Considering the aforementioned and the global requirement for clean water combined with the fact that microalgae are recognized as a cheaper, more efficient, and sustainable alternative wastewater treatment (WWT), the aim of this study was to investigate the potential of the *Scenedesmus obliquus* microalga for the treatment of poultry wastewater. Furthermore, processing of the obtained biomass was also conducted to determine its potential for further applications and the presence of valuable bioactive compounds in the microalga.

The removal of metals, phosphorus, ammonia, and chemical oxygen performed by *S. obliquus* was investigated. The concentration of iron which was 4040 μg/L in wastewater underwent multifold reduction, achieving 511 μg/L in treated water. Also, the content of lead was reduced approximately twofold, while the content of zinc was decreased from 1140 to 128 μg/L. Furthermore, manganese and nickel were significantly reduced, while the presence of arsenic, cadmium, and cobalt was below 1 μg/L. As a consequence of exposing the microalga biomass to extraction with subcritical water under pressure (30 bar) and temperature of 170 °C, there was an increase in the content of individual metals compared to wastewater and treated wastewater. Hence, the content of iron increased to 7890 μg/L compared to poultry effluent and treated water (4040 and 511 μg/L). The same trend was recorded with chromium, nickel, copper, and lead.

After treatment with subcritical water, the obtained liquid extracts and residues as well as the initial biomass were investigated. Compared to the initial biomass, the contents of iron, copper, cadmium, and zinc decreased, while the content of lead, nickel, and manganese increased. Furthermore, according to limit values for residues from purification of communal waste water, the obtained residues fit into the range of values that can be applied in agriculture and are considered safe.

References

1. Ferreira, A., Reis, A., Vidovic, S., Vladic, J., Gkelis, S., Melkonyan, L., Avetisova G., Congestri R., Acién G., Muñoz R., Collet P., Gouveia L. (2019). Combining microalgae-based wastewater treatment with biofuel and bio-based production in the frame of a biorefinery. In Grand Challenges in Algae Biotechnology (pp. 319-369). Springer, Cham.

P46

Application of high pressure technologies for processing of microalgae biomass from brewery wastewater treatment

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Microalgae have proven to be an excellent alternative to conventional secondary and/or tertiary wastewater treatments (WWT) because of their numerous benefits, such as: minimization of mechanical aeration as the O_2 for bacterial activities could be supplied by the photosynthetic microalgae with a consequent reduction of greenhouse gases emissions, avoidance of toxic solid sludge formation, lower energy consumption and overall costs. Biomass produced during the WWT can be successfully valorized in different areas considering the potential presence of important bioactive components in microalgae [1].

Beer is the third most commonly consumed beverage in the world and the first alcoholic one. During production of beer, it has been estimated that 3-10 L of wastewater is generated per 1 L of produced beer [2]. Therefore, the aim of this study was to investigate the impact of high pressure technologies on microalgae biomass which is generated as a result of treatment of brewery wastewater with the final goal of producing microalgae derived products for human use, respecting all safety concerns.

Scenedesmus obliquus was efficiently used for brewery WWT in terms of removing the nitrogen, ammonia, phosphorus and chemical oxygen demand. The obtained biomass was submitted to subcritical water extraction (SWE) at 120, 170, and 220 °C for 10 min and 30 bar. To determine the impact of SWE on biomass, the starting biomass along with obtained liquid extracts and residue after filtration were investigated in terms of organic and microbiological profile and the content of metals. Furthermore, the presence of bioactives was also investigated.

The starting biomass had a content of proteins around 30% and the presence of chlorophylls and sugars was also determined. Gas chromatography–mass spectrometry screening analysis of liquid extracts indicated that unsaturated hydrocarbons were the dominant group of identified compounds, while alkylated and saturated hydrocarbons, phenols, esters, and ketones were present in lower percentage. The content of metals in liquid extracts is dependent on SWE conditions. In particular, the content of Cr in liquid extracts increased with SWE temperature. At 220 °C, it was about 5 times higher compared to the lowest temperature applied. The same trend was noticed with the content of Mn.

Compared to the starting biomass, the content of metals was increased in residues and the increase of their content with the increase of SWE temperature was recorded as well. The most significant increase was recorded with Fe, from 480 to 1537 mg/kg (220 °C).

References

1. Ferreira, A., Reis, A., Vidovic, S., Vladic, J., Gkelis, S., Melkonyan, L., Avetisova G., Congestri R., Acién G., Muñoz R., Collet P., Gouveia L. (2019). Combining microalgae-based wastewater treatment with biofuel and bio-based production in the frame of a biorefinery. In Grand Challenges in Algae Biotechnology (pp. 319-369). Springer, Cham

2. Simate, G. S., Cluett, J., Iyuke, S. E., Musapatika, E. T., Ndlovu, S., Walubita, L. F., & Alvarez, A. E. (2011). The treatment of brewery wastewater for reuse: State of the art. *Desalination*, *273*(2-3), 235-247.

P47

Diversity and plant growth promoting properties of endophytic bacteria from organic and conventional vineyard

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Endophytes are asymptomatic microorganisms, which colonize plant tissue. These microorganisms can affect plant growing directly by production of phytohormones, antioxidants, ACC deaminase, by phosphate solubilization and nitrogen fixation, or indirectly by production of siderophores and antifungal agents. The most common colonizers of plant tissue are bacterial and fungal microorganisms. Their diversity varies by place of colonization, plant condition or agriculture method.

This work was focused on assessment of bacterial endophyte diversity and their plant growth promoting properties in conventional vineyard in Prague and organic vineyard in Kutna Hora.

Endophytic bacteria were isolated from leaves and canes of grapevine and were identified by MALDI-TOF MS (Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry). Further, isolates were tested for their ability of indol-3-acetic acid production, ACC utilization, nitrogenase and antioxidant activity.

There were 40 various bacterial species isolated from conventional vineyard in Prague and 49 from organic vineyard in Kutna Hora. Different activity of endophytes depending on vineyard was observed only in some tests. There are 88 % of bacteria from conventional vineyard that was able to produce antioxidants above 10 mgAA/l and 74 % were in organic vineyard. IAA production above 5 μg/l was detected in 76 % of isolates from conventional and 92 % from organic vineyard. ACC utilization was detected in 14 % of isolates and 25 %, respectively. Nitrogenase activity of endophytes from both vineyards were at the same level.

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P48

Glutamine depletion may contribute to the therapy of solid tumors

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One of the important hallmark of cancer cells is aerobic glycolysis, known as Warburg effect, which is tightly connected to increased glutamine metabolism, that compensates lower production of ATP from glucose. Glutamine is the most abundant amino acid in plasma with critical roles in supporting macromolecule biosynthesis, maintaining redox homeostasis and regulating signaling pathways, all of which contribute to cancer cell proliferation and survival. Most cancer cells are dependent on glutamine, despite the fact it is a nonessential amino acid that can be synthesized from glucose, and cannot survive in the absence of exogenous glutamine. This phenomenom is called „glutamine addiction" [1].

The aim of this project was to study the effect of asparaginase-mediated glutamine depletion on oxidative stress and apoptosis of cell lines derived from solid tumors and on control fibroblasts. Asparaginase is an approved drug for the long-term therapy of acute lymphoblastic leukemia, which decreases the blood levels of asparagine and glutamine [2].

Our results show, that the acute depletion of glutamine and asparagine is accompanied by increased apoptosis, decreased antioxidant capacity, lower activity of mTORc1 and changes in levels of intracelular amino acids and intermediate metabolites. Also combined depletion of glutamine and asparagine is senzitizing cancer cells not only to hydrogen peroxide, but also to some chemotherapeutics headed by pharmacological doses of ascorbate, that has recently being used for treatment of various malignancies [3]. All the described phenomena were more pronounced in case of cancer cells compared to the control fibroblasts.

In conclusion while asparaginase is clinically used for the long-term chemotherapy of leukemia, this study suggests a potential role of its acute exposure as a chemosensitizing factor in the therapy of solid tumors.

References

1. Altman B.J., Stine Z.E., Dang C.V. From Krebs to clinic: glutamine metabolism to cancer therapy. Nat. Rev. Cancer 2016, 16(10) 619-634.

2. Lanvers-Kaminsky C. Asparaginase pharmacology: challenges still to be faced. Cancer Chemother. Pharmacol. 2017, 79(3) 439-450.

3. Schoenfeld J.D., Sibenaller Z.A., Mapuskar K.A., Wagner B.A., Cramer-Morales K.L., Furqan M., Sandhu S., Carlisle T.L., Smith M.C., Abu Hejleh T., Berg D.J., Zhang J., Keech J., Parekh K.R., Bhatia S., Monga V., Bodeker K.L., Ahmann L., Vollstedt S., Brown H., Shanahan Kauffman E.P., Schall M.E., Hohl R.J., Clamon G.H., Greenlee J.D., Howard M.A., Schultz M.K., Smith B.J., Riley D.P., Domann F.E., Cullen J.J., Buettner

G.R., Buatti J.M., Spitz D.R., Allen B.G. $O_2^{(-)}$ and H_2O_2 - mediated disruption of Fe metabolism causes the differential susceptibility of NSCLC and GBM cancer cells to pharmacological ascorbate. Cancer cell 2017, 31(4) 487-500.

P49

Investigation of microbiomes of carotenogenic microalgae *Bracteacoccus aggregatus* and *Coelastrella rubescens*

Anna Zaytseva[1], Daria Bakhareva[1], Konstantin Chekanov[1], Elena Lobakova[1]

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The carotenogenic microalgae *Bracteacoccus* and *Coelastrella* (Chlorophyceae) are able to accumulate carotenoids in their cells, such as astaxanthin and β-carotene, both valuable for biotechnology. In natural habitats and in a laboratory they form robust communities with bacteria. Analysis of bacterial satellites for the creation of taxonomically stable artificial associations is a matter of the research. Obtained associations could be attractive for biotechnology in terms of their productivity and stress resistance.

Microbiome of the strain *Bracteacoccus aggregatus* BM5/15 (IPPASC-2045) and the natural strain *Coelastrella rubescens* R1 was evaluated by light and electron microscopy, conventional microbiological and metagenomic methods. For growth of bacteria the BG-11 mineral medium, the universal media (UM) for heterotrophic bacteria and selective solid BG-11 medium containing 2% CH_3COONa were used. Bacterial composition of algal cultures and bacterial enrichment cultures was evaluated by 16S rRNA metabarcoding.

Light and scanning electron microscopy revealed bacterial cells attached to the *C. rubescens* surface by apical ends, whereas bacteria in the phycosphere of *B. aggregatus* were attached both apically and horizontally. In the *B. aggregatus* culture, bacterial diversity was higher, than in *C. rubescens* in terms of genera abundance: 25 and 10 genera, respectively. In *B. aggregatus* Proteobacteria and Bacteroidota were most abundant, whereas in *C. rubescens* Proteobacteria and Actinobacteriota dominated. 16S rRNA metabarcoding of washouts from the medium agar surface confirmed higher bacterial taxa abundance in the *B. aggregatus* culture.

Collectively, bacterial component of two microalgal cultures was elucidated. A lower biodiversity was detected in lately isolated culture *C. rubescens*, that could be related to bactericidal activity of microalgal or its satellite metabolites.

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Flocculation of *Chlorella vulgaris* microalgae by polystyrene particles

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The harvesting of unicellular algae involves a lot of equipment that is demanding in terms of initial and operating costs,

and the cost is about 30% of the total operating costs. The flocculation process, which is used to reduce the volume of liquid to be processed, can make algae harvesting significantly cheaper, thus eliminating the need for such expensive biomass separation equipment. Flocculants are already in use today, but they are expensive and difficult to regenerate. Polystyrene microparticles offer the possibility of a cheap and effective flocculant that will facilitate the aggregation of algal cells. The alga *Chlorella vulgaris* CCALA 256 was used for this work. The aim of this work was to determine under which conditions (pH, stirrer rotation frequency) the algae flocculation process is most efficient. Suspensions of microparticles A and B were used, with the particles varying in size. The batches of microparticle suspension were characterized by equal numbers of particles A and B. Flocculation was carried out in the pH range 3-7. The highest efficiency of the process was achieved at pH 4 and 5, up to 99%. Future potential of this work is the use of polystyrene microparticles with magnetic core, for their easy separation from biomass, and surface modifications of the material, which will positively affect the efficiency in dose reduction.

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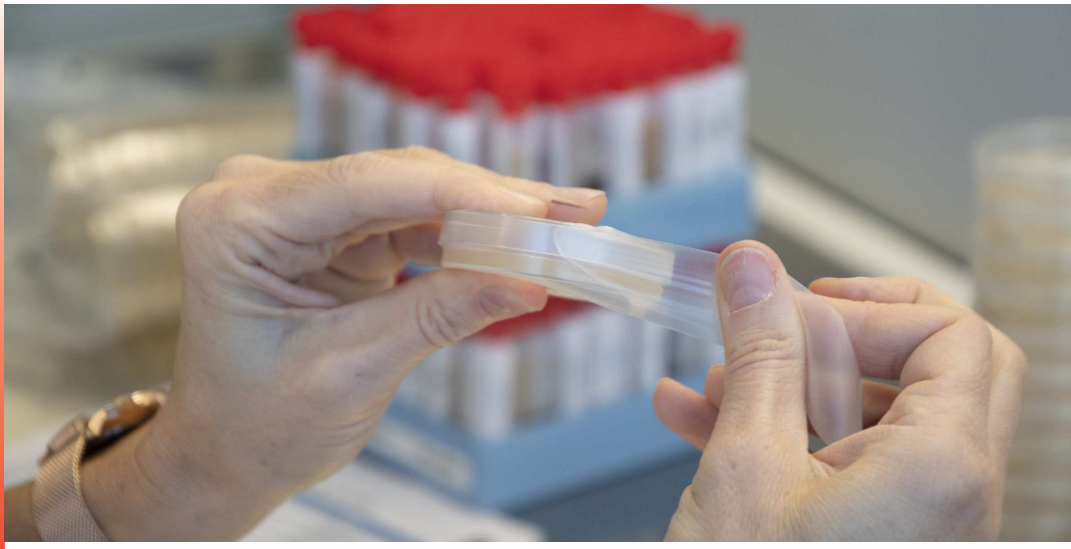

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