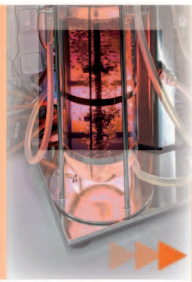
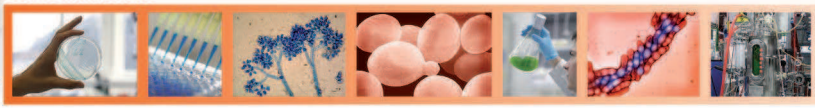


BioTech 2014
and 6th Czech-Swiss Symposium
with Exhibition



Book of Abstracts

Editors:
Vojtěch Spiwok, Zoran Šućur, Olga Schreiberová, Leona Paulová,
Karin Kovar, Jan Káš

ISBN 978-80-7080-887-0

June 11-14, 2014
Prague, Czech Republic

Institute of Chemical Technology Prague
Institute of Biotechnology, LSFM ZHAW, Wädenswil

BioTech 2014
&
6th Czech-Swiss Symposium with Exhibition

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Printing of the Book of Abstracts
was fully sponsored by Novartis

Edited by Vojtěch Spiwok, Zoran Šućur, Olga Schreiberová,
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Published in 2014

Published by the Institute of Chemical Technology Prague,
ICT Prague Press
Technická 5
166 28 Praha 6
Czech Republic

ISBN 978-80-7080-887-0

Printed in the Czech Republic by VENICE Praha s.r.o.

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Institute of Chemical Technology Prague, Czech Republic

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Czech Biotechnology Society

Biotechnet Switzerland

CzechInvest

Swiss Biotech Association

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Welcome Message by Jan Káš and Hans-Peter Meyer

Ladies and gentlemen, colleagues, students and friends,
it is our very great pleasure to warmly welcome you to the BioTech 2014 conference that is taking place in the heart of Europe, in Prague, the Golden City with one hundred spires.

The tradition of this series of symposia was born out of the willingness of Czech and Swiss biotechnologists to share experiences and knowledge, and to stimulate progress in biotechnology in favor of both countries. The first event took place in Prague at the Institute of Chemical Technology Prague (ICT) in 1999 and was a great success. At this meeting, we decided to get together every three years, either in the Czech Republic or in Switzerland, where we would be hosted in Wädenswil by the Institute of Biotechnology of the School of Life Sciences and Facility Management at the Zurich University of Applied Sciences (ZHAW). Since 2005 we have welcomed biotechnologists from other neighboring and distant countries and have created a platform for a larger international event.

This year, for the first time, we have prepared a program with parallel sessions, offering more opportunities for oral presentations and selecting 'easy-to listen to' lectures well-suited to the professional interests of individual participants. The poster program is topic-orientated and all participants have the opportunity for active involvement. We hope that young scientists will use this opportunity to present their research and to compete for one of the several best poster awards. For the first time this year we also have oral presentations for selected posters (on Thursday before the Gala Dinner). Booths and information spots will offer further opportunities for networking and industry promotion. The active exchange between industry practitioners and researchers from academia is a unique, much appreciated feature of this event.

An exciting social program has also has been prepared. The Gala Dinner will take place in the Břevnov monastery, which was founded by the second Prague bishop, Saint Vojtěch, with the Czech Prince Boleslav II. The existence of a brewery was documented in the 13th century, and there will be a demonstration of the beer-making process used in those times. For those who would like to know Prague better, a sightseeing tour has been organized. Saturday is reserved for a visit to the famous Czech brewery, Budvar (original Budweiser), in České Budějovice and then to explore the city of Český Krumlov (UNESCO heritage). We wholeheartedly recommend that you join us for this memorable trip.

Finally, we would like to express our

gratitude to all sponsors, supporters and unnamed scientists who have contributed substantially to this event. The younger generation is now taking over many critical responsibilities and we are happy to see that our tradition, now seeded, is growing and flourishing.

We wish you all a happy and fruitful stay in the Czech Republic.

Jan Káš (*Czech Society for Biotechnology, CZ*) and Hans-Peter Meyer (*Commission for Technology and Innovation, CH*)

Welcome Address by Karel Melzoch, Rector of ICT Prague

Distinguished Guests, Ladies and Gentlemen!

As Rector of the Institute of Chemical Technology Prague, I have great pleasure in welcoming you to the BioTech 2014 symposium. Prague, and particularly to our Institute, which has hosted this bilateral Czech-Swiss scientific event since 1999. In 2005 and 2008 we adopted the BioTech brand and then passed the Czech-Swiss/ Swiss-Czech Symposium to Wädenswil in Switzerland, to our much appreciated partner and co-organiser of this year' event, the Institute of Biotechnology at the School of Life Sciences and Facility Management of the ZHAW. Over the years, we have welcomed many distinguished guests and speakers who have presented significant and interesting lectures. At the same time, BioTech symposia have brought together many people during informal discussions that have helped to grow a unique network of experts in biotechnology within academia and industry.

The Institute of Chemical Technology Prague (ICT Prague), the hosting institution of the BioTech 2014, is a public university providing education and pursuing scientific research and development, and its implementation. It is known for both the depth and breadth of its education and research in almost all branches of chemistry, including chemical engineering, food chemistry and technology, biochemistry, refining, water-treatment, power, biological sciences and technologies, as well as environmental protection, material sciences and other chemistry-based fields of study. Our university, as organised today, was founded in 1952, but its roots date back to 1807 when the first course in chemistry was delivered at the Prague Polytechnic. The ICT Prague consists of four faculties, Chemical Technology, Environmental Technology, Food and Biochemical Technology, and Chemical Engineering. With about 3,800 students, of which approximately 800 are PhD students, ICT Prague is the world's largest, independent university specialising in chemical education. Outstanding chemists graduated by ICT Prague include Professor Otto Wichterle, the inventor of soft contact lenses in the 1950s and Vladimír Prelog, Professor at the ETH

Zürich, who was awarded the Nobel Prize for Chemistry in 1975. Currently, our university has a rich network of international ties, including activities within EU programmes. Research teams are involved in more than 30 FP7 projects, including frontier and collaborative research, and Marie Curie mobility and career development grants. I am keen to promote new projects and collaborations in research and education from ideas that will be seeded within this scientific conference on biotechnology, an essential field in the modern chemistry portfolio, enabling applications in medicine and pharma, cosmetics, food, feed, and chemical synthesis.

Dear visitors and participants of BioTech 2014! I wish you a pleasant stay in the city of Prague and I hope you find here many fruitful and inspiring ideas that would further enhance your scientific work and biotechnology in general!

Karel Melzoch

Rector of the Institute of Chemical Technology Prague

Welcome Address from the Management of the School of Life Sciences and Facility Management in Wädenswil

It is our honour and great pleasure to deliver the welcome address to scientists, industrial partners, students and guests at the BioTech 2014 and the 6th Czech-Swiss Symposium.

Many Czechs reside in Switzerland, and most Swiss people recognise the names of famous sportsmen and women, doctors and musicians who have Czech roots. Switzerland and the Czech Republic are small countries, each unique in their history and culture, yet they share many common values, particularly in education. These attitudes are reflected by the proud history of the Czech Republic. Everywhere we can see examples of the power of the written word and music in the societal change that has shaped the modern Czech nation. This country is no stranger to intercultural collaboration, as can be seen, for example, with Thomas Mann, who was awarded Czech citizenship, enabling his emigration to Switzerland; the late president, Vaclav Havel, a poet and dramatist, was well-known in Switzerland through his friend, the Swiss dramatist Friedrich Dürrenmatt; and Rafael Kubelík, the world-famous Czech conductor (the Czech Philharmonic Orchestra among others), who settled in the Swiss village of Kastanienbaum in Canton Lucerne for the last period of his life.

As scientists, we are familiar with the Czech Republic's many contributions to science and innovation. Nevertheless, world-leading science is not carried out in isolation and we, as representatives of the Management of the School of Life Sciences and Facility Management (LSFM) in Wädenswil, also strive to introduce our students and academic staff to new intercultural experiences. Since the roots of our school go back to 1942, we have strong links with industry and have developed focussed, application-orientated study programmes. In the spirit of the highly successful 2011 Swiss-Czech Symposium, a memorandum of understanding was initiated between ICT Prague and the LSFM to encourage talented students to achieve scientific excellence through application-orientation and intercultural experiences. We have a common vision and are keen to pursue its realisation, particularly with our support of the concept of the Industrial Postgraduate Research Programme (IPRP). In addition to standard PhD work, gifted students who enrol in the future IPRP programme will be choosing to engage in communication, management/business and cross-disciplinary activities to enhance their future career prospects. We are pleased to help them fulfil their aspirations by developing a long-term strategy that con-

tributes to the realisation of this goal. The workshop, planned for Wednesday 11 June 2014, will be an important step forward in the future collaboration between ICT Prague and the LSFM at the ZHAW.

On behalf of the ZHAW management, professors, co-workers and students, we wish you every success at the BioTech 2014 conference.

Urs Hilber (*Dean*) and Daniel Baumann (*Vice-Dean*)

Message from Marie Kousalíková, Mayoress of the Municipal District Prague 6

I always take great delight in seeing our municipal district to facilitate a project, which either contributes to establishing and strengthening international cooperation in science or allows to link research to practice.

I believe that not only science itself but it is this very collaboration on scientific achievements, mutual experience and information exchange that is truly beneficial and moves mankind forward. Therefore I would never hesitate to support the BioTech 2014 & 6th Czech-Swiss Symposium since this event stands for both the information exchange among participants from different countries and the research results application into practice.

The Prague 6 District, as a seat of five universities and many of the top research institutions, undoubtedly belongs to the centers of education, development and innovation not only in Prague but I believe that also within the national and European level. And the BioTech 2014 & 6th Czech-Swiss Symposium contributes to this reputation to a considerable extent.

Biotechnology is one of the oldest fields of human activities and it is also a very dynamic field with enormous potential. I am thoroughly confident that BioTech 2014 & 6th Czech-Swiss Symposium would be a significant contribution to the development of this field and namely to the cooperation among people in this area.

Marie Kousalíková

Mayoress of the Municipal District Prague 6

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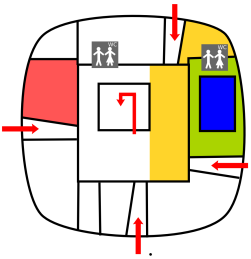
Programme at Glance

Program at Glance

	Balling Hall	Hall 01	Hall 02
	We 11/6/14		
	9:00-18:00 - Registration		
9:00		Pre-Conference Workshop: Biotechnology for controlled remediation of sites contaminated with chlorinated ethenes: Czech-Swiss teamwork joins research	Pre-Conference Workshop: International Postgraduate Research Programme Concept Meeting
13:00	Pre-Conference Workshop: How to get manuscript published?		
15:00	**Conference Opening Welcome messages**		
16:30	Plenary lecture		
17:30	Presentation of Main Sponsors		
18:30	**Welcome Party - Gallery**		
	Th 12/6/14		
8:30	Large and Small Molecules for Pharma	Environmental Biotechnology	Biorefinery
10:45		Coffee Break & Poster Session	
11:15	Large and Small Molecules for Pharma	Environmental Biotechnology	Biorefinery
12:30		Lunch & Poster Session	
13:30	Food, Feed and Nutrition	Environmental Biotechnology	Biorefinery
15:15		Coffee Break & Poster Session	
15:45	Food, Feed and Nutrition	Environmental Biotechnology	Biorefinery
17:00	Short Presentations of Highlighted Posters		
19:30	**Gala Dinner - Břevnov Monastery**		
	Fr 13/6/14		
8:30	Large and Small Molecules for Pharma	Microalgae Biotechnology	
10:45		Coffee Break & Poster Session	
11:15	Large and Small Molecules for Pharma	Microalgae Biotechnology	
12:30		Lunch & Poster Session	
13:30	Biomaterials and Biochemicals	Microalgae Biotechnology	
15:15		Coffee Break & Poster Session	
15:45	Biomaterials and Biochemicals	Microalgae Biotechnology	
16:35	Poster Awards		
17:00	**Closing Ceremony**		
18:00		Guided Prague Sightseeing	
	Sa 14/6/14		
8:30	Excursion to Budweiser Budvar with Český Krumlov Sightseeing		

Venue



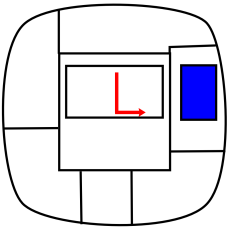
Ground floor

Balling hall

Exhibition

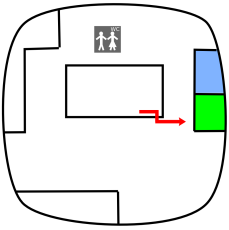
Poster sessions
and Lunches

Welcome party



1st floor

Balling hall



2nd floor

Hall 01

Hall 02

Program

Pre-Conference Events

Wednesday, June 11, 2014
Venue: National Technical Library, Hall 01

09.00 - 12.30 **Pre-Conference Workshop: Biotechnology for controlled remediation of sites contaminated with chlorinated ethenes: Czech - Swiss teamwork joins research**

Chairperson
Mária Brennerová, *Institute of Microbiology, ASCR, CZ*

Remark: Open for all conference participants and students of ICT Prague

09.30 - Informal discussion with refreshment

10.00 - TechTool project Integrative technology for assessment and enhancement of complete removal of chloroethenes from groundwater
Maria Brennerova, *Institute of Microbiology, AS CR, CZ*

10.20 - Remediation companies' perspective on the stimulated reductive dehalogenation of chlorinated ethenes. Case studies
Monika Sťavelová, *AECOM CZ, CZ*

10.40 - Use of structured nanofiber yarns in environmental biotechnologies
Tomas Lederer, *AQUATEST, Technical University Liberec, CZ*

11.00 - 11.20 *Coffee Break*

11.20 - Assessment of bioremediation potential and monitoring of biological reductive dechlorination in sites contaminated with chlorinated ethenes. The overall approach
Christof Holliger, *École Polytechnique Fédérale de Lausanne, CH*

11.40 - Assessment of bioremediation potential and monitoring of biological reductive dechlorination in sites contaminated with chlorinated ethenes. Case studies
Sonia Tarnawski, *École Polytechnique Fédérale de Lausanne, CH*

12.00 - Removal of chlorinated ethenes by anaerobic microorganisms
Jiri Mikeš, *EPS, CZ*

12.20 - Discussion

10.00 - 14.30 **Pre-Conference Workshop: International Postgraduate Research Programme Concept Meeting**

Venue: National Technical Library, Hall 02

Chairperson

To be nominated

Remark: SIG (Special Interest Group)

10.00 - Concept and Unique Benefits of International and Industrial PhD Programme

12.30 - Lunch & Informal Discussion

13.30 - Round Table Discussion and Future Steps

13.00 - 15.00 **Pre-Conference Workshop: How to get manuscript published?**

Venue: National Technical Library, Balling Hall

Chairperson

Murray Moo-Young, *Editor in Chief of Biotechnology Advances, Elsevier*

Remark: Open for all conference participants and students of ICT Prague

13:00 - An introduction to publishing and the journal publishing cycle including vital tips on how to prepare your manuscript and structure your article including facts about plagiarism, the Impact Factor, Open access and Innovation in the research and publishing landscape

Bart Wacek, *publisher from Elsevier*

14:00 - The pleasure and pain of being a JBA editor

Murray Moo-Young, *Editor in Chief of Biotechnology Advances*

Conference

Wednesday, June 11, 2014

Venue: National Technical Library, Balling Hall

15.00 - **Conference Opening**

Chairpersons

Tomas Brányik, *ICT Prague, CZ*
Karin Kovar, *ZHAW, Wädenswil, CH*

15.10 - **Welcome by rectors and officials supporting the Czech-Swiss collaboration**

Urs Hilber, *Director LSFM of the ZHAW, Wädenswil, CH*
Karel Melzoch, *Rector ICT Prague, CZ*
Marie Kousalíková, *Mayoress of the Metropolitan District Prague 6*
Werner Bardill, *Embassy of Switzerland in the Czech Republic*

16.00 - **Opening lectures**

Biotec from velvet revolution until today: A personal account from the Swiss side
Hans-Peter Meyer, Co-founder of the Czech-Swiss Symposia, *Visp, CH*

Biotec from velvet revolution until today: A personal account from the Czech side
Jan Káš, Co-founder of the Czech-Swiss Symposia, *ICT Prague, CZ*

16.30 - **Plenary Lecture**

On the origin of life on earth
Václav Pačes, *Institute of Molecular Genetics, ASCR, CZ*

17.30 - **Presenation of Main Sponsors**

Robert Kužela, *Site manager, Lonza Biotec, CZ*
Christoph Bremus, *Director Sales Bioprocess - Europe Eppendorf, Germany*
Dirk Hebel, *Infors HT, Switzerland*

18.30 - **Welcome Party**

Venue: National Technical Library, Gallery

Thursday, June 12, 2014
Session 1: Large and Small Molecules for Pharma
Venue: National Technical Library, Balling Hall

Chairpersons
Hans Peter Kocher, *icb-partners, Basel, Switzerland*
Peter Šebo, *Institute of Microbiology, ASCR, CZ*

08.30 - Microbial production of antibody fragments for ophthalmic use
Peter Steiner, *Novartis, Basel, CH*

09.00 - Recombinant scaffolds derived from *Streptococcus* ABD domain as potential therapeutics for autoimmune disorders
Petr Malý, *Institute of Biotechnology, ASCR, CZ*

09.25 - DARPins: Design, bacterial expression and bioorthogonal coupling for novel therapeutic concepts
Hannes Merten, *Department of Biochemistry, University of Zürich, CH*

09.50 - Optimization of batch time/cycle time for industrial pharma processes
Zdeněk Cakl, *Lonza Biotec, Kouřim, CZ*

10.15 - Glycotargets: novel vaccines for veterinary use
Christine Neupert, *MALCISBO, Zürich, CH*

10.45 - 11.15 *Coffee Break & Poster Session*

11.15 - Streptavidine-based system for antigen delivery and vaccination
Ondřej Staněk, *Institute of Microbiology, ASCR, CZ*

11.40 - Recombinant protein tools & examples in bioanalytics
Roland Tynes, *FHNW, Muttenz, CH*

12.05 - Recombinant proteins for diagnostics
Mojmír Ševčík, *Biovendor, CZ*

12.30 - 13.30 *Lunch & Poster Session*

Session 2: Food, Feed and Nutrition
Venue: National Technical Library, Balling Hall

Chairpersons
Milan Čertík, *Slovak University of Technology, Bratislava, SK*
Othmar Käppeli, *ABAC R&D AG, Schlieren, CH*

13.30 - Baker's yeast for production of sustainable ingredients in health, nutrition and wellness
Jørgen Hansen, *Evolva SA, Reinach, CH*

14.00 - Microbial Production of the Vitamins B_2 and C
Hans-Peter Hohmann, *DSM Nutritional Products, Kaiseraugst, CH*

14.25 - Biotransformations of prenylated hop flavonoids
Pavel Dostálek, *ICT Prague, CZ*

14.50 - The many faces of microbial laccases
Linda Thöny-Meyer, *Laboratory for Biomaterials, EMPA St. Gallen, CH*

15.15 - 15.45 *Coffee Break & Poster Session*

15.45 - From bacterial genomics to human metabolism
Guy Vergères, *Functional Nutritional Biology Research Group, Agroscope, Bern, CH*

16.10 - Biotechnically enriched cereals with polyunsaturated fatty acids as functional food and feed additives
Milan Čertík, *Department of Biochemical and Food Technology, Slovak University of Technology, Bratislava, SK*

16.35 - Present and Future of Nutrition & Biotech
Othmar Käppeli, *ABAC R&D AG, Schlieren, CH*

17.00 - 19.00 **Poster Session - short presentations of highlighted posters**
Venue: National Technical Library, Balling Hall

Chairpersons
Hans-Peter Meyer, *Commission for Technology and Innovation CTI, Bern, & HES-SO Valais, Sion, CH*
Karel Melzoch, *Rector of Institute of Chemical Technology Prague, CZ*

19.30 **Gala Dinner (with a medieval brewing demonstration)**
Venue: Břevnov Monastery

Thursday, June 12, 2014 - Parallel Sessions
Session 3: Environmental Biotechnology
Venue: National Technical Library, Hall 01

Chairpersons
Olga Valentová, *ICT Prague, CZ*
Ivo Frébort, *Palacký University, Olomouc, CZ*

08.30 - The Bio-nanocapsules. Versatile liposomes armed with virus-derived functional domains
Katsuyuki Tanizawa, *Centre of the Region Haná for Biotechnological and Agricultural Research, Palacký University, Olomouc, CZ*

08.55 - Nanostructured and functionalized surfaces for biocompatibility improvement and bactericidal action
Petr Slepička, *Department of Solid State Engineering, Institute of Chemical Technology Prague, CZ*

09.20 - Multicomponent biosurfactants - A "Green Toolbox" extension
Vladimír Jirků, *Department of Biotechnology, Institute of Chemical Technology Prague, CZ*

09.55 - Magnetic nanoparticles in biotechnologies and biosensing
Kateřina Holá, *Regional Centre of Advanced Technologies and Materials, Palacký University, Olomouc, CZ*

10.20 - New perspectives in antimicrobial peptide production in plants
Tufan M. Öz, *Centre of the Region Haná for Biotechnological and Agricultural Research, Palacký University, Olomouc, CZ*

10.45 - 11.15 *Coffee Break & Poster Session*

11.15 - Biotechnological aspects of cytoskeletal regulation in plants
Jozef Šamaj, *Centre of the Region Haná for Biotechnological and Agricultural Research, Palacký University, Olomouc, CZ*

11.40 - Barley with engineered drought resistance
Ivo Frébort, *Centre of the Region Haná for Biotechnological and Agricultural Research, Palacký University, Olomouc, CZ*

12.05 - Induced resistance as a bio-based strategy for plant protection against pathogens
Lenka Burketová, *Institute of Experimental Botany, Prague, CZ*

12.30 - 13.30 *Lunch & Poster Session*

13.30 - Biological treatment of wastewater from chemical industry with high concentration of nitrates and sulphates
Jana Zábranská, *Department of Water Technology and Environmental Engineering, Institute of Chemical Technology Prague, CZ*

14.00 - Electrochemically-enhanced bioremediation of groundwater in a microbial fuel cell
Petra Hedbavna, *Department of Civil and Structural Engineering, University of Sheffield, UK*

14.25 - Geochemistry and microbiology of the slag leachate remediation with permeable reactive barriers
Karel Waska, *Department of Geology and Environmental Geosciences, Northern Illinois University, DeKalb, IL, USA*

14.50 - Production technology of fermentation accelerator and experience in sustainable use of organic animal waste
Alexander Ivanov, *Federal Center for Toxicological, Radiation, and Biological Safety, Kazan, Russia*

15.15 - 15.45 *Coffee Break & Poster Session*

15.45 - Towards haloalkane dehalogenases suitable for industrial applications
Veronika Štěpánková, *Masaryk University, Brno, CZ*

16.10 - Simultaneous production of bioelectricity and docosahexaenoic acid (DHA) from glycerol by the photomicrobial fuel cell
John Chi-Wei Lan, *Department of Chemical Engineering and Materials Science, Yuan Ze University, Chung-Li, Taiwan*

16.35 - Continuous cultivation of *Azohydromonas australica* for production of copolymer Poly (3-hydroxybutyrate-co-3-hydoxyvalerate)
Ashok K. Srivastava, *Indian Institute of Technology Delhi, New Delhi, India*

Thursday, June 12, 2014 - Parallel Sessions
Session 4: Biorefinery
Venue: National Technical Library, Hall 02

Chairpersons
Petra Patáková, *ICT Prague, CZ*
Urs Baier, *ZHAW, Wädenswil, CH*

08.30 - Biomass refineries for a clean environment
Murray Moo-Young, *University of Waterloo, Canada*

09.20 - Lignocellulosic biomass utilization toward biorefinery: Technologies, products and perspectives
Solange Mussatto, *Centre of Biological Engineering, University of Minho, PT*

09.55 - Perspectives of applied microbiology with purple bacteria driven by systems biology
Hartmut Grammel, *Biberach University of Applied Science, Biberach, Germany, Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg, Germany*

10.20 - Applied biotechnology for the production of biofuels and bioproducts from sugarcane bagasse
Silvio da Silva, *Department of Biotechnology, University of Sao Paulo, Brazil*

10.45 - 11.15 *Coffee Break & Poster Session*

11.15 - Fungi-based biorefinery: Ethanol, biogas, fish feed and superabsorbents from lignocellulosic materials
Mohammad J. Taherzadeh, *Swedish Centre for Resource Recovery, University of Borås, Sweden*

11.40 - From biogas to biorefinery through added value chains
Urs Baier, *ZHAW, Wädenswil, CH*

12.05 - Bioinformatics for biotechnology research: data mining of *Clostridium pasteurianum* genome
Karel Sedlář, *Department of Biomedical Engineering, Brno University of Technology, CZ*

12.30 - 13.30 *Lunch & Poster Session*

13.30 - Renewable chemicals by design
Sean Sutcliffe, Edward Green, Patrick Simms, Timothy Davies, *Green Biologics Ltd., UK*

14.10 - Bioengineering Clostridia. A road map for gene system development
Ying Zhang, *Centre for Biomolecular Sciences, University of Nottingham, UK*

14.35 - Immobilized biocatalysis for biofuel production
Martin Rebroš, *Institute of Biotechnology and Food Science, Slovak University of Technology, Bratislava, SK*

14.55 - In situ product removal from improved isopropanol, butanol and ethanol production by fermentation
Ana M. López-Contreras, *University of Wageningen, Netherland*

15.15 - 15.45 *Coffee Break & Poster Session*

15.45 - The production of aminolevulinic acidand biohydrogen in a biorefinery concept using metabolically engineered *Rhodobacter sphaeroides* O.U.001
Gökhan Kars, Ümmühan Alparslan, *Selçuk University, Konya, Turkey*

16.10 - New microorganisms for wastes upgrading. The case of organic fraction of municipal solid wastes and brewer's spent grain within Bioassort project
Antonella Amore, *University of Naples, Italy*

16.35 - Parallel cultivation of microorganisms using rigid wall single-use bioreactors
Sebastian Kleebank, Anne Niehus, *Eppendorf AG, Germany*

Friday, June 13, 2014
Session 1: Large and Small Molecules for Pharma
Venue: National Technical Library, Balling hall

Chairpersons
Diego Schmidhalter, *Lonza AG, CH*
Kateřina Petříčková, *Institute of Microbiology, ASCR, CZ*

08.30 - Structure and function of a human heteromeric amino acid transporter
Dimitrios Fotiadis, *Institute of Biochemistry and Molecular Medicine, University of Bern, CH*

09.00 - Recombinant production of peptides
Sarah Wegmüller, *HES-SO Valais, Sion, CH*

09.25 - Microbial expression of human enzymes: from biocatalysis to drug design
Andrea Camattari, *Institute of Molecular Biotechnology, TU Graz, A*

09.50 - Sampling enhancement in biomolecular simulations
Vojtěch Spiwok, *Department of Biochemistry and Microbiology, Institute of Chemical Technology Prague, CZ*

10.15 - Using nature's chemical diversity for drug discovery
Kathrin Buntin, *Novartis, Basel, CH*

10.45 - 11.15 *Coffee Break & Poster Session*

11.15 - Gene shuffling for synthesis of novel lincosamid antibiotics
Lucie Najmanová, *Institute of Microbiology, ASCR, CZ*

11.40 - Production of highly active compounds by fermentation
Diego Schmidhalter, *Lonza AG, Visp, CH*

12.05 - Manumycins reloaded: from feeble antibiotics to promising anti-inflammatory agents
Kateřina Petříčková, *Institute of Microbiology, ASCR, CZ*

12.30 - 13.30 *Lunch & Poster Session*

Friday, June 13, 2014
Session 5: Biomaterials and Biochemicals
Venue: National Technical Library, Balling hall

Chairpersons
Linda Thöny-Meyer, *EMPA St. Gallen, CH*
Ivana Márová, *Brno University of Technology, CZ*

13.30 - Polymeric biomaterials based on collagen for bone regeneration
Lucy Vojtova, *CEITEC, Brno, CZ*

14.00 - The scientific approach and methods for mitigation of a large-scale limitations in isolation and purification of active pharmaceutical ingredients
Pavel Havelka, *Lonza Biotec, Kouřim, CZ*

14.25 - Bioactive bacterial exopolysaccharides. modification, characterization and chondrogenic potential for cartilage regenerative medicine
Sylvia Colliec-Jouault, *IFREMER, France*

14.50 - Use of immobilized *Lactobacilli* to pharmaceutical and cosmetics products
Petr Ryšávka, *Helvetia Pharma Prague/Favea, Ltd., Kopřivnice/ Pharmaceutical Biotechnology, CZ*

15.15 - 15.45 *Coffee Break & Poster Session*

15.45 - Utilization of various waste substrates for biotechnological production of biopolymers and other high value products
Stanislav Obruča, *Materials Research Centre, Brno, CZ*

16.10 - Production of poly(4-hydroxybutyrate) (P4HB) in recombinant *Escherichia coli*. Identificationof the factors stimulating P4HB synthesis
Qun Ren, *Laboratory for Biomaterials, EMPA St. Gallen, CH*

16.35 - **Poster Awards**

17.00 - **Closing Ceremony**

18.00 - **Guided Prague Sightseeing**

Friday, June 13, 2014 - Parallel Sessions
Session 6: Microalgae Biotechnology
Venue: National Technical Library, Hall 01

Chairpersons
Kateřina Bišová, *Institute of Microbiology, ASCR, CZ*
Yusuf Chisti, *Massey University, NZ*

08.30 - Products from microalgae
Yusuf Chisti, *Massey University, NZ*

09.20 - Bridging the lipid yield gap
Packo Lamers, *Wageningen University*

09.55 - Bioethanol production from green microalgae; from theory to practice
Giuliano Dragone, *Institute for Biotechnology and Bioengineering, University of Minho, PT*

10.20 - The development of microalgal biotechnology in the Czech Republic
Jiří Masojídek, *Laboratory of Algal Biotechnology, Institute of Microbiology ASCR, Třeboň, CZ*

10.45 - 11.15 *Coffee Break & Poster Session*

11.15 Growing Green Electricity: A new use for *Cyanobacteria*
Barry Bruce, *University of Tennessee, Knoxville, USA*

12.05 Harvesting microalgae with novel agents using non-covalent interactions
Gita Procházková, *Institute of Biotechnology, Institute of Chemical Technology Prague, CZ*

12.30 - 13.30 *Lunch & Poster Session*

13.30 Controlled synthesis of energy reserves in green algae; from cell cycle regulation to production
Vilém Zachleder, *Laboratory of Cell Cycles of Alga, Institute of Microbiology ASCR, Třeboň, CZ*

14.00 Phenotypic spectrum and 3D/TEM analysis of *Parachlorella kessleri* mutants produced by heavy-ion irradiation
Shigeyuki Kawano, *University of Tokyo, Japan*

14.25 The open thin-layer photobioreactor at the Zürich University of Applied Sciences: Experiences after a year of continuous operation
Dominik Refardt, *Institute of Natural Resource Sciences, Wädenswil, ZHAW, CH*

14.50 Bioremediation of contaminated agricultural land by combined effect of agriculture, biogas and microalgae production
Miroslav Kajan, *Czech Biogas Association, CZ*

15.15 - 15.45 *Coffee Break & Poster Session*

15.45 Toward the sustainable cultivation of microalgae to produce renewable biofuels and fine chemicals
Jean-Paul Schwitzguébel, *Laboratory for Environmental Biotechnology, EPFL, Lausanne, CH*

16.10 The importance of basic genetic experiments for improvement of microalgal biotechnology
Kateřina Bišová, *Laboratory of Cell Cycles of Alga, Institute of Microbiology ASCR, Třeboň, CZ*

Saturday, June 14, 2014
Excursion to Budweiser Budvar with Český Krumlov sightseeing

Social Program

Welcome Party in Gallery of National Technical Library
Wednesday, June 11, 2014, beginning at 18:30
Fee – included in registration fee

Gala dinner in Břevnov Monastery with the medieval brewing demonstration
Thursday, June 12, 2014, beginning at 19:30 in Břevnov Monastery
Shuttle bus is leaving from the main entrance of NTL at 19:00
Fee €50

The history of the Břevnov brewery is inseparably connected with the history of the monastery. It has been founded by St. Adalbert and the Count Břetislav II in the year 993 and soon after its origin the brewery came to existence too, as a part of its economic facilities. It was in operation with several pauses to the year 1889 when it was closed. The newly built St. Adalbert Monastic Brewery is picked up on this long tradition again. It is located in a beautiful baroque object of the former stables, its modem equipment having been largely produced by the Czech industry. The capacity of the brewery is 3000 hectolitres in a year.

The shuttle bus will drive you from the main enterance of National Technical Library to Břevnov Monastery at 19:00. On the way back you can go take tram No. 22 or 25 (stop Břevnovský klášter) to metro A Hradčanská (25) or Malostranská (22). The journey takes approximately 15 minutes (4 km).

Guided Prague Sightseeing
Friday, June 13, 2014, beginning at 18:00 in front of National Technical Library
Fee €20

This sightseeing tour will familiarise you with the main sights of Prague. A bus leaving from the main entrance of National Technical library at 18:00 will drive you to the Prague castle. Prague castle started being built in the 9th century and used to be the seat of Czech Kings. At present, there is located the main office of Czech president. Visitors of Prague castle can also admire three courtyards and famous St. Vitus cathedral. The tour will continue through the Lesser town with many beautiful palaces and gardens up to the Charles Bridge, the oldest and most beautiful of Prague's bridges. Afterwards we continue to the Old Town crossing the Charles Bridge. The sightseeing will be finished in the Old-town squarewith world-famous Old-town hall with Astronomical clock approximately at 20:30.

Excursion to Budějovický Budvar with Český Krumlov sightseeing
Saturday, June 14, 2014
The bus is leaving from the main entrance of NTL at 8:30
Fee €50

A bus leaving from the National Technical Library at 8:30 will drive you to the South of Bohemia. The first stop is in České Budějovice where you can enjoy the excursion to Budweiser Budvar Brewery, lunch is included. The trip then continues to Český Krumlov, a town located in the South of Bohemia. In 1992 the historical

core and Krumlov castle were entered into the UNESCO list of World cultural and natural heritage. The central part of Krumlov is located on both embankments of the meandering Vltava river. The guided-tour will walk you through medieval streets with old decorated renaissance houses; from the vantage point you can enjoy a panoramic view of Krumlov castle. Then you cross the river to reach the second largest castle in the country. You will visit castle courtyards, the baroque theatre and if you are lucky you can catch sight of bears, kept in the castle moat. You will arrive back to Prague in the late afternoon.

Poster Awards

ELSEVIER

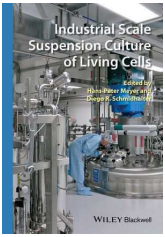
Elsevier supported poster awards by € 1,000

DSM
BRIGHT SCIENCE. BRIGHTER LIVING.

DSM supported poster awards by € 1,000

WILEY

Wiley supported poster awards by three books



Lectures
Symposium Opening

L01

Biotec from the velvet revolution until today - a personal account from the Swiss side

H. P. Meyer

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The purpose of the Czech-Swiss Symposia is the advancement of biotechnology by exchanging knowledge and experience to support collaboration. The potential for biotechnological manufacturing was, and still is, far from being realized, both in the Swiss and Czech industries. Moreover, the "buzzword" biotechnology has led to strange priorities and to community finances being deployed too thinly. This dilution of available resources, and misplaced priorities in times of financial constraints, is a toxic cocktail. New models of highly focussed collaboration/networking are necessary to facilitate progress and to drive the implementation of biotechnologies [1,2]. The question is what have we achieved since the beginnings of these symposia, some 15 years ago, and is the scope of the conference still adequate? Shortly after the non-violent transition of political power (the Velvet Revolution or Sametová Revoluce) in 1989, I had the opportunity to play an active role in a team acquiring and integrating a Czechoslovak industrial site into a global Swiss company [3]. This lecture gives an account of how biotechnology has become a key manufacturing technology and to what extent the political, social and economic environments have changed. How has cooperation and collaboration between Swiss and Czech groups been fostered by these symposia, and what remains as wishful thinking?

References

1. Meyer, H.-P., Eichhorn, E., Hanlon, S., Lütz, et al.: The use of enzymes in organic chemical synthesis and the life sciences: perspectives from the Swiss Industrial Biocatalysis Consortium (SIBC). *Catal. Sci. Technol.*, (2013) **13**, 29-40. 2. Meyer, H.-P.: Sustainability and Biotechnology. *Org. Proc. Res. & Dev.* (2011) **15**, 180-188. 3. Meyer H.-P.: Anlagebau und Technologietransfer biotechnischer Anlagen in Tschechien, *Schweizer Ingenieur und Architekt* **35**, 656-660 (1994).

L02

Origin of Life on Earth: Natural Cause or Intelligent Design?

V. Pačes

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Creationism, or hypothesis of intelligent design, is one view of the origin of life. Another view is based on application of natural laws. Although this also still remains a hypothesis many experiment and the-

oretical reasonings support the possibility that life emerged without any unnatural forces. Recently, discovery of ribozymes led to the conception of "RNA world". Comparative genomics shows that genetic information needed for basic life features is realively low. And numerous biochemical experiments using various natural catalysts and variety of conditions hint to how "molecules of life", polymeric structures included, may have changed pre-biotic to biotic.

L03

Microbial production of antibody fragments for ophthalmic use

P. Steiner

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Antibody fragments are an emerging area of focus within the monoclonal antibody market and represent a new class of therapeutic proteins. Due to their smaller molecular size, antibody fragments have a number of advantages over full-size antibodies and thus render these molecules a well suited format for local therapies. Specifically for local treatment of ophthalmic diseases, highly-stable, well soluble and high affinity single-chain antibody fragments (scFv) are developed at ESBATech and have been successfully tested in clinical trials. Due to the lack of glycosylation, scFv's can be produced in microbial production system. A robust, fast and scalable high-cell density *E. coli* fermentation process has been developed for high yield production of the fragments. Subsequently, native protein is obtained from insoluble Inclusion bodies by an efficient refolding process. These refolding processes and the following chromatographic purification steps are developed and characterized at different scales using high throughput screening and quality by design approaches. To meet the high quality requirements for ophthalmic products, removal of host cell impurities, especially of bacterial endotoxins, is one of the key challenges for microbial production of antibody fragments. Furthermore, the excellent solubility of the small fragments also allows for high protein concentrations and thereby reaching higher effective drug concentrations upon local administration. In summary, single-chain antibody fragments can be produced very efficiently in microbial production systems with the highest quality standard required for treatment of ophthalmic diseases.

L04

Targeting Human Interleukin-23 Receptor Signaling by Novel Antagonists Derived from an Albumin-binding Domain Scaffold

M. Kuchař[1], L. Vaňková[1], H. Petroková[1], J. Černý[2], R. Osička[3], O. Pelák[4], H. Šípová[5], B. Schneider[2], J. Homola[5], P. Šebo[1,3], T. Kalina[4] and P. Malý[1]

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Engineered combinatorial libraries derived from small protein scaffolds represent a powerful tool for generating novel binders with high affinity, required specificity and designed inhibitory function. This work was aimed to generate a collection of recombinant binders of human interleukin-23 receptor (IL-23R), which is a key element of pro-inflammatory IL-23-mediated signaling. A library of variants derived from the three-helix bundle scaffold of the albumin-binding domain (ABD) of streptococcal protein G and ribosome display were used to select for high-affinity binders of recombinant extracellular IL-23R. A collection of IL-23R-binding proteins (called REX binders), corresponding to 18 different sequence variants, was used to identify a group of ligands that inhibited binding of the recombinant p19 subunit of IL-23, or the biologically active human IL-23 cytokine, to the recombinant IL-23R or soluble IL-23R-IgG chimera. The strongest competitors for IL-23R binding in ELISA were confirmed to recognize human IL-23R-IgG in surface plasmon resonance experiments, estimating the binding affinity in the sub- to nanomolar range. We further demonstrated that several REX variants bind to human leukemic cell lines K-562, THP-1 and Jurkat, and this binding correlated with IL-23R cell-surface expression. The REX binders also competed with the p19 protein for binding to THP-1 cells. Moreover, the presence of several REX variants significantly inhibited the IL-23-driven expansion of IL-17-producing primary human $CD4^+$ T-cells. Thus, we conclude that unique IL-23R antagonists were generated that might be useful in designing novel anti-inflammatory biologicals. Novel binders of ILP series, raised against p19/IL-23 cytokine, will be also presented.

Reference:

Kuchař M, Vaňková L, Petroková H, Černý J, Osička R, Pelák O, Šípová H, Schneider B, Homola J, Šebo P, Kalina T, and Malý P. Human IL-23 receptor antagonists derived from an albumin-binding domain scaffold inhibit IL-23-dependent ex vivo expansion of IL-17-producing Tcells. *Proteins: Structure, Function and Bioinforma-*

tics. 2013; 00:000–000 **(in press).** DOI: 10.1002/prot.24472

L05

DARPins: Design, bacterial expression and bioorthogonal coupling for novel therapeutic concepts

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The design of antibody drug conjugates (ADC) which deliver highly cytotoxic payloads to malignant cells is a promising concept for future cancer treatment. Recent developments in the field of bioorthogonal chemistry have simplified the conjugation of different drugs to generate ADCs. However, antibodies suffer from an inherent complexity in protein manufacture, as they are glycosylated and carry disulfide bridges. This results in undefined stoichiometries as well as elaborate and expensive production and conjugation processes [1]. Designed Ankyrin Repeat Proteins (DARPins) are novel binding proteins which overcome these shortcomings [2,3]. These proteins are very stable, easily produced in bacteria in high yields and devoid of cysteines. The scaffold allows the site-specific incorporation of the non-natural amino acid azidohomoalanine in *E. coli* at any desired position, as well as of cysteine [4,5]. Hence, the simultaneous site-specific and stoichiometrically defined bioorthogonal conjugation of a whole series of effector or half-life extension molecules as well as their combination becomes possible [5-7]. Here, click chemistry and thiol-maleimide coupling are applied. This results in novel therapeutic concepts and flexible, tailor-made applications of DARPins in biomedicine.

References

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L06

Optimization of batch time/cycle time for industrial Pharma processes

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Custom Manufacturing Organizations (CMOs) in the pharmaceutical industry are under pressure to effectively operate their plants while avoiding large capital investments. Utilization of multipurpose facilities brings advantageous solutions to accommodate various custom processes and flexibly reflects new business opportunities. However, this concept has its challenges. New adaptations may be beneficial for one process while others may be negatively impacted – typically in the throughput parameter. This conflict requires innovative solutions, specifically in situations when different clusters of active pharmaceutical ingredients (API) and different downstream operations are projected to the same facility. Process cycle time duration is the key to economically successful operations. The first molecule (1) is a very complex inclusion body-based process with a final fermentation volume of 55,000 kg. Downstream processing is comprised of 9 different unit operations and is limited by volume constraints in refolding tank(s) (max 88,000 kg). The amount of final product is 3,000 g with batch time 26.6 days and a cycle time 24.3 days. The second molecule (2) is produced by biotransformation and the product is further purified in 7 downstream operations which are different from molecule (1). The amount of product (2) is 1,600 kg with a batch time of 15 days and cycle time 10.3 days. Optimization requires complex and detailed analysis of utilities, process flow, CIP loops and vessel occupancy for both molecules. The essential aspect to address is the reduction of equipment idle times resulting in non-effective plant utilization directly impacting project profitability while also ensuring product quality which must not be compromised. Major bottlenecks in manufacturing of molecule (1) can be reduced by improved CIP loop organization and implementation of 3 new vessels. Cycle time is subsequently reduced to 15 days. Molecule (2) production can be significantly improved without any investment by utilizing an already existing second fermenter. This, together with the CIP improvements reduces the cycle time of molecule (2) to 5.8 days. Further cycle time reduction is connected with the purchase of new equipment. For this type of plant optimization, a number of software tools can be used. This case study has been performed using SuperPro Designer® and SchedulePro® from Intelligen, Inc.

L07

Glycotargets: Novel vaccines for veterinary use

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Malcisbo develops novel carbohydrate-based vaccines for animal use to prevent parasitic diseases. As a proof of concept a vaccine against the blood-sucking nematode *Haemonchus contortus* has been selected. It is well known that purified proteins of *H. contortus* can be used as

vaccines. However, recombinant proteins are not effective while specific lectins (carbohydrate-binding proteins) are very effective in killing the parasites *in vitro* [1]. Using our "glycotarget discovery platform" we have identified glycan targets for vaccine development. Such glycotargets have been validated in the model nematode *Caenorhabditis elegans* leading to the discovery of nematode-specific glycosyltransferases. Subsequent engineering of insect cells allowed reproduction of nematode-specific glycans on recombinant proteins. For testing their efficacy such antigens were tested in sheep, the natural host of *H. contortus*. Animals immunized with our novel glycoproteins displayed a reduction of parasitic infestation. Besides having a potential vaccine against *H. contortus* infection the results indicate the importance of glycans in effective anti-parasitic vaccine development. These results may open also new avenues to fight human parasitic diseases such as hookworm.

References

1. A lectin-mediated resistance of higher fungi against predators and parasites. Bleuler-Martinez, S. et al. 2011 *Mol Ecol.* (14):3056-70

L08

Streptavidine based system for antigen delivery and vaccination

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For various diagnostic and vaccinal applications, it is important to stimulate antigen-specific immune responses of T lymphocytes. This remains a technical challenge, as it depends on targeted delivery of antigens into cytosol or endosomes of professional antigen presenting cells (APCs), such as dendritic cells, which process antigens and present them to T cells in complex with MHC class I and II molecules, respectively. Here we describe a novel system for antigen delivery into APCs that offers high flexibility of targeting of various endocytic receptors. The system is based on genetic fusion of the antigen of choice with streptavidin that in its tetrameric form binds a biotinylated targeting antibody recognizing an endocytic receptor. Upon endocytic uptake the antigen is processed for presentation on the surface of APC in complex with MHC molecules. We used here the mycobacterial low-molecular weight proteins of the 6-kDa Early Secreted Antigenic Target (ESAT-6) protein family (ESX) antigens for the evaluation of the novel vaccine delivery strategy that enables versatile *in vivo* targeting of antigens into specialized dendritic cell (DC) subsets. When directed through the CD11b or CD11c β 2-integrins or diverse C-type lectins, the ESX-SA:biot-antibody complexes were efficiently captured and presented on major histocompatibility complex molecules of DCs to spe-

cific T-cell receptors. Robust ESX specific T-cell responses were induced by immunization with as little as several picomoles of ESX-SA targeted to DC subsets. Moreover, directing of TB10.4-SA to airway $CD205^{+}$ cells enabled the induction of mucosal T-cell responses and provided significant protection against virulent *M. tuberculosis*.

L09

Recombinant Protein Tools & Examples in Bioanalytics

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Purified, reproducible and high definition recombinant proteins have proved indispensable for bioanalytics, discovery and diagnostics. It has been the responsibility of our protein expression laboratory to produce these highly demanding biomolecules for functional utility and application in these and related fields. Recombinant proteins include enzymes such as those involved in a recently developed enzyme-based vitamin B6 diagnostics assay. Additional examples of recombinant proteins produced include recombinant antibodies, antibody fragments, antigens, virus proteins and membrane receptors. Human antibodies produced include disease-specific autoimmune antibodies of both IgM and IgG isotypes. Recombinant antibody Fab fragments along with corresponding recombinant cytokine ligands represent a further example where structural co-crystallization activities have led to improved antibody engineering and design. Plant pathogen antigens for grapevine virus diagnostics represent an application where the antigens have additionally been successfully applied for antibody discovery through phage display technology. Membrane proteins include exploratory development into the integrin-family receptor class. These applications will be discussed particularly as viewed from the standpoint of modern bioanalytics and diagnostics.

L10

Animal cell derived recombinant proteins for diagnostics

M. Ševčík[1], E. Motyčáková[1]

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An expanding number of protein targets for diagnostics and therapies have stimulated a demand for production of thousands of recombinant proteins that are used as immunizing antigens for raising antibodies, assay standards, or drugs. Along with well established production in bacteria and yeast, animal cell expression is often used for this purpose today. At BioVendor, a Brno based company specializing in the production of *in-vitro* immunodiagnostics, Human Embryonic Kidney 293 cells are exploited for production of near-native

recombinant proteins. The process of cell culture preparation, transfection, transient expression and the downstream processing will be presented. Considering the unique nature of proteins, each molecule represents a new challenge in terms of a careful fine tuning of preexisting protocols. Protein oligomerization, aggregation, glycosylation pattern, stability and process yield all are the common issues that will be further discussed in this presentation.

L11

Baker's yeast for production of sustainable ingredients in health, nutrition and wellness

J. Hansen

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Most valuable small molecules are made from fossil oils, from extraction of plant or other natural materials or obtained through specialized agriculture. The use of fossils for manufacturing has its obvious drawbacks, but less appreciated is the fact that extraction from natural sources may lead to exhaustion of these, and though some agricultural production systems are perfectly sustainable, not all are. Thus, growing the plant or raising the animal may take more land, more water or more energy than it really should. Finally, any extraction process may require solvents or other processes which generate significant waste.

Making the compounds by fermentation instead can improve the product's sustainability greatly, avoiding deleterious use of fossils, natural source extraction or freeing land or other resources for other uses. Evolva's Genetic Chemistry technologies [1,2] allow for this. State-of-the art methodologies for establishment of heterologous biosynthesis pathways in Baker's yeast allow for fast development of fermentation-based sustainable manufacturing routes. Added benefits are higher product quality (only one product formed at a time), improved supply chain stability (no seasonal variation) and the possibility for customization (blends of single components). Production of vanillin, resveratrol and Stevia sweeteners will be discussed.

References

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L12

Microbial Production of Riboflavin (Vitamin B2) and L-Ascorbic Acid (Vitamin C)

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Thirteen natural chemical compounds with vital catalytic, regulatory, and protective functions for the cellular metabolism are recognized as vitamins. They are produced by microorganisms, plants, and animals. Having lost the capabilities to synthesize these compounds humans rely on dietary vitamin intake. Farm animals like poultry, swine, and to a lesser extent cattle are depending on dietary vitamin supply as well. Medical conditions or nutritional habits of humans and the need for an increasing efficiency in animal husbandry generated a market for synthetically produced vitamins with a value above 2 billion Euros *per annum*. Until the 1980s industrial vitamin production was within the realms of organic synthesis. Only the production routes of vitamin B12 and vitamin C involved fermentation and biocatalytic steps with natural isolates as production strains, which were improved by random mutagenesis and selection procedures. With the advance of genetic engineering techniques the targeted and purposeful alteration of the genetic makeup of chosen production strains became possible. Consequently, industrial riboflavin production switched around the year 2000 almost completely to microbial processes based on genetically engineered production strains. Very effective D-pantothenic acid (vitamin B5) fermentation processes based on recombinant strains were developed as well. The lecture focus on the development of microbial strains and processes for riboflavin and L-ascorbic acid production.

L13

Biotransformations of prenylated hop flavonoids

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Many studies have confirmed that some hop prenylflavonoids effectively inhibit the proliferation of tumor cells and prevent cancer growth and metastasis. Another important effect of some hop prenylflavonoids is their estrogenic activities that mimic human steroid hormones - estrogens, and thus suppress critical symptoms or reduce the risks of hormone-associated cancers. Prenylflavonoid 8-prenylnaringenin has been identified as the most potent phytoestrogen known to date. The level of xanthohumol, the major prenylflavonoid of hop cones in beer is usually about 0.1 mg/l, while in fresh hops, this value is an order of magnitude higher. This is due to the isomerization of xanthohumol into isoxanthohumol at high temperatures during wort boiling. Therefore the predominant prenylflavonoid in beer is isoxanthohumol, whose

concentration can be up to 2 mg/l. Levels of other prenylflavonoids in beer, such as 8-prenylnaringenin, is unfortunately almost negligible. Therefore moderate beer consumption has very few prenyflavonoid-related positive effects on health. However, an interesting fact is that prenylflavonoids ingested via beer consumption interact with the human gut microflora, particular the anaerobic bacterium *Eubacterium limosum*, which is capable of O-demethylation of isoxanthohumol into 8-prenylnaringenin, with up to 80% efficiency. The amount of 8-prenylnaringenin in the blood thus is increased up to 10 times compared with the amount obtained directly via beer consumption. Unfortunately, this biotransformation occurs in only one-third of the human population due to inter-individual differences in intestinal microbiota. Moreover, the rate of conversion is variable between individuals, depending on factors such as age, genetic makeup, and the medical condition of the individual. This biotransformation reaction is interesting due to the estrogenic properties of 8-prenynaringenin and the administration of probiotic preparations may be one way to increase uptake of 8-prenylnaringenin into the human body.

L14

The many faces of microbial laccases

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Laccases (E.C.1.10.3.2) are members of the multi copper oxidase enzyme family. For the first time isolated more than 120 years ago from the sap of the lacquer tree *Rhus vernicifera* they have nowadays become industrially interesting biocatalysts with a many different applications. They catalyse the oxidation of various types of substrates, including (poly)phenols such as lignin and its derivatives, thereby forming the corresponding radicals that can undergo further reactions. The catalytic centre comprises a (blue) type I copper that serves as an electron acceptor at the substrate binding site and a tri-nuclear copper centre buried in the enzyme. In our laboratory we use laccases for different purposes: (i) Bacterial laccase from *Bacillus pumilus* serves as a biocatalyst in so-called laccase mediator systems, where a substrate is oxidized by the enzyme. This coupled reaction can be used in efficient oxidative bio-transformations. We have explored a

number of commercially available as well as in-house prepared microbial laccases in combination with 91 potential laccase substrates to find promising laccase mediator couples. (ii) Directed evolution of *B. pumilus* laccase for improved oxidation of guaiacol was performed by two different shuffling approaches involving active enzyme variants carrying mutations close to the type I copper in the active site, resulting in two candidates with improved activity. (iii) In a green chemistry approach *Trametes versicolor* laccase was used for iodination of spruce wood in order to apply an antimicrobial surface protection. We showed by HPLC-MS analysis the conversion of the lignin derivative vanillin to iodovanilline, which inhibits growth of wood degrading fungi. (iv) *T. versicolor* laccase was used as an atom radical polymerization catalyst for the synthesis of well-defined poly(met)acrylates. The polymerization can be carried our both in homogeneous and heterogeneous phase. Our work exemplifies the broad applicability of these promising biocatalysts.

L15

From bacterial genomics to human metabolism

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The Human Genome Project has revolutionized life sciences by crystalizing the fast development of holistic tools of molecular analysis. The integration of 'omics' data derived from these strategies into biological models will ultimately contribute to a systemic understanding of human physiology. With some delay, compared to the pharmaceutical sector, food and nutritional sciences have now embraced these research strategies (1). The processing of food along the chain 'fermentation by technological microorganisms' → 'digestion by the human microbiota and the digestive tract' → 'intestinal transport by enterocytes' → 'metabolic activation by the human organism' offers a unique sequence of biochemical modifications of the food matrix that are mediated by both prokaryotic and eukaryotic cells. These modifications can be efficiently investigated using a combination of bacterial genomics, foodomics and nutrigenomics. This strategy promises to accelerate the translation of the molecular knowledge available on fermented foods into information that is relevant to human health (2). Milk is a natural vector to deliver bacteria and products of fermentation with specific health benefits to humans. We have therefore undertaken a research program, which will ultimately link the genome of lactic acid bacteria, to the metabolome of the corresponding fermented dairy products and, ultimately, to the phenotype (blood cell transcriptome, serum metabolome) of the organisms having ingested these products (3, 4). Selected *in vitro* and *in vivo* examples of these applications along the food processing chain are presented in this lecture.

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L16

Biotechnically enriched cereals with polyunsaturated fatty acids as functional food and feed additives

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Polyunsaturated fatty acids (PUFAs) are indispensable for human well-being due to their healthy, dietary and functional properties. Cereals as the major food supply are deficient in PUFAs. However, cereals could be considered as challenging sources of these compounds if they are naturally modified with the aim to enrich them with PUFAs. To reach the goal, biotechnological technique based on fungal prefermentation of cereals by solid state fermentation (SSF) has been developed. During the process, Zygomycetous fungi (*Thamnidium* sp., *Cunninghamella* sp., *Mucor* sp., *Mortierella* sp.) utilized cereals and converted them to new cereal-derived bioproducts with a high content of various PUFAs. Depending on the strain, type of cereal substrates and cultivation conditions, a range of cereal-based bioproducts enriched with PUFAs (up to 2.4 % gamma-linolenic acid, 4.2 % arachidonic acid, 2.1 % dihomo-gamma/linolenic acid, 2.3 % eicosapentaenoic acid) have been prepared [1]. In general, these bioproducts should be considered safe for the production of food or feed ingredients/supplements. Therefore, SSF-based cereals have been successfully applied for making cereal goods (rolls, bread, pasta) as well as a feed additive for animal diet. Elevated amounts of PUFA-cereals in wheat flour has changed rheological properties of the dough (increased water absorption, reduction of dough-rise, prolonged dough development time, higher softening degree of dough) and sensorial properties of the final cereal goods (brownish color, crackness, accepted fungal flavour) as well. Thus, biotechnologically prepared PUFA-enriched cereals may open novel prospects for the market of functional PUFA-riched cereals.

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The work was supported by grant VEGA 1/0975/12 and by grants APVV-0662-11 and APVV-0294-11.

L17

Present and Future of Biotech & Nutrition

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The biotech era of nutrition is upon us: The human genome and several plant genomes have been characterized yielding information on how constituents of the diet interact with genes. Metabolic adaptation of metabolism in response to variations in the nutrient supply has first been well characterized in uni-cellular organisms e.g. nutrient dependent regulation of the lactose, histidine and tryptophan operons by their respective substrates. The gene expression in response to changes in the nutritional status is one of the well-established events In multi-cellular organism, the control of gene expression by nutrition differs in many aspects from that operating in single cell organism, and involves complex interactions of hormonal, neural and nutritional factors. The study of how constituents of the diet interact with genes, and their products, to alter phenotype and, conversely, how genes and their products metabolize these constituents into nutrients, anti-nutrients, and bioactive compounds is referred to as nutrigenomics. It is used to address issues important to nutrition and health and may further revolutionize wellness and disease management. Historically different phases in the elucidation of the nutrient – health relationship occur. They reach from the empirical knowledge (detection / prevention of deficiencies) to the present introduction of nutrients for health. Besides health main trends for the future of nutrition are convenience and sensory features under conditions of safety and sustainability. The breakdown of results from basic biotech research into commercial products has become a Herculean task. Challenges range from the discovery of ingredients, mechanisms and benefits over processing to evidencing nutritional claims and price. Development of products improving the host's resistance against viral and bacterial infections will be presented together with approaches to enhance bioavailability and convenience.

L18

The Bio-nanocapsule: Versatile liposome armed with virus-derived functional domain

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We previously developed 'Bio-nanocapsule (BNC)' that is useful as a novel nano-carrier for the tissue/cell-specific gene/drug delivery systems (GDS/DDS). The initially-developed BNC was a hollow liposome (LP) embedded with hepatitis B virus (HBV) surface antigen (HBsAg) L proteins, which possess HBV-derived machineries capable of human hepatocyte-specific recognition, membrane fusion, and evasion from the reticuloendothelial system [1, 2]. The cellular uptake of BNC was observed only with human hepatic cells, the rate of which was similar to that of HBV [3]. Subsequently, we have constructed the BNC-LP complex using LPs pre-loaded with various materials. The BNC-LP complex has been shown to deliver genes and drugs more efficiently than the previous BNC *in vitro* and *in vivo* in a human hepatocyte-specific manner [4]. It has been demonstrated that conventional nano-carriers used in GDS/DDS, such as LPs, synthetic polymers, and nano-micelles, enter into the cells *via* the endocytosis cascades and substantial portions of incorporated nano-carriers are sorted to lysosome where undesired degradation occurs. In contrast, most viruses, known as naturally occurring nano-carriers, are able to establish their infections by avoiding lysosomal degradation. Recently, it was revealed that the BNC moiety of the BNC-LP complex mediates the cellular uptake of LPs on the cell surface and subsequent cytoplasmic release of the payloads from LPs. These findings have thus led us to engineer nano-carriers by mimicking viruses, especially the surface structure of viruses. Based on the detailed investigation of functional domains of not only HBV but also many other viruses, the domains needed as nano-carriers were displayed outwardly on the surface of the LP-based nanocapsules, in consideration of their spatial arrangement. These BNCs are expected to exhibit various features such as cell specificity, cell entry activity, stealth function, and regulatory functions for intracellular dynamics, as effectively as those of viruses.

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L19

Nanostructured and functionalized surfaces for biocompatibility improvement and bactericidal action

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Different types of polymer carriers were modified (plasma and laser treatment). Commonly used polymers (e.g. HDPE, PS, PTFE, PEN), biopolymers (e.g. PLLA, PMP) or fibrous biopolymers (cellulose) were treated in the present study. Pristine and modified surfaces were consequently used for procedures such as nanoparticle grafting. Surface physico-chemical properties (surface chemistry, morphology) of treated surfaces were determined. The enhancement in cell adhesion and proliferation on modified substrates was studied in vitro. Bactericidal action of noble metal nanoparticles (Au, Ag) and its size dependence was characterized.

L20

Multicomponent biosurfactants – A ”Green Toolbox” extension

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The unflagging interest in surfactants of biological origin, representing ecological alternatives to their synthetic counterparts, has been marked with increasing R&D efforts to increase yields, enlarge sourcess and identify new congeners, among others. In this context, rhamnolipids (RLs), offering (in a view of their natural function) a relatively large scale of surface activities as well as stability towards the extremes of environment, logically attract attention. In the background of fixed topics (variety of RL congeners, diversity of microbial producers, factors affecting production, roles

attributed to RLs for the producing cell...) this overview will cover the topic of multicomponent RLs mixtures in general and "tailor made" mixtures in particular, their biological effects and potential application as additives of bioremediation.

L21

Magnetic nanoparticles in biotechnologies and biosensing

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Nanotechnologies based on the application of magnetic nanoparticles (MNPs) offer a great potential in biomedicine, biotechnologies and sensing applications. In this review, the synthetic approaches enabling to control the size, morphology and surface functionalization of magnetic nanoparticles are described along with properties of biogenic magnetite nanoparticles separated from magnetotactic bacteria. Various biotechnological and biomedical applications of MNPs will be discussed including cell separation processes, enzyme and protein immobilization procedures, magnetic sensing, and MRI contrast enhancement. Hybrid systems combining magnetic nanoparticles with fluorescent carbon dots or silver nanoparticles will be emphasized for their use in advanced bio-analytical and medical technologies. [1, 2]

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L22

Antimicrobial Peptide Production in Plants for Medical and Agricultural Biotechnology

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Antimicrobial peptides (AMPs) are vital components of the innate immune system of nearly all living organisms. They generally act as the first line of defence against various pathogenic bacteria, parasites, enveloped viruses and fungi. These low molecular mass peptides are considered prospective therapeutic agents due to their

broad-spectrum rapid activity, low cytotoxicity to mammalian cells and unique mode of action which hinders emergence of pathogen resistance. In addition to medical use, AMPs can also be employed for development of innovative approaches for plant protection in agriculture. Conferred disease resistance by AMPs might help us surmount losses in yield, quality and safety of agricultural products due to plant pathogens.

Heterologous expression in plant-based systems, also called plant molecular farming, offers cost-effective large-scale production which is regarded as one of the barriers to medical or agricultural use of AMPs. Although AMP production in plants, specifically cereals, holds great promises for medicine and agriculture, certain technical limitations regarding product yield and downstream processing still remain. Therefore, this study was aimed at expression of AMPs in barley endosperm and evaluation of related parameters to boost expression level and yield, improve stability and facilitate product purification. Additionally, fast and promising tools for evaluation of plant-based expression strategies and functional assessment of the heterologously produced AMPs were investigated.

The rapidly increasing need for recombinant peptides in medicine and agriculture requires improvement of plant expression systems and related biotechnological tools. Overall, barley grains are considered reliable platforms for large-scale production of AMPs which are attractive candidates for molecular farming and plant protection.

L23

Biotechnological Aspects of Cytoskeletal Regulation in Plants

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The plant cytoskeleton comprises of dynamic actin and tubulin polymers. Through their dynamic properties and organizational patterns, both cytoskeletal elements fulfil a pleiade of functions pertinent to plant cell growth and morphogenesis, proliferation and cellular interactions with abiotic and biotic factors. Interphase cortical microtubules control deposition of cellulose microfibrils and consequently determine cell shape by dictating cell growth directionality [1]. During cell division, mitotic microtubular arrays are necessary for cell division plane determination, faithful chromosome segregation, and finally the partitioning of the two daughter cells [2]. Actin microfilaments undertake intracellular transport tasks and organelle positioning functions during intercalary and tip growth of plant cells [3]. Finally, both cytoskeletal elements are responsive to extracellular stimulation [4] and as such they are remodelled after challenges by abiotic or biotic stresses. Due to the importance of cytoskeletal functions at the cellular level and their impact at the whole plant level, both actin filaments and microtubu-

les have big potential for biotechnological applications. This is substantiated by following facts:

1. Both cytoskeletal arrays are important for cell wall deposition (including cellulose and lignin) and biomass production for biotechnology applications (e.g. for biofuel).

2. Microtubules are associated with cold hardiness of commercially important cereal cultivars. This property is associated with tubulins themselves, thus engineering of tubulins may improve crop yields at suboptimal temperatures.

3. Microtubules are associated with herbicide resistance, again through tubulin itself. Thus engineering of tubulins may generate crop lines that can selectively grow under a limiting herbicide load.

By focusing on central mechanisms of cytoskeletal regulation and on new high resolution imaging technologies to address such mechanisms in unprecedented details, we wish to highlight the plant cytoskeleton as a platform of biotechnological applications relevant to agriculture, food and textile industry and drug development.

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L24

Barley with engineered drought resistance

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Barley is an agriculturally important crop and the production of plants with enhanced stress tolerance is one of the important goals in barley breeding. New techniques of molecular cloning and plant transformation accelerate classical breeding techniques and help to produce barley plants with required enhanced traits [1]. Morphology and development of the barley plants can be altered by genetic manipulation with genes coding for cytokinin dehydrogenase (EC 1.5.99.12; CKX), a principal enzyme controlling cytokinin levels in plants [2]. Three unique transgenic barley lines (*Hordeum vulgare* cv. Golden Promise) transformed with an expression cassette consisting of β-glucosidase root specific promoter, a variant of CKX1 gene from *Arabidopsis thaliana* with engineered protein targeting to either cytoplasm, vacuoles or apoplast, and NOS terminator were prepared and T2 generations of homozygous plants were analyzed. Selected transgenic lines, distinctive with

altered morphology of the root system, showed higher resistance to drought conditions than wild type plants, both when grown in soil or in hydroponic culture. This method of conveying drought resistance may be further exploited in order to create a usable trait that can be transferred to commercial cultivars of barley.

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L25

Induced resistance as a bio-based strategy for plant protection against pathogens

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Increasing demand for environmentally friendly substitutes of traditional pesticides motivates scientists to turn back to natural rudiments of plant resistance to pathogens. Plants are basically resistant to the most of pathogens since they dispose of both preformed and inducible defence mechanisms developed during their coevolution with natural enemies. Recognition of the invading pathogen by plant is followed by activation of highly effective defence mechanisms, which inhibit pathogen spread from the infected cell and activate signal transduction within yet uninfected parts of the plant. Besides pathogens themselves, the same mechanisms can be triggered by pathogen-derived compounds, such as bacterial and fungal pathogen-associated molecular patterns (PAMPs), pathogen-secreted molecules, etc., signalling molecules involved in plant resistance to pathogens as salicylic acid and jasmonic acid or even by the wide range of unrelated compounds.

Mycelium of a serious fungal pathogen *Leptosphaeria maculans* was used as a source of fungal cell carbohydrate [1] and polypeptides recognized by *Brassica napus* plants as microbe-associated molecular patterns. Correlation NMR experiments defined trisaccharide bound to O-3 of serine residue α-D-Glc*p*-(1→2)-β-D-Gal*f*-(1→6)-α-D-Man*p*-(1→3)-L-Ser. The obtained results strongly support the conclusion that these carbohydrates induce defence response in *B. napus* plants.

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L26

Biological treatment of wastewater from chemical industry with high concentration of nitrates and sulfates

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Industrial wastewaters coming from production of nitro-organic compounds contain high concentration of nitrates and sulfates from 1.5 to 2 g/l and 3 to 6 g/l, respectively, pH 1, temperature 15 °C and no organic matter. Chemical treatment of this type of pollution is difficult, therefore biotechnological approach was chosen. Biological reduction of nitrates by denitrifying bacteria and sulfates by sulfate-reducing bacteria needs some reducing equivalents, mainly readily degradable organic compounds. Results of preliminary batch experiments with activated sludge indicated strict succession of biological denitrification and sulfate reduction and possibility to restrict the process only to denitrification by limitation of substrate. Batch experiments were carried out with different kind of organic substrate (methanol, ethanol, acetic acid and distillery slops)[1]. Based on measured reaction rates ethanol was chosen as the best substrate and technological parameters were set down for a continuous process. The laboratory scale process line was designed consisting of neutralization step, denitrification reactor inoculated with activated sludge [2], degasification reactor, sedimentation tank, pumps for wastewater and substrate, sludge recirculation and controlling sensors for temperature, pH and ORP. The laboratory reactor was operated 120 days with stepwise increasing loading rate to 90 $g.l^{-1}.h^{-1}$ ($N\text{-}NO^{3-}$). Efficiency of nitrogen removal reached 98 % with a high specific denitrification rate 15.6 $mg.g^{-1}.h^{-1}$ ($N\text{-}NO^{3-}$, VSS). Nitrate reduction to nitrogen gas results in evolving alkalinity, and therefore input wastewater could be neutralized only to pH 2.5 with operational pH in the reactor 6 - 8. The results of the long-term operation of the laboratory model have led to a design of optimal operational conditions and technological parameters of biological denitrification of examined wastewater. Based on this data the full-scale treatment plant was constructed and has already been in successful operation for two years.

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L27

Electrochemically-enhanced bioremediation of groundwater in a microbial fuel cell

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The *in situ* biodegradation of organic contaminants in groundwater is often limited by a lack of electron acceptors for microbial metabolism. Some bacteria have the ability to exchange electrons with solid materials and this unique feature can be used to enhance bioremediation of organic pollution. Electrodes of microbial fuel cells (MFCs) installed in the ground may serve as an inexhaustible electron acceptor. The possibility of electricity production linked to the biodegradation of organic substrates makes this novel technology highly sustainable. An electrically conductive permeable reactive barrier is one possible design solution for *in situ* application; however, this technology is still under-developed. The current study focuses on treatment of groundwater contaminated by phenolic compounds (phenol, cresols, xylenols) under laboratory conditions. Initial laboratory tests were performed in a double-chamber MFC. Biodegradation rate of phenols in these MFCs was compared with the anaerobic experiments while electricity production was measured. Preliminary results suggest that electricity up to 2.0 mW/m^2 of electrode surface area can be produced in a double-chamber MFC using groundwater contaminated by phenolic compounds. Biodegradation of phenols is enhanced above that occurring in the absence of the treatment. Further analysis will include microbial community characterization using molecular biology methods identifying microbial species important for degradation of phenols and electricity production. The study is expected to provide design specifications for field-scale applications which will be illustrated.

L28

Geochemistry and Microbiology of the Slag Leachate Remediation with Permeable Reactive Barriers

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Many industries produce wastes that are highly alkaline by nature, or wastes with highly alkaline weathering products. Depo-

sition of such materials represents a serious threat to the environment, and remediation measures often require high maintenance and cost. In this study, a column experiment was used to examine three different permeable reactive barriers (PRB; dolomite, quartz, and Apatite-II™) for the remediation of highly alkaline pH in slag leachate. The effects on the pH neutralization and the subsequent response of heavy metal concentrations and microbial communities were compared between different positions of the PRB inside the column and between two different incubation temperatures. Regular monitoring of physicochemical and geochemical conditions in the columns revealed no significant influence of the quartz PRB, an intermediate influence of the dolomite PRB, and a strong influence of the Apatite-II ™PRB. In the dolomite remediation, a moderate decrease of pH was observed, indicating the dissolution of the PRB and precipitation of calcite, a process previously described as dedolomitization. No significant mobilization of heavy metals was observed during the same period in the dolomite PRB effluent, suggesting coprecipitation with the carbonate minerals. In the Apatite-II ™remediation, the pH neutralization was accompanied by substantial changes in the geochemistry by microbially-mediated production of organic acids. This in turn led to significant dissolution of the remediated sediment and consequent increase in total dissolved solids. The comparison of microbial communities among the different columns showed the greatest change in the Apatite-II ™incubations, corroborating the level of environmental changes caused by the remediation. This study provided a novel insight into the neutralization of hyperalkaline slag leachates with a great potential for the utilization of fermenting alkalitolerant microorganisms to enhance the remediation effect.

L29

Production Technology of Fermentation Accelerator and Experience in Sustainable Use of Organic Animal Waste

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Currently, anthropogenic environmental pollution has become a major problem and has reached global scale, increasing every year. The serious hazard constitute livestock waste, household, agricultural wastewater and sediments demanded as organic fertilizers but having limitations in

the application due to the presence of toxic compounds (heavy metals), pathogenic microorganisms, etc. Addressing environmental issues are directly related to the development of applied biotechnology aimed at the establishment of the natural environment rehabilitation means. Using biotechnology techniques microorganisms-destructors of organic waste (Bacillus, Streptomyces, Pichia) were selected and biological fermentation accelerators for utilization of manure, litter, sewage sludge were designed. The following technological schemes of their production were developed: cultivation on nutrient media, biomass accumulation, stabilization, and product quality control. The technological process lies in stratified introducing of bioproduct over the entire volume of stockpiled substrate (litter and semisolid non-litter manure with humidity 86-92 %). In the case of non-litter liquid manure with humidity 92-97 % and dung runoff with humidity over 97 % the biological product to be poured in directly into the collector, drain pit, treatment facilities reservoir. Due to the high biological activity of microorganisms that are part of the biological products the process of organic substances destruction accelerates, and the substrate neutralization and deodorization occur. Adding of this fertilizer obtained by treatment of manure/litter, improves the physical, chemical and biological properties of the soils that positively affect the yield and quality of crop production.

L30

Towards haloalkane dehalogenases suitable for industrial applications

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Haloalkane dehalogenases are hydrolytic enzymes which act to cleave carbon-halogen bonds and to convert halogenated compounds into corresponding alcohols. Many of their substrates belong to hazardous environmental pollutants or warfare chemicals and some of the products are valuable building blocks in organic and pharmaceutical synthesis, making these enzymes attractive for various practical applications: (i) biocatalytic preparation of optically pure compounds; (ii) bioremediation of toxic environmental pollutants; (iii) decontamination of chemical warfare agents; and (iv) biosensing of environmental pollutants [1]. Nevertheless, further extension of haloalkane dehalogenase uses in practice require the most limiting factor – low stability under harsh reaction conditions typical of industrial processes – to be overcome. In this sense, the strategies based on the protein engineering combined with in-silico prediction tools proved to be very useful. We have designed and constructed highly

stable mutants of technologically interesting haloalkane dehalogenase DhaA from *Rhodococcus rhodochrous* [2-4]. In contrast to the wild-type enzyme, our newly obtained mutants are stable in the presence of denaturing organic co-solvents, such as DMSO or acetone, and their melting temperature increased by 21 °C. Such a remarkable stabilization converts haloalkane dehalogenases into commercially viable biocatalysts, broadening their usability in industrial biotechnologies.

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L31

Simultaneous Production of Bioelectricity And Docosahexaenoic Acid (DHA) From Glycerol By The Photomicrobial Fuel Cell

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The current world energy crisis and global warming have yielded an ever desperate search for sustainable green energy sources. Microalgae present a potential biochemical/bioenergy source of both renewable and sustainable qualities. It is understood that ”Photomicrobial fuel cells (PMFCs)” generate electricity from both photosynthesis and catabolism of endogenous carbohydrates in the light and from catabolism alone in the dark circumstance [1.2]. Crude glycerol, a major by-product from the downstream processes of biodiesel, has been used as a feedstock for the fermentation of algae *Schizochytrium limacinum* to produce docosahexaenoic acid (DHA; 22:6 n-3). This paper investigates the potential of using photomicrobial fuel cell to generate bioelectricity and DHA simultaneously. The microalgae strains, *Schizochytrium limacinum* and *Chlamydomonas reinhardtii transformation F5*, were conducted and operated in a three-chambers design of PMFC under several conditions.

Crude glycerol was fed to dark chamber as well as carbon dioxide was introduced to photo-chamber. The bioelectricity, DHA and final biomass were analyzed. The first anode-chamber cultivated with *S. limacinum* generated a maximum power density of 179 uW m^{-2} characterized with internal resistance of 24 kohm by feeding crude glycerol as electronic donor. The second-chamber loaded with *C. reinhardtii transformation F5* utilized carbon dioxide produced from first-chamber yielded a maximum power density of 23 uW m^{-2}. The conversion of crude glycerol in PMFC operation achieved 96 % after 96 hours. The total lipid and DHA content was found 38 % w/w DCW and 26 w/w lipid, respectively. Our results indicated that operation of PMFC can effectively utilize crude glycerol and carbon dioxide and be a potential system to produce bioenergy and biochemical. Such strategy not only increased the yield of DHA product but also produced bioelectricity.

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L32

Continuous cultivation of *Azohydromonas australica* for production of copolymer Poly (3-hydroxybutyrate-co-3-hydoxyvalerate)

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Conventional polymers are produced from the depleting petroleum resources and are a major source of environment hazard. Biotechnological protocols are, therefore, desperately required for production of biodegradable polymers/copolymers from renewable resources. Bacteria *Azohydromonas australica* has a unique ability to grow on sucrose and accumulate bio/copolymers during the growth phase of cultivation particularly under excess availability of carbon source & severe nitrogen limitations. The batch kinetics of the cultivation was used to develop a mathematical model which was then extrapolated to simulate feeding strategies of carbon and nitrogen source for highly productive fed-batch cultivation to accumulate significantly high biomass & PHB concentrations. Extrapolation of the selected fed-batch cultivation was done to attempt continuous production of copolymer Poly (3HB-co-3HV) at high concentration and productivity. Continuous cultivation was initiated as batch during 0 - 22 h using statistically optimized medium thereafter 125 g/L sucrose and 2.8 g/L

nitrogen (along with rest of the medium components) were fed continuously at a dilution rate of 0.09 h^{-1}. Valeric acid feeding (as guided by the batch experiments) was also initiated at 22 h and it was done in the form of pulses (1g/L each) to exponentially growing culture. This was continued till the end of fermentation. Continuous cultivation was continued for 6 days without any contamination problem. The steady state concentrations of biomass and Poly (3HB-co-3HV) accumulations were 32.42 g/L and 24.65 g/L respectively. The hydroxybutyrate (HB) and hydroxyvalerate (HV) content of copolymer Poly (3HB-co-3HV) in the bioreactor at the harvest time were observed to be 56 % and 17 % of DCW respectively. This cultivation strategy yielded an overall Poly (3HB-co-3HV) productivity of 2.18 g/L/h which was significantly higher than batch cultivation.

L33

Biomass refineries for a clean environment

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There is a universal dream of an eco-utopia whereby oil refineries are eventually replaced by biomass refineries. The dream envisages a bio-economy which reverses the ongoing damages of climate change by promoting a clean environment while maintaining national energy security. Many researchers are trying to prevent this dream to become a nightmare. There are difficult challenges. In these deliberations, we first review the envisaged eco-friendly idealized world, then point out some major scientific bottlenecks and geopolitical constraints. In addition, we reflect on the need for a lifestyle change of our current "throw-away" rather "recycling" community behavior. Clearly, the collective research must be necessarily multidisciplinary to address the diversity of societal constituents. This brief overview examines what biotechnology can contribute to the international scenario. Within context, we relate the relevance of our own research on the processing strategies for the production of drugs, foods and fuels primarily from renewable agricultural biomass resources. Quantitative design correlations for pneumatically aeration-agitation bioreactor operations are being developed for applications with genomic and metabolic engineering to microbial fermentation processes. Finally, we express our dismay of the media hype which has prevented more cooperative government policies worldwide on strategic priorities within national commercial interests.

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L34

Lignocellulosic biomass utilization toward biorefinery: technologies, products and perspectives

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Lignocellulosic biomass wastes (LBW) are generated and accumulated in large amounts around the world every year. The disposal of large amounts of such wastes in the nature may cause environmental problems, affecting the quality of the soil, lakes and rivers. In order to avoid these problems, efforts have been directed to use LBW in a biorefinery to maximize the reutilization of these wastes with minimal or none production of residual matter. Through biorefinery, the biomass is submitted to different conversion processes that may include biological, physical and chemical technologies to produce fuels, power, heat, food, feed, and value-added chemicals. The products that can be obtained as well as the technologies that can be used for biomass disruption and conversion vary according to the characteristics of the LBW, such as the physical properties and chemical composition in terms of cellulose, hemicellulose, lignin and protein contents [1]. By producing multiple products through a biorefinery, the value of the biomass feedstock is maximized, which can be of particular interest in countries with abundance of these wastes and also in the underdeveloped countries that can explore better their resources and waste materials. In the future, a significant increase in the implementation of biorefinery processes for biomass valorisation is expected motivated by the desire in reducing the impact and toxicity of these wastes to the environment and at the same time to produce compounds of industrial interest from competitive technologies based on the use of low-cost raw materials. The creation of more jobs is another positive and important aspect related to the implementation of these processes on industrial scale.

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L35

Perspectives of Applied Microbiology with Purple Bacteria driven by Systems Biology

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Anoxygenic photosynthetic purple bacteria are well-known to offer highly attractive opportunities for industrial applications. Potential products derived from intracytoplasmic photosynthetic membranes (ICM) include pigments, coenzymes, biohydrogen, biopolymers and recombinant membrane proteins. Since high levels of ICM are formed anaerobically at low-light intensities, most attempts to exploit purple bacteria were so far conducted phototrophically, using light as energy source. However, mass cultivation of photosynthetic microorganisms is generally inefficient due to the inevitable limitation of light when cell densities become very high. It is thus interesting that in *Rhodospirillum rubrum* the high-level production of ICM can be completely separated from light, when the bacteria are grown microaerobically in the dark with a two-carbon substrate growth medium. On the basis of this cultivation process, we applied a systems approach using a combination of bioreactor cultivations, metabolomics and computational modelling to develop *R. rubrum* for biotechnological applications. This work is intended to open a new perspective for utilizing photosynthetic bacteria in biotechnology. The presented examples include the production of biohydrogen, industrially relevant carotenoids and the utilization of carbon dioxide as a feedstock for bioprocesses.

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L36

Applied biotechnology for the production of biofuels and bioproducts from sugarcane bagasse

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Copious amounts of sugarcane bagasse (SB) are generated every year in Brazil during the production of sugar and alcohol by the industries. SB contains an appreciable amount of carbohydrates which is readily available on-site for the production of several value-added products like fuel ethanol, xylitol, organic acids, 2,3-butanediol, industrial enzymes and others by biochemical means. The main process steps involved for bioconversion of SB into these products are: pretreatment, detoxification of sugar syrups, cellulose-mediated hydrolysis, microbial fermentation and product recovery [1]. The xylan extracted from the SB hemicellulose is of particular interest since it is the source of xylose which can be converted into D-xylitol by fermentative or enzymatic routes. Xylitol is a polyol widely used as a sweetener in the food industries, being indicated for consumption by diabetic people due to its insulin-independent metabolism. It also holds applications in caries prevention and leading to the re-mineralization of teeth enamel. The hemicellulose-derived xylose can also be used for the production of ethanol, a renewable liquid fuel. Looking at the fast depletion of fossil fuel and a regular price hikes worldwide, ethanol production from lignocellulose has gained a significant momentum. In this line, Brazil has shown a considerable progress towards the development of bioethanol as a sustainable alternative of gasoline. Total bioethanol production for 2012/2013 was 23.64 billion liters in 405 sucrose processing plants [2]. Various biotechnological methods have been developed in laboratories in past for the production of such products harnessing SB, however these technologies need to be proven successful at industrial level. Our laboratory is dedicated towards the development of successful technological platform for the sustainable production of xylitol, ethanol and industrial enzymes. This presentation deals with important aspects for the biotechnological production of ethanol and xylitol from sugarcane bagasse and developments happened at our laboratories.

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L37

Fungi-based biorefinery: Ethanol, biogas, fish feed and superabsorbents from lignocellulosic materials

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Zygomycetes and ascomycetes are two large and wide-spread groups of filamentous fungi. Many species of these fungi have great use in food applications e.g. in tempeh, red rice, Quorn, etc. Zygomycetes are primarily saprophytic fungi, capable of assimilating lignocelluloses and agricultural waste materials and producing a variety of metabolites. Since 1999, we have studied physiology and metabolite formation of several zygomycetes and ascomycetes strains from *Rhizopus, Mucor, Rhizomucor, Fusarium* and *Neorospora genera*. The growth of these fungi were investigated on spent sulfite liquor, lignocellulosic hydrolyzates, rice straw, orange peels, etc. These fungi are generally able to take up hexoses and pentoses, and even dimers such as cellobiose, and some of them are even superior to baker's yeast in terms of ethanol yield and productivity. We approach an integrated application of these edible fungi, where they are cultivated on lignocellulosic materials for ethanol production. The biomass of the fungi will then be used as fish- and animal feed or further processed to produce biological superabsorbents, which can be converted to biogas at the end of its lifetime. It means fungi-based biorefinery produces several products simultaneously, which makes ethanol process more feasible.

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From Anaerobic Digestion to Biorefinery through added Value Chains

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Biogas production from renewables as well as from wastes and manure is on the rise throughout Europe since over two decades. Due to the rising demand of renewable energy with a carbon neutral footprint, anaerobic digestion (AD) of organic material is often treated equivalent to biogas production alone, disregarding the fact, that AD offers a plethora of other routes to gain

improved value from organic input material.

Arguing from a Swiss background, with wastes and not renewables as a raw substrate for AD and based on a reference scenario with 20'000 t/a of OFMSW (organic fraction of municipal solid waste) & Manure, both offering by far the largest mass potential of organics in the country, 9 theoretical extensions for AD technology with a potential to add new value chains to existing biogas schemes will be presented.

L39

Bioinformatics for biotechnology research: data mining of *Clostridium pasteurianum* genome

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Today's research relies more and more on interdisciplinary cooperation. The utilization of next-generation sequencing technologies brings possibility of performing a research on molecular level. Such an example can be given in data mining of *Clostridium pasteurianum* NRRL B-598 genome we sequenced recently [1]. The first step of bioinformatics analysis consists in assembling of short reads into longer sequences. The most suitable algorithm for assembling could be selected according to genome's features and sequencing technology. The second step involves a genome annotation in a public database because no bioinformatics analysis can be published without publicly available data. There are 3 interconnected databases (GenBank/EMBL/DDBJ) to choose. These databases provide automatic annotation and gene prediction. The algorithms perform similarity searches utilizing previously annotated genes of similar organisms or they predict new genes by open reading frame (ORF) detection. For our *Clostridium*, we reached draft genome comprising of 6,041,878 bases that are split into 138 contigs (unplaced genomic sequences). Despite draft sequence, the genome annotation predicted 5,547 genes, including 5,367 protein coding sequences (CDSs). On the other hand, an automatic annotation can bring errors in correct gene name assignment because of similarity of different genes. These errors can be spread across whole databases that are not moderated. Thus, a further manual analysis using BLAST searches is needed. Manually selected sequences can be processed by other tools of bioinformatics, e.g. phylogenetic analysis, entropy analysis, protein conformation prediction, etc. as was provided for our *Clostridium* [2]. Such in silico analysis can be used as a base for further experimental work e.g. rtPCR, microarray or western blot analysis, that can lead to signal pathways reconstruction utilizing tools of systems biology.

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L40

Renewable Chemicals by Design

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Green Biologics Ltd. (GBL) has developed an advanced fermentation platform for renewable chemicals (specifically n-butanol and acetone) that integrates best in class microbiology with high productivity fermentation and innovative solvent recovery. This platform will be enhanced using synthetic biology to develop microbial chassis for a wider range of "Renewable Chemicals by Design". GBL is headquartered in Oxford, UK but operates globally and has pilot plant and demonstration facilities in North America. We report on GBL's commercial progress in China and North America. The presentation will focus on some of the key technical and commercial challenges associated with solvent production using clostridia and how these have been overcome using novel fermentation technology. In addition, we will also describe progress on utilising low cost cellulosic feedstocks. Overall, these developments have the potential to transform process economics.

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Bioengineering Clostridia: A Road Map for Gene System Development

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The genus *Clostridium* is one of the largest amongst prokaryotes. An extremely diverse assemblage of species, they are composed of Gram-positive, anaerobic, endospore-forming, rod-shaped bacteria. The antics of a few (for example, *Clostridium difficile, Clostridium botulinum* and *Clostridium tetani*) have given the genus a bad name. The vast majority of the species, however, are entirely benign, and exhibit extreme biocatalytic di-

versity. Indeed, the propensity of many of the solventogenic species to produce organic solvents, and in particular the alcohols ethanol and butanol is of great interest to the industrial biotechnologist. Prominent amongst the species being investigated are those clostridia able to produce the superior biofuel butanol, such as *Clostridium acetobutylicum* and *Clostridium beijerinckii*, as well as cellulosic species able to produce ethanol from renewable biomass, typified by *Clostridium thermocellum* and *Clostridium phytofermentans*. More recently, there has been an upsurge in activity directed at exploiting acetogenic species, such as *Clostridium ljungdahlii* and *Clostridium autoethanogenum*. They grow on a spectrum of waste gases from industry (eg., steel manufacturing, oil refining, coal and natural gas) as well as 'synthesis gas' (CO & H_2) produced from renewable and sustainable resources, such as biomass and domestic/ agricultural wastes. This enables low carbon fuels and chemicals to be produced in any industrialized geography without consumption of valuable food or land resources. The Clostridia Research Group have established a Road Map for the implementation of tools and procedures with which forward and reverse genetic strategies may be applied to any clostridial species and beyond.

L42

Immobilized biocatalysis for biofuel production

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Immobilized living cell and enzymes offer several important advantages compared to conventional free cell/enzyme processes. Possibility of continuous operation at high dilution rates with no wash out danger, higher reaction rates at increased cell/enzyme concentrations and higher yields. Application of both, enzymes and cells immobilized in polyvinyl alcohol lens shaped capsules – LentiKats will be demonstrated on biofuel production.

Acknowledgement: This work was supported by GRAIL (Grant agreement no: 613667), project co-financed by the European Commission under the 7th Framework Programme.

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L43

"In-situ" product removal for improved isopropanol, butanol and ethanol production by fermentation

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The production of acetone, n-butanol and ethanol (ABE) or isopropanol, n-butanol and ethanol (IBE) by anaerobic bacteria from biomass resources, are processes with a long industrial history. Both processes are considered to have great potential for the commercial production of butanol, an important bulk chemical with a large market which also presents good properties to be used as biofuel from renewable resources. The IBE process is of interest since isopropanol represents an important bulk chemical as well which can be a precursor of propylene. The current butanol-producing microorganisms utilize all sugars in lignocellulosic biomass (both C6, C5), which make the IBE processes very suitable to be a part of lignocellulosic biorefineries for the efficient conversion of biomass-derived sugars into chemicals and fuels. One of the major bottlenecks of these processes is the high cost of the separation of the products. Due to product toxicity (especially of butanol), the end-concentrations of IBE reached in batch fermentations are limited to approx. 2 % (v/v). Currently there is an interest in the use of *"in-situ"* product recovery methods in the ABE or IBE processes because these offer two main advantages compared to conventional down-stream techniques: i) these methods could be suitable for continuous and selective toxic product recovery. Therefore, relieving product inhibition and enhancing substrate utilisation. *"In-situ"* removal offers the potential of using concentrated feed solutions, resulting in an important reduction of process streams, and ii) reduction of the costs of product recovery if the separation technique is competitive with distillation. In this presentation, the results of using gas-stripping and of adsorption (using a zeolite and activated carbon) as *"in-situ"* recovery methods for IBE production in different process configurations will be described. The IBE productivity of cultures where any of these methods was applied improved at least in 50 % compared to the control cultures.

L44

The production aminolevulinic acid and biohydrogen in a biorefinery concept using metabolically engineered *Rhodobacter sphaeroides* O.U.001

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5-Aminolevulinic acid (ALA) having very important application areas in medicine, agriculture and biotechnology is

a high value-added product. The biosynthesis of 5-ALA in living things occurs either by using succinyl-CoA and glycine (Shemin or C-4 pathway) or using glutamate (C-5 pathway). *Rhodobacter sphaeroides* synthesize ALA using C-4 pathway. The *glutamyl tRNA reductase* gene was cloned and expressed in *R. sphaeroides* so that C-5 pathway was also enabled to function upon assembling all the genes of C-5 pathway. Consequently, a new and unique bacterial strain producing more ALA at different conditions was developed. The production of biohydrogen was also investigated in the same bioprocess within a biorefinery approach. For a feasible process, sugar beet molasses was used as a substrate. After the introduction of *glutamyl tRNA reductase* gene into *R. sphaeroides*, transcription of glutamyl tRNA reductase gene was detected by RT-PCR. Then, the amount of ALA produced by mutant strain (38.75 mM) was found to be higher compared to the wild type cells (23.34 mM). In addition, 1.2 L H_2 / L culture was produced in the same bioreactor using molasses. The results showed that additional ALA production pathway was successfully introduced into *R. sphaeroides*. In this way, an enhanced ALA production was achieved in addition to biohydrogen production within a biorefinery context.

L45

New microorganisms for wastes upgrading: the case of Organic Fraction of Municipal Solid Wastes and Brewer's spent grain within Bioassort project

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The aim of this work was the development of new tools and processes for upgrading two wastes - Organic Fraction of Municipal Solid Wastes (OFMSW) and agro-industrial wastes, Brewer's spent grain (BSG) - that are abundantly discharged in EU. New microorganisms were selected as a source of new (hemi) cellulases to be used as components of the enzymatic cocktails applied in the conversion of the selected wastes into fermentable sugars for production of second generation ethanol or lactic acid. Around 100 novel bacteria were isolated from Western Ghats (India), a rich biodiversity area inhabiting several unexploited microbes. 12 bacteria were selected for cellulase/xylanase activity production.

A screening in liquid culture was performed, selecting the best producer of xylanase in the presence of xylan 1 % as sole carbon source. Characterization of the extracellular enzyme mixture of the selected xylanase producer was carried out in order to define the best conditions needed for its application in the conversion of OFMSW hemicellulose portion into fermentable C5 sugars. Six fungal strains potentially applicable to OFMSW conversion were also selected among 14 fungi belonging to DEBB collection shown to produce both cellulase and xylanase activity on solid medium. A lactic acid bacterium of DEBB collection was also selected and studied for lactic acid production using BSG as raw material. The pre-treated BSG was saccharified with a commercial cellulase complex and directly used for setting up the lactic fermentation, showing to be a good raw material for lactic acid production.

This research was supported by a Marie Curie International Research Staff Exchange Scheme Fellowship within the 7th European Community Framework Programme: "Improvement of technologies and tools, e.g. biosystems and biocatalysts, for waste conversion to develop an assortment of high added value eco-friendly and cost-effective bio-products" BIOASSORT (318931).

L46

Parallel Cultivation of Microorganisms using Rigid Wall Single-Use Bioreactors

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Single-use bioreactor solutions have been successfully established in animal and human cell culture in the last years. Currently this technology is investigated for microbial applications. The following case study demonstrates that reproducible process control could be achieved using single-use bioreactors operated in parallel.

E. coli K12 (DSM 498) was cultivated in a fully-instrumented Eppendorf BioBLU® 1f Single-use Vessel. This rigid wall stirred-tank single-use bioreactor was specifically designed for microbial applications, and is equipped with two Rushton-type impellers, four baffles and liquid-free Peltier exhaust condensation. The magnetic coupled overhead drive safely operates up to 1600 rpm, ensuring k_La values of up to 4150 h^{-1}. A 4-fold DASGIP parallel Bioreactor System with DASGIP® Control Software was used for precise process control. Starting with a working volume of 0.7 L the cultures were grown in PAN medium with an initial glucose concentration of 40 g/L. DO-based feeds started simultaneously in all 4 units 11.3 h (± 0.1 h) after inoculation. The temperature

was set to 37 °C and the pH was adjusted to 6.8 using 25 % ammonia solution. The cultures were submerged aerated through integrated dip tubes with a constant rate of 42 sL/h (1 vvm). Dissolved Oxygen concentration was regulated to 30 % with the stirrer speed ranging from 600 rpm to 1600 rpm (tip speed of 1.34 m/s to 3.56 m/s). Oxygen transfer rates (OTR) were automatically calculated via an exhaust analysis module GA4. OTR values of up to 230 mmol/L/h were observed in the single-use bioreactors. Biomass was determined offline as cell wet weight and reached maximum values of 163 g/L. These results are comparable to results that have been obtained in glass bioreactors.

L47

Human heteromeric amino acid transporters

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Membrane proteins fulfill innumerous key functions in all living cells and account for more than 30 percent of the eukaryotic proteomes. However, the number of membrane proteins for which high-resolution 3D structures have been published is still very low amounting to 440 unique structures in contrast to >15'000 structures of water-soluble proteins. Among these unique structures, most are of bacterial membrane proteins overexpressed in bacteria or of eukaryotic membrane proteins naturally expressed at unusually high levels in the tissues of certain organs. Only a relatively small number of structures are of eukaryotic, recombinant membrane proteins. The major bottleneck for structural studies of mammalian membrane proteins is the production of microgram to milligram amounts of highly pure and correctly folded protein. This makes heterologous overexpression of such proteins in host cells (e.g., yeast, insect and mammalian cells) unavoidable and remains nowadays a real challenge.

Human heteromeric amino acid transporters (HATs) are membrane proteins involved in renal aminoacidurias, cocaine relapse, herpesvirus infection and tumor growth. HATs are composed of two subunits, the heavy and the light. The light subunit is the catalytic one and consists of 12 transmembrane domains. Heavy subunits are type II membrane N-glycoproteins with a large extracellular domain. Structural information of HATs is scarce because of the lack of recombinant protein.

Recently, we had a major breakthrough with the overexpression of a recombinant HAT in the methylotrophic yeast *Pichia pastoris* [1]. Furthermore, we could gain first structural information using purified protein and transmission electron microscopy [2]. However, for 3D crystallization and high-resolution structure determination by X-ray crystallography milligram amounts of pure protein are needed. Growth of Pichia cells overexpressing HATs in bioreactors is an excellent solution for the yield of large amounts of cells for subsequent purification and crystallization.

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L48

Recombinant Production of Peptides

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Peptides have applications ranging from diagnostics and therapeutics to natural inhibitors of bacterial growth in food industry. Recent technological advances in delivery and formulation of peptides have rekindled the interest of the pharmaceutical industry for peptide therapeutics resulting in 6 new drugs approved in 2012 alone. Recombinant production is an interesting alternative to the chemical synthesis, in particular for longer peptides/short proteins and peptides containing post translational modifications such as multiple disulfide bonds. Such peptides can be found in animal venoms, a yet largely untapped source of lead molecules for drug discovery. Their interesting properties include high selectivity and potency at low doses, as well as being very stable and poorly immunogenic. Different expression systems were tested for the production and purification of snake-derived three-finger toxins containing four disulfide bonds. In order to express the peptides in the cytoplasm of *E. coli*, where the reductive environment normally prevents the formation of disulfide bridges, several strains with mutated reductases and expressing inducible auxiliary proteins to aid correct S-S bridge formation were compared. In another approach the peptides are expressed in the oxidative environment of the ER of the methylotrophic yeast *Pichia pastoris* and then secreted into the medium.

L49

Microbial expression of human enzymes: from biocatalysis to drug design

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The application of microbial expression systems for protein production, with its advantages and challenges, has been traditionally exploited for the benefit of various fields in biotechnology: enzymes, expressed as recombinant proteins, represent a product per se (e.g. additive for detergents, waste treatment or natural polymer processing), or are used to perform highly specific and environmental-friendly biotransformations to obtain higher quality or quantity of APIs or bulk chemicals. More recently, the identification of crucial human enzymes, either involved in drug metabolization or targets of genetic diseases, triggered the interest in expressing human complex proteins in microbial systems, paving the way to a formidable tool for identifying new drugs on wild type and (more interestingly) mutant, disease-associated, enzymes. In this respect, examples of interesting enzymatic systems will be provided, expressed as recombinant proteins in *Escherichia coli* and in the methylotrophic yeast *Pichia pastoris*. Given the complexity of the task, novel tools and strategies for expression, screening and biotransformation have to be conceived; this talk will present the results obtained, together with the challenges waiting ahead.

L50

Sampling Enhancement in Biomolecular Simulations

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Computer simulations of biomolecules have a great potential to complement or even replace experiments in structural biology or drug design. However, this ambition is hampered by the fact that corresponding processes, such as protein folding or binding of a drug to its target, are too slow to be efficiently simulated even on the world's largest computers. Design and application of new methods to improve efficiency of biomolecular simulations is a viable strategy to solve this problem, hand in hand with development of new hardware.

The major source of inefficiency in these simulations comes from the fact that the simulated systems tends to stay in various local energy minima (e.g. in protein folding intermediates) instead of going directly to the global minimum (i.e. the native structure). This problem can be addressed by an artificial potential, which disfavours previously visited states of the studied system, as implemented in the method called metadynamics [1,2]. This talk

will present recent applications of this technique in simulation of structural changes in two domains of biotechnology research – carbohydrates and proteins. Namely, conformational studies on glycosaminoglycan building blocks and protein folding simulations will be presented.

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L51

Using nature's chemical diversity for drug discovery

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Approximately 40 % of the pharmaceuticals in clinical use are natural products or their derivatives [1]. The continuing success of natural products is due to their structural diversity which is not available from alternative sources and from their wide variety of biological activities. Since more than 100 years Novartis has a strong emphasis on natural products in drug discovery. By applying chemical as well as biological screening, promising lead compounds are discovered continuously. In addition, novel technologies such as next generation sequencing, synthetic biology and high resolution mass spectrometry will continue to lead natural product discovery into the next decades.

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L52

Gene shuffling for synthesis of novel lincosamide antibiotics – inspiration from nature

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Several approaches can be used to design more efficient derivatives of natural bioactive compounds; combination of appropriate biosynthetic genes is one of them. Genetic engineering of producing strains requires several prerequisites: Availability of gene cluster to be modified as well as gene or set of genes coding for required modification(s) and also the detailed knowledge of characteristics of

all enzymes converting modified precursors to a novel product. Natural evolution of biosynthetic clusters gives us inspiration how to solve this engineering problem. Lincosamide antibiotics biosynthesis could be a good example. Two known natural lincosamides differ in the amino acid building block. The less active celesticetin incorporates proteinogenic L-proline while clinically important lincomycin integrates unusual proline derivative – 4'propyl-L-proline (PPL). The prolonged side chain of proline moiety is a hot spot affecting biological activity of the final product. During the evolution of lincomycine biosynthetic pathway, set of six genes coding for unusual PPL precursor was adopted, probably from producer of structurally and functionally dissimilar group of compounds, pyrrolobenzodiazepines (PBDs) with antitumor activity. The acquisition of new precursor had to be followed by radical change in the substrate specificity of the subsequent enzymatic activity – N-demethyllincosamide synthetase (NDLS), particularly its A-domain activating the modified amino acid precursor for condensation reaction. The specific modification of the NDLS A-domain substrate binding pocket led to enlargement of the cavity enabling the activation of not only PPL, but even larger substrate 4-pentyl-L-proline. The relaxed substrate specificity was successfully used for mutasynthetic production of even more efficient antimicrobial lincosamide derivatives.

L53

Production of highly active compounds by fermentation

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The last 10 years have shown a shift within active pharmaceutical ingredients towards highly potent active pharmaceutical ingredients. A number of such highly active compounds are produced by fermentation. These compounds may be administered as such e.g. as chemotherapeutic agents in cancer treatment or e.g. as highly active cytotoxic compounds bound to an antibody, called antibody drug conjugates. These medicines require lower doses and typically exhibit lower side-effects with a positive effect on safety and well-being of patients. On the other hand and as consequence of their high potency, companies producing such compounds under cGMP are confronted with new manufacturing challenges. Areas that are affected by the high product potency aspect are (1) the manufacturing technology and its related toolbox, (2) manufacturing facility design criteria such as containment and environmental aspects, (3) the supporting infrastructure and (4) access to adequately trained personnel. The presentation discusses the manufacturing of and manufacturing set-up for highly active compounds that belong to the risk class 5 (according to Lonza's internal risk classification) equal to a safe bridge classification of 3-4 and requiring an OEL standard of 20ng per m3

of air. Additional focus will be given to the problems posed by the multiproduct character of the facility.

L54

Manumycins reloaded: From feeble antibiotics to promising anti-inflammatory agents.

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The group of manumycin antibiotics is represented by roughly 30 bioactive metabolites produced by actinomycetes. The structure of them is typical by the presence of two carbon chains (upper and lower) connected in *meta*-fashion to the unique central epoxyquinone moiety (mC_7N). In most manumycins, the second cyclic unit, C_5N, is attached to the lower chain [1]. The biosynthesis of their representative, asukamycin, has been deciphered including the relevant genetic information [2]. Manumycins are poor antibiotics, acting weakly against $Gram^+$ bacteria. However, thanks to multiple enzyme-inhibitory features, they possess potent anti-cancer and anti-inflammatory features. They inhibit activity of Ras farnesyl transferase, caspase 1, Iκ B kinase, neutral sphingomyelinase and acetylcholinesterase. So far, commercially available manumycin A anti-proliferative and pro-apoptotic features were studied most of all using numerous cancer models. In our project we have concentrated on the anti-inflammatory effects of manumycins on human cells. We have isolated several metabolites of the family and also identified some novel ones by means of genetic screening of new actinomycete natural isolates and by genetic and metabolic engineering of characterized producers. Our data show that the structure of the most variable part in their molecule, the upper polyketide chain, determines their particular enzyme-inhibitory features. Whereas some compounds possess strong pro-apoptotic features as manumycin A, in others these are less prominent compared to anti-inflammatory, making them quite promising candidates for future use to cure inflammatory diseases [3]. Supported by IGA MZCR grant NT/13012-4 and LH12191 grant of Ministry of Education, Youth and Sports.

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L55

Polymeric Biomaterials Based on Collagen for Bone Regeneration

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For accelerated healing large bone defects we have recently developed a new 3D biodegradable porous scaffold based on a biodegradable composite of collagen. 3D collagenous scaffolds are highly porous biomaterials with porosity of about 85-90 % providing better nutrition and vascularization of the regenerated tissue. Collagenous scaffolds were modified with both polymeric nanofibers and inorganic microparticles. Polymer nanofibers based on biodegradable polycaprolactone significantly increased the moduli of elasticity, while inorganic hydroxyapatite microparticles improved the strength of collagen scaffolds. Prepared 3D collagen porous scaffolds were seeded with autologous mesenchymal stem cells (MSCs) using the method of tissue engineering. To accelerate the healing of bone tissue, MSCs seeded scaffolds were additionally enriched with thrombocyte rich solution (TRS). Thus prepared polymer/cells temporary biopolymer implants were subjected to preclinical testing on white rabbits. The macroscopic and histological analysis of the regenerated bone tissue was performed 12 weeks after the implantation. The regeneration of femoral condyle defects of white rabbits were compared for the scaffolds described above and those involving only commonly used MSCs or TRS. The Highest volume and the most uniform distribution of newly formed bone were found in bone defects treated with polymeric collagenous scaffolds modified with PCL nanfibers, HAP microparticles and enriched with both MSCs and TRS.

Acknowledgement: This work was supported by the project "CEITEC - Central European Institute of Technology" (CZ.1.05/1.1.00/02.0068) from European Regional Development Fund.

L56

The scientific approach and methods for mitigation of a large-scale limitations in isolation and purification of active pharmaceutical ingredients

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Many APIs are produced via custom manufacturing nowadays. This approach require transferring the technology from a customer to a manufacturer plant. Development, optimization and adaptations of the technologies are very often requested in addition to the transfer. Processes are typically developed and tested at laboratory scale while real manufacturing exploits full-scale equipment with its technical and process limitations, which are very often not encountered under laboratory conditions. Lonza has established procedures, how to transfer, develop, optimize and scale-up biotechnological processes in reliable and timely manner. Basic technology transfer stages will be discussed in the presentation with focus on process understanding in field of downstream processing.

L57

Bioactive bacterial exopolysaccharides : modification, characterization and chondrogenic potential for cartilage regenerative medicine.

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Sulphated polysaccharides have diverse biological functions in the tissues from which they originate especially in the cellular physiology. These bioactive molecules present a great potential for medical, pharmaceutical and biotechnological applications such as wound dressings, biomaterials, tissue regeneration and 3D scaffolds, and even drugs. Marine bacteria from deep-sea hydrothermal vent environments have demonstrated their ability to produce in an aerobic carbohydrate-based medium, unusual extracellular polymers. They present ori-

ginal structural features that can be modified to design bioactive compounds and improve their specificity. In particular, with the aim of promoting biological activities, chemical modifications (depolymerisation and substitution reactions) of one exopolysaccharide produced by *Alteromonas infernus* (GY785 EPS) have been undertaken. The structure of this GY785 EPS has been previously described [1]. Using two different sulphation processes, two types of low molecular weight (LMW) oversulphated derivatives have been isolated from the GY785 EPS. First LMW GY785 EPS derivatives have been obtained by a free radical depolymerisation. Then a sulphation reaction has been developed using either dimethylformamide (DMF) or ionic liquid (IL) as solvent. The two derivatives named GY785 DRS-DMF and GY785 DRS-IL respectively have been characterized and some biological analyses have been initiated in order to compare the potential of these two derivatives for cartilage regeneration [2,3].

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L58

Formation lactic acid bacteria biofilm: changes of VIABILITY of biofilm probiotic cells compared to plantonic form

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Maintenance of lactic acid bacteria (LAB) viability is one of the most important aspects in the production of food supplements. On the other hand, low pH in stomach can induce decrease in viability through the gastrointestinal tract. Nowadays, it is an effort to prepare LAB product, which should be stable (i) during the passage through the gastrointestinal tract and (ii) for a long term shelf live before use. In this work, the ability of LAB to adsorb on the inorganic substrates was evaluated. As a matrix, food carrier Probifix

was used. The adsorption and biofilm forming on the inorganic substrates was observed by using electron microscopy. Further, viability of the free cells form and the biofilm preparative at low pH was compared in time scale. Planctonic and biofilm form of probiotic strain *Lactobacillus acidophilus* was compared according to following properties: the resistance against low pH, the resistance against influence of bile, the adherence to tissue cells and polystyrene, the ability to inhibit growth of pathogenic strains and the resistance to antibiotics. The biofilm form of LAB exhibited increased resistance against low pH as well as to bile salts, increased adherence to polystyrene and affected inhibition of pathogenic strains. The adherence to tissue cells and the resistance to antibiotics have not significantly changed. The results indicate that the biofilm form of LAB is more resistant than free cell form at all tested pH values. The complex view on LAB problematic brings us new possibilities and approaches with the aim to achieve a production of stable and biologically active material for pharmaceutical industry.

L59

Utilization of various waste substrates for biotechnological production of biopolymers and other high value products

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Agriculture and food industry generate large amount of carbon-rich waste products and by-products which disposal represent serious environmental issue. On the contrary, these waste materials can be also considered as inexpensive substrates for various biotechnological processes. This strategy is beneficial for several reasons – waste material is used in environmentally-friendly way and; moreover, utilization of cheap waste substrate might reduce cost of the biotechnological products. Therefore, we investigated waste frying oils (WFO) as substrates for production of polyhydroxyalkanoates (PHA) employing *Cupriavidus necator* H16. VFO are very promising substrates for PHA production, because, unlike other carbon sources, they provide very high yield coefficient ($Y_{PHA/oil}$). Moreover, we observed that application of protease-hydrolysed whole whey as a com-

plex nitrogen source even improve process of PHA production. Furthermore, using random mutagenesis we isolated mutant strain of *C. necator*, which was capable of improved PHA production from WFO in terms of yields as well as mechanical properties of produced materials. Coffee is one of the world's most popular beverages and has been growing steadily in commercial importance. Nowadays, coffee is, after petroleum, the second largest traded commodity in the world. Hence, coffee industry is responsible for the generation of large amounts of waste. The most important are spent coffee grounds (SCG), solid residues after beverage preparation or soluble coffee production. Oil extracted from SCG (SCG contain approx. 15% of oil) can be converted into PHA similarly as WFO. The solid residues after oil extraction can be (chemically and/or enzymatically) hydrolysed and used as substrate for either production of PHA employing *Bacillus megaterium* and *Burkholderia cepacia* or carotenoids using carotenogenic yeasts, in particular *Sporobolomyces roseus*. Solids after hydrolysis still possess high calorific value and can be, hence, used as a fuel in industrial boilers to at least partially cover energetic demands of the process.

L60

Poly(4-hydroxybutyrate) (P4HB): from biosynthesis to applications

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Poly(4-hydroxybutyrate) (P4HB), belonging to the family of bacterial polyhydroxyalkanoates (PHAs), is a strong, flexible and absorbable material which has a wide range of medical applications due to its excellent biocompatibility and biodegradability. It is the first and only PHA-based product approved by the FDA for clinical usage. The most remarkable property of P4HB is its very high elasticity that benchmarks closely to ultrahigh molecular weight polyethylene. Furthermore, its degradation takes place via surface erosion instead of burst degradation of other often used biopolymers such as poly(lactic acid) (PLA) and poly(glycolic acid) (PGA). Thus, P4HB has potential to fill an unmet need as a new absorbable biomaterial offering a set of properties that are not currently available for medical product development. Unfortunately, this potential has been not widely explored due to different limitations. The lack of commercially available P4HB is one of such limitations. Our activities focus on the process engineering of P4HB biosynthesis, especially P4HB from different substrates to increase production yield and reduce the cost.

We also work on optimization of the downstream processing of P4HB to obtain material quality of medical degree. Furthermore, different applications of P4HB have been explored such as using P4HB as an elastomeric scaffold material for tissue engineering.

L61

Products from microalgae

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Microalgae and cyanobacteria are sunlight-driven cell factories that can be used to produce many useful products. This presentation will focus on production of nutraceutical, pharmacological and other products from microalgae. The diverse applications of microalgae will be reviewed. Methods of growing algae for commercial purposes in large-scale photobioreactors and open systems will be discussed. Production of pharmacological and other high-technology products generally requires a contamination-free, controlled and highly consistent production environment. This form of contained culture of light-dependent microorganisms must inevitably use photobioreactors. Although some algae can be grown in bioreactors without light (i.e. heterotrophic growth), this method of production is not applicable to all the species that are of interest. Furthermore, heterotrophic cultures do not always produce the desired metabolites, or produce them in lower concentrations than do equivalent photosynthetic cultures. Photosynthetic culture has been traditionally carried out in open ponds, lagoons and 'raceways'; however, open systems are generally less productive than closed photobioreactors. Furthermore, open culture is possible only for the few species that can be grown in the necessarily selective extremophilic environment of ponds and lagoons. Also, production of highly biologically active compounds and algal toxins dictates the use of a contained culture system. Large-scale closed photobioreactors must be robust, highly productive, efficient, and inexpensive to build and operate. This presentation will discuss advances in engineered design of photobioreactors for large-scale production of phototrophic microorganisms and their products.

L62

Bridging the lipid yield gap

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"The realisation of economic and sustainable production of microalgal bulk lipids requires enhancement of the lipid productivity" is a frequently made statement. But what is the upper limit of microalgal lipid yield, and how big is

the gap with current practice? The present work answers these questions and explores the potential of strain selection, strain improvement, bioreactor design and bioreactor operation for bridging the lipid yield gap. First, the theoretical maximum lipid yield on light was estimated using genome-based stoichiometric modelling of the microalgal metabolism [1]. This was then compared with current best practice, to quantify the lipid yield gap. Subsequently, potential yield gains were determined through strain selection and through controlled-photobioreactor studies on various cultivation modes and cultivation conditions, including temperature, pH and light intensity [2-8]. Finally, a *Scenedesmus obliquus* starchless-mutant was developed and evaluated for improved lipid productivity. The theoretical maximum lipid yield on light, conservatively estimated at 0.81 gram triacylglycerides per mol visible-light-photons, is approximately 8-fold higher than what is currently achieved in year-round outdoor production at demonstration scale. An approximate 3-fold reduction of this yield gap was possible when optimal strains (*Scenedesmus obliquus, Chlorella zofingiensis* and *Neochloris oleoabundans*) were used, combined with optimal photobioreactor design and operation (aimed at low light absorption per cell) [1-8]. Another 1.5-fold reduction was achieved through improved carbon partitioning towards lipids in a starchless mutant. There is large potential to improve the lipid yield of outdoor microalgal cultures. This work shows that a multidisciplinary approach can substantially bridge the lipid yield gap.

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L63

Bioethanol production from green microalgae: from theory to practice

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Increasing global energy demand and environmental concerns have led to a growing interest in the replacement of fossil fuels by renewable energies. Among the existing energy alternatives, biofuels have emerged as one of the most important sustainable fuel sources. Recent studies have shown that microalgae can be used as potential feedstock for biofuel production due to several advantages in comparison to other energy crops. These photosynthetic microorganisms are able to biosynthesize large amounts of lipids and carbohydrates during short periods of time that can be subsequently processed into biodiesel and bioethanol, respectively [1]. However, so far, most of research has focused on the use of microalgal biomass for biodiesel production rather than on its bioconversion into bioethanol. This issue could be related to several challenges that still have not been solved, such as finding a cost-effective cell disruption method to ensure a complete hydrolysis of intracellular starch granules or determining process conditions to maximize product yield and to enable a high ethanol concentration at the end of fermentation, among others [2]. In this presentation, a general overview of the process of producing bioethanol from microalgal biomass will be given and recent achievements in this research field will be summarized. Future research directions and obstacles will also be discussed.

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L64

The development of microalgal biotechnology in the Czech Republic

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Microalgae are an excellent source of biomass rich in proteins, oils and antioxidants as well as many valuable secondary metabolites with potential medical use. Over the last 60 years, microalgal biotechnology has shown a range of applications: from the traditional biomass production for human and animal nutrition, soil conditioning in agriculture, technologies for waste-water treatment, products for cosmetics and medicine, and most recently to the possible production of a 'third' generation of biofuels. In the former Czechoslovakia, microalgal biotechnology developed rapidly in the 1960s to provide source of microalgal biomass. Unique thin-layer outdoor cascades for microalgae cultivation have been developed. The Laboratory of Algal Biotechnology in Třeboň has been focused on screening and characterisation of microalgal strains for application in nutrition and industry, development of cultivation procedures and analytical techniques, identification and characterisation of bioactive compounds for pharmacology as well the development and construction of cultivation units. Many operations were often carried out semi-empirically and disputes between biotechnologists vs. physiologists were settled when modern diagnostics was introduced to control growth of microalgal cultures. In the 1990s, based on solid photosynthesis background, we've pioneered the use of chlorophyll fluorescence – non-invasive, fast and sensitive technique – to monitor changes of photosynthesis and physiological status of microalgal mass cultures with the aim to estimate growth rate and productivity in large-scale microalgal biotechnology. Chl fluorescence can be used for (i) screening of microalgal strains, (ii) adjustment of culture conditions (irradiance, temperature, nutrient availability, biomass density, etc.) and (iii) optimization of cultivation regimes of microalgal mass cultures in various cultivation units.

L65

Applied Photosynthesis: Putting PSI to Work

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Oxygenic photosynthesis is driven via sequential action of PSII and PSI reaction centers via the Z-scheme. Both of these pigment-membrane protein complexes are found in cyanobacteria, algae, and plants. Unlike PSII, PSI is remarkably stable and does not undergo limiting photo-damage. This stability, as well as other fundamental structural differences, makes PSI the most attractive reaction centers for applied photosynthetic applications. These applied applications exploit the efficient light harvesting and high quantum yield of PSI. Yet in these devices the PSI particles are redeployed to provide electrons either directly as a photocurrent or, via a catalyst to yield H_2. In photocurrent producing devices, PSI has been immobilized onto various electrode substrates with a continuously evolving toolkit of strategies and novel reagents. It has already been shown that the photosynthetic complexes

can be integrated into devices that have been shown to function for many months. We will advance applied photosynthesis by extracting the most efficient components of natural photosynthesis and integrate them into novel devices using synthetic biology to create a new paradigm of bioenergy. We have designed novel coupling chemistries for biomolecular complexes that can yield hydrogen and yield photocurrrents. Our ability to "grow once and use many" is fundamentally different to other biomass based energy solutions, which are all single harvest based processes. As an environmentally benign and renewable resource, PSI may provide a new sustainable source of bioenergy. Although this technology is still emerging, the ability to couple the current advanced algal/cyanobacterial bioreactors with the rapidly evolving areas of biotechnology and nanotechnology suggest that one day we may in fact be able to grow green electricity or environmentally benign fuels.

L66

Harvesting microalgae with novel agents using non-covalent interactions

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Microalgal biomass represents an attractive biotechnological commodity, nevertheless high production costs can slow down the applications of variable large-scale cultivations. Downstream processing encompasses up to 30 % of those costs and so cost-effective alternatives to conventional cell-separation methods are sought. The presented work focuses on testing two novel harvesting agents, i.e. modified yeast cell walls (MYCWs) and iron oxide magnetic microparticles (IOMMs). Both agents were chosen as they can be easily produced at low cost. In order to estimate the conditions (pH, presence of specific ions) favourable for harvesting, the surface charge (zeta potential) of microalgae (*Chlorella vulgaris*) and agents were measured in various environments. The obtained results revealed a negative surface charge of *C. vulgaris* and positive surface charge of MYCWs independent on the tested conditions, whereas the surface charges of IOMMs were dependent upon the conditions. Subsequent harvesting experiments were designed based on the surface properties of interacting particles and resulted under model conditions in separation efficiencies of > 90 % at a ratio of MYCWs to microalgal biomass equal to 0.03 (g/g) at pH 7 and pH 10. In the case of the IOMMs the same efficiencies were obtained only for a mass ratio of IOMMs to microalgal biomass equal to 0.4:1 (g/g) at pH 4. Furthermore, same experiments were performed under culture conditions (various media compositions, pH 6.8) and separation efficiencies of > 90 % were achieved in all situations for MYCWs at a mass ratio of MYCWs to microalgal biomass in the range of 0.01 to 0.04 (g/g). In the case of the IOMMs, the same efficiencies were obtained only for a mass ratio of IOMMs to microalgal biomass equal to 3.0:1 (g/g)

upon omitting phosphorous from the medium. Thus, although both agents were successful in harvesting microalgae, MYCWs are more effective compared to IOMMs.

L67

Controlled synthesis of energy reserves in green algae; from cell cycle regulation to production

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Increased interest has been focused on microalgae not only due to their high growth rate and high photosynthetic efficiency, but particularly due to the possibility of controlling their metabolism to produce relatively high content of energy-rich compounds, either starch [1] and/or lipids [2]. Under unfavourable environmental or stress conditions, many algae either accumulate starch or alter their lipid biosynthetic pathways towards the synthesis and accumulation of neutral lipids [2,4] mainly in the form of triacylglycerols that are typically stored as cytoplasmic lipid bodies. Interestingly, the algal strains appropriate for overproduction of starch are not usually suitable for overproduction of lipids and vice versa [1-4]. Based on our experience in the cell cycle study both starch and lipids are readily consumed by the cell cycle processes. The amount of overproduced lipids or starch was dependent on light intensity and the cell cycle phase, e. g. they were used as the source of carbon and energy for the cell division. Therefore to produce starch- or lipid-enriched biomass it is crucial not only to support starch or lipids synthesis but also to suppress all cell division events. The cell division can be blocked by various treatments from the application of inhibitors to nutrient limitation. For the overproduction of starch can be used both application of cycloheximide inhibition and sulfur limitation. In the case of controlled overproduction of lipids the most effective treatment was limitation with any macroelement (nitrogen, sulfur, or phosphorus); nitrogen limitation was the most effective. Moreover, diluted nutrient media (5- or 10-fold) were identified as the best method to stimulate lipid overproduction (60 % of DW). To verify the laboratory results for potential industrial use, starch and lipid production was tested in algal cultures grown in an outdoor scale-up thin layer photobioreactor.

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L68

Phenotypic spectrum and 3D/TEM analysis of *Parachlorella kessleri* mutants produced by heavy-ion irradiation

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Heavy-ion beams have higher linear energy transfer (LET) than gamma rays and X-rays; consequently, these beams are used as mutagenic agents in plant breeding programs. Because of its elevated LET, heavy-ion radiation at relatively low doses induces mutations without excrescent abnormalities or other negative phenotypic effects in plants. A major effect of heavy ion beams is pronounced induction of DNA double-strand breaks, which is not the case for gamma- or X-rays; high-LET heavy-ion beams also induce large deletions. This study demonstrates that algal breeding using a unicellular alga, *Parachlorella kessleri*, by heavy-ion mutagenesis can improve lipid yield in laboratory and outdoor experiments. The primary screening yielded some mutants among which a secondary screening yielded several strains, which were subjected to phenotypic assays including 3D/TEM. This is 3D reconstruction of transmission electron microscopy (TEM) based on over hundred serial sections per cell to visualize the dynamics of starch and lipid accumulation and subcellular changes in microalgae [1]. *P. kessleri* strains produced by heavy-ion irradiation spanned a broad spectrum of phenotypes that differed in lipid content and fatty acid profiles. Starch grain morphology was distinctively altered in one of the mutants. The growth of strain PK4 was comparable to that of the wild type under stress-free culture conditions, and the mutant also produced large quantities of lipids, a combination of traits that may be of commercial interest. Thus, heavy-ion irradiation is an effective mutagenic agent for microalgae and may have potential in the production of strains with gain-of-function phenotypes [2].

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L69

The open thin-layer photobioreactor at the Zürich University of Applied Sciences: experiences after a year of continuous operation

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Research on phototrophic cultivation of microalgae has witnessed a sharp rise in the last decade. In Switzerland, this research has typically been performed based on laboratory experiments. Larger-sized photobioreactors were lacking, which hindered the execution of more R&D-oriented projects. The Zurich University of Applied Sciences ZHAW has therefore decided to acquire a photobioreactor that allows experimentation on a pilot-scale. This was achieved within the framework of the EUREKA project 'Alganol' and in cooperation with the Laboratory of Algal Cell Cycles (Institute of Microbiology, ASCR) and the Company BCS Engineering in Brno. The reactor, which is in operation since spring 2013 at the Institute of Natural Resource Sciences of the ZHAW, is an open thin-layer photobioreactor, equipped with various sensors and a PLC control system, which together allow a tight control of the cultivation process and a fine-grained, detailed collection of data. A year later, the reactor has already a firm place in the curriculum where it is used for teaching in different classes as well as for bachelor and masters theses. It has also sparked several research projects, which span from metagenomics to feed production. Due to its uniqueness in the Swiss research landscape, the reactor also allowed the ZHAW to join the 'biomass' group of the Swiss Competence Centers of Energy Research SCCER, which have been funded by the Swiss government to promote energy research over the coming years.

L70

Bioremediation of contaminated agricultural land by combined effect of agriculture, biogas and microalgae production

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Diverse natural phenomena (earthquakes, typhoons) as well as human activities (nuclear power plant accidents) occurring locally or on a larger area may lead to a

temporary degradation of agricultural land. Although agricultural production is sustainable the products may be unacceptable to the market. Ultimately, it may cause restrictions or even stop of agricultural activities in the damaged area consequently leading to various negative effects such as increasing unemployment and drop in the life quality. Alternative use of agricultural products represents a solution for such a region. The unacceptable agricultural product can be subjected to microbial degradation in oxygen free environment for the production of biogas, which is then used to generate electricity and heat in a cogeneration unit. Flue gas (containing carbon dioxide) exhausted by the cogeneration unit will serve as a carbon source for the growth of algae. Algal biomass will be comprehensively utilized to produce bioethanol, biodiesel, biogas and/or used as feed. Post-fermentation residues (digestate) containing mineral nutrients will be used as organic fertilizer for both the damaged soil and algal cultivation. Thus a closed energy independent cycle is formed; the commercial product of such cycle is rather energy (electricity, bioethanol, biodiesel, heat) than agricultural crops. The key advantage of the proposed solution is that the necessary technological elements of the model are established, validated and can be used immediately.

L71

Toward the sustainable cultivation of microalgae to produce renewable biofuels and fine chemicals

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Microalgae are considered as renewable feedstock for the production of next-generation biofuels. However to achieve the financial sustainability to the algal biofuel production, it will be necessary to integrate it with the processing of high-value products in the biorefinery concept. Carotenoids have been proposed as added-value compounds that could contribute to make microalgal biofuel production economically feasible. Therefore, the viability and sustainability of extracting carotenoids before the hydrothermal treatment of the remaining biomass to produce syngas were investigated. On the other hand, harvesting microalgae cells remains a technical challenge and typically contributes to 20-30 % of biomass production costs and represents more than 50 % of the total cost of algal biofuels. The potential of co-culture of filamentous fungal species with microalgae in a lichenization process as a strategy to reduce the cost and energy consumption of harvesting and of the whole process seems promising and was thus investigated. In submerged cultures, filamentous micro-

organisms actually aggregate and grow as loosely packed pellets or compact granules. Microalgae cells were immobilized in these pellets and easily removed as an aggregate with the fungal cells. Pellet formation is strain specific and highly dependent on operational conditions during cultivation. This study especially was focused on lichen pellet formation during the co-culture of *Chlorella sorokiniana* and of an unidentified filamentous fungus, which was eventually characterized. While algae growth was optimal between pH 6 and 10, the highest pellet formation was observed in the pH range of 4-7, thus requiring a strict control of pH during the whole cultivation. The effect of such a co-cultivation on the production of added-value chemicals (carotenoids) and on the biofuel potential of the remaining biomass is under evaluation, and will be compared to results obtained with cultivation of microalgae only.

L72

The importance of basic genetic experiments for improvement of microalgal biotechnology

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Microalgal biotechnology is traditionally performed with wild isolates of different algal species. Thus a new task for biotechnology often involves an isolation, characterization and optimization of a new strain or species of algae. In agriculture, breeding have been successfully used for centuries to improve traits of well established variants of organisms. Since the rules governing classical genetics were discovered they have been used extensively in breeding. Although many biotechnologically relevant algae do not posses sexual development required for breeding/crossing they still can be modified by mutagenesis. Whereupon the resultant mutants are not considered GMO and their cultivation is therefore not limited by legislation. The approach can be used to manipulate any algal strain in order to isolate mutant cells with desired phenotype. Here, we used classical genetics approach of mutagenesis by ethylmethanesulphonate (EMS) to produce mutants with desired properties of haploid unicellular green alga *Chlamydomonas reinhardtii*. After screening approximately 20 000 mutants we isolated eight stable temperature sensitive mutants able to grow and divide at permissive (24 °C) and unable to do so at restrictive (36 °C) temperature. The mutants show interesting growth phenotypes and different gene expression. While primarily isolated for basic research the mutants have potential as stocks for biotechnology. I will discuss the importance of basic research in developing new well characterized biotechnologically interesting strains.

This work was supported by AS CR (M200201205) and by project Algain.

Posters

P001

Biodiesel Production by *Chlorella sp* strain isolated from Djurdjura national park (North Algeria)

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Continued use of petroleum sourced fuels is now widely recognized as unsustainable because of depleting supplies and the contribution of these fuels to the accumulation of carbon dioxide in the environment [1]. Biodiesel is defined as a fuel comprised of mono-alkyl esters of long-chain fatty acids from mostly plant oils, as a clean and renewable alternative to the traditional fossil fuel, has attracted more and more attention in recent years [2]. Nowadays, there has been an increasing interest in looking for new oil feedstock for biodiesel production [3].Among them, microalgae have the highest oil or lipid yield among various plant oils, and the lipid content of some microalgae has up to 80 % and the compositions of microalgal oils are mainly triglyceride that can be applied to form biodiesel through transesterification [4].

The present work is a contribution to the fatty acid analysis of *Chlorella sp.* (green microalgae), strain isolated in a pond at the National Park Djurdjuran (northern Algeria), for use in the production of biodiesel.

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P002

Determination of Cr (III) in Environmental Samples by Flame Atomic Absorption Spectrometry after Preconcentration with PPyCl Polymer

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The application of conducting polymers, such as Polyaniline (PAN), Polypyrrole (PPy), Poly o Phenylenediamine (POPD), Poly (aminophenol) (PAP) have recently been paid a lot of attention for its application in biochemistry and biotechnology [1]. Polypyrrole is one of the most extensively used conducting polymers. Polypyrrole is obtained by chemical or electrochemical oxidation of pyrrole monomer. During chemical polymerization of pyrrole, physical, chemical or electrical properties of polypyrrole can be altered by doping polypyrrole with various dopants or agents [2].

The aim of this study, determination of chromium (III) in some natural and mineral water and domestic and industrial waste water samples are carried out by determination of Cr (III) using PPyCl as solid phase. PPyCl was synthesized from pyrrole by chemical oxidation–polymerization method, with Fe(III) as oxidant [2]. Polymerization was carried out in aqueous solution. Polypyyrol-chloride packed cartridges were prepared by an empty SPE tube (6 mL). Polypyrrole-chloride was studied as sorbent for preconcentration of Cr (III) using solid-phase extraction prior to determination by flame atomic absorption spectrometry. The preconcentration procedure is based on retention of the chelates on PPyCl resin column after formation of chelate between analyte ion and pyrocatechol violet. The analyte retained were eluted from the resin by using 2 M HCl. The influences of the analytical parameters including amounts of reagents, pH of the solutions and type of eluent and sample volume were also investigated. The recoveries were generally found in the range of 90-100 % with <6.6 %, RSD.

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P003

Biodegradation of Reactive Dyes by Free and Immobilize *Coprinus plicatilis*

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Wastewater from the textile industry is one of the most problematic to treat due to its color, high chemical oxygen demand (COD), biochemical oxygen demand (BOD), suspended solids, turbidity and toxic compounds [1]. Dye color interferes with penetration of sunlight into waters, retards photosynthesis, inhibits the growth of aquatic biota and affects gas solubility in water bodies [2]. White-rot fungi have

been considered by far the most efficient dye decolorizing microorganisms [3]. In this study, four different reactive dyes (Reactive red 123, Everzol Red 3BS, Remazol violet 5R, Golden Yellow RNL) were investigated decolorizing abilities by *Coprinus plicatilis*. Reactive red 123 was selected as the best degration. Reactive red 123 was completely degraded by free *Coprinus plicatilis*. The white rot fungus *Coprinus plicatilis* was immobilized onto several carrier matrices: amberlite XAD7, Ca- alginate, sand, kaolin and gelatin. Then immobilized cells were used for degradation of Reactive red 123. Ca-alginate was selected as the best immobilization matrice. The maximum dye decolorization rate was calculated as 98 % (dye concentration; 10.0 mg/L). At the end of biodegradation process, the metabolites of the dye were analyzed via FT-IR. It was concluded that the decolorization of dye by immobilize and free *Corinus plicatilis* was achieved.

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P004

Solid Phase Derivative Extraction of Some Chlorophenols in Water Samples

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The harmful effects of aromatic phenolic compounds in different enviroments have been investigated. Chlorophenols (CPs), which are frequently found in environmental water and soil samples, are classified as toxic and potentially carcinogenic pollutants, [1]. The main sources of CPs are effluent discharge of plastic, dye, leather, textile, paper, pesticide industries, and chlorination process [2]. CPs determination at trace level is substantially important, because of their naturally low concentrations in environmental water samples. Therefore, for current study prior to GC-MS determination, preconcentrations of 2,4-dichlorophenol, 2,6-dichlorophenol, 2,4,6-trichlorophenol, 2,3,4,6-tetrachlorophenol, pentachlorophenol were performed with solid phase derivative extraction. The CPs adsorbed on resin as phenolate ions were derivatizated with methyl chloroformate, then the derivatizated phenols were eluated with hexan and determined by GC-MS. Effects of several factors including, concentration of NaOH, type and volume of derivatizing re-

agent, reaction time, sample volume and amount of resin have been evaluated.

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P005-S

Fungal β-rutinosidases: Structure-function study

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Fungal β-rutinosidases (α-L-rhamnosyl-β-D-glucosidases) are specific glycosidases, which cleave the disaccharide rutinose from rutin [1]. Very little information on these enzymes is available: neither primary nor tertiary structures are known. On the other hand, rutinosidases have high biotechnological potential in the drink- processing industry.

Two rutin-hydrolyzing enzymes from *Penicillium chrysogenum* and one from *Mucor circineloides* were isolated. After trypsin treatment, several partial peptide sequences were obtained by mass spectrometry. Based on these sequences, degenerate primers were designed and used for the PCR-based amplification of the gene fragments with genomic DNA as the template. The PCR products were sequenced and a BLASTX alignment analysis of the *Mucor*-derived sequence revealed a 98 % identity to a putative exo-glucanase from *Penicillium oxalicum*, whose nucleotide sequence is not disclosed. To obtain the upstream and downstream coding sequences, we performed 5' and 3' RACE experiments (Rapid Amplification of cDNA Ends). Both *Penicillium* sequences had 100 % identity with the putative exo-glucanase from *P. chrysogenum* Wisconsin 54-1255 (entire genome sequence). Based on these BLAST and RACE results, we designed specific primers and amplified the full-length genes.

Currently, we are testing *Pichia pastoris* for the functional expression of our fungal enzymes.

Acknowledgement: Czech Science Foundation P207/11/0629 and 7AMB13AR005 grants are acknowledged.

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P006-S

Degradation of different forms of PHA materials by thermophilic microorganisms

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Polyhydroxyalkanoates (PHA), which are produced from renewable carbon resources by many microorganisms, are environmentally compatible polymeric material and can be processed into films and fibers. Mechanical properties of PHAs make them suitable replacements for petrochemically produced bulk plastics (polyethylene, polypropylene etc.), but in contrast to these commodity plastics PHA are completely biodegradable to carbon dioxide and water without formation of any toxic by-product.The work was aimed at biodegradability of bacterial natural and modified polyesters and their composites. Four types of PHA materials were tested. Poly(3-hydroxybuytyrate), produced from waste frying oils by *Cupriavidus necator* H16, was tested in following forms i. PHB nanofibers formed by electro-spinning. ii. PHB processed into film by extrusion iii. PHB processed by extrusion and modified by commercially available plasticizer iv. PHB used as partial replacement of polyether polyol in polyurethane.

The biodegradation test was performed under thermophilic conditions described in norm IS/ISO 20200, all of the samples were completely degraded during three weeks of standard biodegradation test. The only exception was modified polyurethane sample, however, also in this case we observed significant degree of degradation, especially decrease of weight and elongation to break and Young modulus. Thus, PHA materials can be considered as highly biodegradable materials regardless of their form of processing and; moreover, their addition into originally none-biodegradable materials significantly increases bioavailability of the materials.

Acknowledgments: This work was supported by project „Centre for Material Research at FCH BUT“ No. CZ.1.05/2.1.00/01.0012 from ERDF.

P007-S

Evaluation of Autochthonous selected yeasts from grapes and cellar in winemaking of Aglianico vine

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Aglianico cultivar is an ancient black grape variety native of Campania region of

the Southern of Italy. For many years, wines were produced by natural fermentation carried out by autochthonous yeasts present on the grapes and in the cellar. The grapes epiphytic microflora is prevalently composed of apiculate yeasts, with a poor fermentative power and by oxidative yeasts, belonging to non-*Saccharomyces* species. On the other hand, almost all the yeast strains present in the cellar, such as "winery yeast" belong to *Saccharomyces* "sensu stricto" [1].

The aim of our work was the selection and the employment of new combinations of yeasts, obtained from the native microflora of Aglianico grapes and cellar located in the Irpinia Area, to improve the organoleptic and sensory peculiarities of the wine obtained.

The yeasts isolated from grapes and cellar, were characterized by morphological, biochemical, and technological analysis. They belong mainly to the genus *Saccharomyces spp., Hanseniaspora spp., Rhodotorula spp., Metschnikowia spp.* and *Candida spp.* according to Beltran et al. 2002 [2]; between these yeasts were selected two strains (AGSW15 and FLOSW4) that shown the best winemaking performance. They were identified by the sequence analysis of the 26S rDNA D1/D2 region they results belonging to species *H. uvarum* and *S.cerevisiae*. They were used in semi-industrial-scale fermentations to confirm if they could be good candidates as autochthonous fermentation starters.

The fermentation carried out with the new starter, showed positive differences compared to commercial yeasts: in fact the analysis of the produced wine demonstrated that they were efficient in completing fermentation and could positively affect the wine quality. In order to evaluate the aromatic profiles the obtained wine samples were characterized by SPME-GC/MS technique [3].

The results shown that the yeasts selected successfully dominated the fermentation process and contributed to increasing the organoleptic quality preserving the peculiarities of this typical regional wine.

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P008

Does the RED mean DEAD?

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Fluorescence determination of microbial cell viability became a routine method

for detecting influence of a variety of antimicrobial agents, stress conditions and environmental impact to different cell communities. The most commonly used dead indicator is undoubtedly propidium iodide (PI) that stains cells red based on the disintegrity of cytoplasmatic membrane and/or their inability to exclude this positively charged dye. The red stained cells are generally considered as dead whereas nonstained or counterstained with another dye as alive. Employing flow cytometry (FC) enables high throughput analysis of thousands cells within seconds with high reproducibility. We have tested method robustness for both yeast and bacterial representatives, namely: *Saccharomyces cerevisiae, Pichia pastoris, Clostridium pasteurianum* and *Thermus aquaticus*. The number of stained cells was constant within the concentration range 10-50 μg/ml and incubation interval 5-30 minutes with the relative standard deviation (RSD) 0.40 % for *S. cerevisiae*, 0.75 % for *P. pastoris*, 3.63 % for *C. pasteurianum* and 1.52 % for *T. aquaticus.*

Although it is believed that the red cells are definitely dead and cannot recover, we have found at least two stages of different staining pattern in bacterial populations. First, the absolute one corresponding to the intensity of fixed cells and the second, cells with lower red fluorescence intensity that mostly exhibit also the ability to be stained with vital indicators e.g. carboxyfluorescein diacetate. This was not observed for yeast cells. Stationary culture of *T. aquaticus* consisting of 99.8 % red cells with different red fluorescence intensity revealed no reproduction during plate count tests (zero CFU) whereas inoculation of liquid media resulted in a cell proliferation and growth.

Comparing to the traditional plate count method PI together with FC represent fast and precise tool for viability determination and simultaneously enable deeper insight into the cell vitality, but still they do not give the definitive answer whether the cells are dead or not.

P009-S

Production of a heteromeric amino acid transporter with *Pichia pastoris*

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Most therapeutic targets are membrane proteins. However, the amounts required for functional studies are not commercially available or readily synthesised in the laboratory. For rational drug design, the structure of the target is crucial, and pharma companies are therefore interested in obtaining human proteins for structural and functional analyses (i.e. several hundred milligrams of protein which are necessary for crystallography).

The heteromeric amino acid transporter (HAT) is a membrane protein [1] which is involved in several pathologies and whose

structure is not fully elucidated, comprised of heavy (4F2hc) and light (LAT2) subunits that are linked by a conserved disulfide bridge. The production of HAT was studied in batch and fedbatch cultures of the recombinant yeast *Pichia pastoris* (KM71H), and critical factors affecting the heterodimerisation of HAT were analysed.

Shake flask cultivation has typically led to aggregates (i.e. dimerisation of single subunits) and non-specific product variants. During fedbatch cultivations in bioreactors under well-controlled process conditions, such phenomena were not observed. Maximum heterodimer formation in fedbatch processes, (as detected with α-4F2 and α-StrepTagII antibodies), was established by temperature reduction (from 30°C to 25°C) during production or by cultivation at a high cell density, and maintaining a residual methanol concentration between 1.5 and 7.0 $g.l^{-1}$.

Undesirable product variants, such as single subunits, occurred due to molecular strain construction, and as a result of process control. Nevertheless, further experiments are necessary to elucidate the influence of different cultivation conditions on heterodimerisation of HATs.

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P010-S

Comparison of enzyme immobilization methods to enhance cellulolytic digestion of biomass in bioethanol production

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In recent years, petrol crisis and subsequent environmental problems focused attention of society on renewable energy sources used in transportation. It is necessary to find out a new economic way for production of 2^{nd} generation of biofuels and reduction of cost of existing technologies. Immobilization of cellulolytic enzymes enables long term recycling of biocatalysts in production of cellulosic ethanol. Paramagnetic iron particles (0.5-1.0 μm) coated with different carriers (SiMAG-Cyanuric and SiMAG-Carboxyl), polyacrylamide gel and sodium alginate beads have been tested as carriers of cellulolytic enzymes to enable their recovery in the process of bioethanol production. The cellulolytic activity of free and immobilized enzyme was tested using filter Paper N°1 and AVICEL as standard substrates. The binding yield of paramagnetic microparticles was improved from 42.2 % to 82.3 % (1,5 mg of proteins on 50 μg of par-

ticles) compared to initialworking condition. Unfortunatelly, immobilization affected the activity of enzyme, the amount of glucose released form cellulose was 62 % lower compared with the free enzyme. In the other set of experiments, enzyme was treated in two different ways: entrapped into the mesh of the polymer and attached over the external surface of the supports already polymerized. The best results (maximum conversion of cellulose into glucose) were achieved at 55°C at pH 5 with the enzyme bounded on the surface of alginate beads (3,37 mg/0,5 mL of glucose released from a initial concentration of 10 g/L of AVICEL) and the enzyme could be used for four cycles before it was damaged.

P011

Lignocellulosic ethanol production from chicken manure using *Saccharomyces cerevisiae* TISTR 5048 via simultaneous saccharification and fermentation (SSF)

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Chicken manure is one of the abundant waste materials in livestock industry. This mostly contain residual chicken feed such as corn, soybean and rice bran which is lignocellulosic materials. The management for chicken manure can bring to produce biogas and fertilizer [1-3]. Therefore, the chicken manure could be used to the raw materials for some value added product in this industry and also reduce global warming. The aim of this research was to study lignocellulosic ethanol production from chicken manure using *Saccharomyces cerevisiae* TISTR 5048 via SSF. The main components in chicken manure were monitored by a differential/thermal gravimetric analyzer (TG/DTA). Lignin in chicken manure was separated by modified from TAPPI T203 method. The hemicellulose was then pretreated to produce total reducing sugars with 1.5 % (v/v) sulfuric acid using an autoclave at 121°C, 15 psi for 90 min and follow by enzymatic hydrolysis process. Total reducing sugars were determined with dinitrosalicylic (DNS) acid method [4]. The compositions of reducing sugars was identified by silylation method by a GC-FID [5]. The hydrolyzed were fermented using *S. cerevisiae* TISTR 5048 at 40°C on a rotary shaker for 72 h to produce lignocellulosic ethanol and determined by GC-FID. The results showed that the main components in chicken manure consist of moisture content, cellulose, hemicelluloses, lignin and rock. The percentage of each components were 7.21, 24.67, 23.74, 13.81 and 10.64, respectively. The maximum of total reducing sugars in the hydrolyzed was 0.28 g/g substrates (cellulose). Identification of reducing sugars by a GC-FID was found that glucose and xylose are major components. Finally, the average ethanol yield obtained was 0.10 g/g of glucose with a theoretical ethanol yield to be

78.63 %.

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P012

Biosynthesis of Nylon precursor Dodecanedioic acid in *Yarrowia lipolytica*

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Aliphatic α,ω-dicarboxylic acids (DCA) of the type addressed by this program are used in a wide variety of plastics and other chemical applications. The DCA12 produced in the largest quantity (>40 MM lb/yr) as a pure chemical intermediate is dodecanedioic acid (C_{12}); it is used in polyamides such as nylon 6,12, which is noted for high moisture resistance. The DCA produced in the largest quantity (>40 MM lb/yr) as a pure chemical intermediate is dodecanedioic acid (C_{12}); it is used in polyamides such as nylon 6,12. The dodecanedioic process is based on non-renewable petrochemical feedstocks. The multi-step conversion process produces unwanted by-products such as cyclooctadiene and vinyl cyclohexene, which result in yield losses. The nitric acid oxidation step yields NOx, which is either released to the atmosphere or must be destroyed in a reduction furnace. Biotechnology offers an innovative way to overcome the limitations and disadvantages of the chemical processes to make diacids. *Yarrowia* biocatalyst are able to convert long-chain fatty acids directly to long-chain diacids. In this research, the results demonstrated that the pox2 deletion strain, enhance fatty acid production about 20 wt % which overexpress the ω-oxidation pathway genes can enhance dodecanedioic acids production. In addition, In order to enhance DCA_{12} content, the *Yarrowia*-codon acyl-carrier protein thioesterase gene, BTE from *Umbellularia californica*, FatB3 from *Cocos nucifera* were also expressed. The results demonstrated that expressed BTE and FatB3 can enhance DCA_{12} content from 12.9 % to 51.2 %. Finally, the RNA inference technology can also enhance DCA_{12} production from 1.23 g/L to 2.35 g/Li n this research.

P013

Cyclizing 5-Aminolevulinate Synthases in the Biosynthesis of Actinomycete Secondary Metabolites: Outcomes for genetic screening techniques

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The aim of this project was to retrieve new putative producers of attractive secondary metabolites from nature. Genetic screening was based on the recent knowledge [1], indicating that certain actinomycetes possess 2 biosynthetic pathways for 5-aminolevulinate (ALA) synthesis (C4 and C5), and use the first one strictly for biosynthesis of secondary metabolites harbouring ALA-derived moiety (the C_5N unit). Among the metabolites, linear polyketides (manumycins, ECO-02301), glycolipids (moenomycins), macrolides (bafilomycin A1, virustomycin A), polyenes (annimycin), and others (reductiomycin) were recognized. These metabolites show pharmaceutically attractive biological activities: cancerostatic, immunomodulatory, anti-protozoal, and anti-mycotic. We screened altogether 1500 natural strains, originating from soils and sediments of some unique habitats deposited in the Culture Collection of Soil Actinomycetes in České Budějovice (http://www.actinomycetes.cz) [2]. The occurrence of the cALAS gene, coding for unique ALA synthase responsible for formation of the C_5N unit, was determined. At first, the genetic screening protocol based on Southern blot hybridization with a cALAS specific probe, revealed in retrieval of 98 putative producers out of 700 screened. Analyses of the relevant sequence data from our screening project together with the growing data from actinomycete genome-sequencing projects enabled us to design a simpler PCR screening method, which we used further on. Amplifying 519 bp fragment of cALAS genes from the set of degenerative primers designed to target conservative regions, we obtained another set of putative producers (107 strains of 800 screened). Based on relatedness of 16S rRNA sequences, new producers represent diverse group of *Streptomyces* (40 species, separated in 14 clades) and related genera, indicating that biosynthetic gene operon may be spread horizontally. Phylogeny of cALAS gene and the information on characterized biosynthetic gene clusters encoding formation of C_5N-carrying secondary metabolites gave us valuable hints on the evolution of the novel cyclization function of cALAS. The study was supported by the project MYES-LH12191.

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P014

Genome sequence and annotation of Streptomyces sp. W6 – putative natural producer of annimycin antibiotic

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Streptomycetes represent important source of promising natural metabolites used in i healthcare. Here we present a draft genome sequence of the *Streptomyces sp.* strain W6. W6 strain was isolated from 6 years old recultivated site after black coal mining covered by grass and herbs (Wyoming, USA) by using standard microbiological techniques. The strain W6 was cultivated in M2, Mueller-Hilton, and GYM media. The strain is deposited in the Culture Collection of Soil Actinomycetes in České Budějovice (http://www.actinomycetes.cz) [1]. The strain W6 showed antibiotic activity against *Bacilllus subtilis* subsp. *subtilis* CCM 2217, therefore it was screened for presence of biosynthetic gene cluster for secondary metabolites harbouring 5-aminolevulinate -derived moiety, the C_5N unit [2]. This genetic screening was focused on retrieval of new putative producers of secondary metabolites with anti-inflammatory activities, which are highly promising for pharmaceutical purposes. Genetic screening resulted in the presence of genes involved in synthesis and cyclization of C_5N unit. Pilot sequence prospecting indicated the synthesis of moenomycin-type of metabolite. According to 16S rRNA sequence similarity, strain W6 is related to *Streptomyces sioyaensis*. The whole genome sequencing was performed via Roche 454 GS Junior sequencer and reads were assembled using Newbler software . Resulting contigs were screened for the presence of biosynthetic gene clusters. Most importantly, the W6 strain encodes for annimycin gene cluster similar to the recently identified region in *Streptomyces calvus*.

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P015-S

Polyphenol esterase in commercial enzyme preparations and their use in regioselective acylation of glycosides

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Enzymes, due to their abilities like high selectivity (chemo-, regio-, stereo-) and catalysis in mild reaction conditions, are the useful tool in modification of structurally complicated carbohydrates. Reduced costs and increasing availability of a wide scale of enzyme preparations allow researchers to search for new catalytic activities and their application in the preparative scale.

In our work we focused on search for polyphenol esterase activities – caffeoyl esterase and tannase. For such purpose, two synthetic chromogenic substrates, namely 4-nitrophenyl caffeate and 4-nitrophenyl gallate were prepared and were used to screen the hydrolase activity in selected commercial enzyme preparations intended primarily for the food industry and for laundry purposes. Such preparations include mostly liquid or water-soluble lipases, proteases, esterases and raw glycanases. The highest caffeoyl esterase activity was found in PDN N1/11 (Biocatalysts) and Ultraflo L (Novozymes) and the highest tannase activity was observed for Novozym 735 (Novozymes), Viscozyme L (Novozymes) and Peclyve LVG (Lyven). Moreover, commercial immobilized enzymes were tested in the regioselective acylation of methyl-β-D-glucopyranoside using the activated vinyl esters of caffeic acid and gallic as donors. Among the tested enzyme preparations, Lipex 100 T (Novozymes) and Lipozyme TL IM (Novozymes) were effective in the studied reaction.

Acknowledgment:
This work was supported by the Slovak Research and Development Agency under the contract No. APVV-846-12 and by the Slovak Grant Agency for Science VEGA under the project No. 2/0138/12.

P016-S

Ergosterol, a product of *Saccharomyces cerevisiae*, stimulates plant defence reaction

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Ergosterol is a main sterol of higher fungi that plays an essential role in membrane stabilization. Being a precursor of D2 vitamin, ergosterol is also an economically important metabolite. It is commercially produced by two-stage fed-batch fermentation using *Saccharomyces cerevisiae*. Here we show that ergosterol is perceived by tobacco (*Nicotiana tabacum*) as a molecule originating from a pathogen. Ergosterol is shown to trigger defence responses, including accumulation of reactive oxygen species, pathogenesis-related proteins or phytoalexins, in tobacco, without influencing plant cell viability. Ergosterol produced by *S. cerevisiae* could be therefore used in agriculture to stimulate plant resistance against pathogens.

This work was supported by the Grant agency of the Czech Republic (P501/12/0590) and by the Masaryk University Program, Project No MUNI/A/0981/2013.

P017

Level of heavy metals monitoring in street dust of Denizli, Turkey

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Traces of heavy metal ions play important roles in human health. While some trace elements including copper, selenium, zinc are necessary for life, some of them like arsenic, and lead are hazardous for life [1]. The determination of the levels of trace heavy metals in the various environmental samples including natural waters, geological and biological samples, dusts, soils, sediments are continuously performed by researchers, in order to monitor heavy metal pollution in the environment.

Street dusts are environmental materials for monitoring the levels of heavy metal ions [2]. Two main factors known to influence the levels of trace element in dust have been reported as traffic and industry. Monitoring the trace heavy metal contents of dust samples is an efficient way of obtaining information on the actual environmental state of large areas and of surveying its development.

According to our literature review, no studies have been reported on the heavy metal levels in dust samples from Denizli city center. In the present work, the monitoring of toxic heavy metal such as Mn, Cu,

Pb, Ni, Cr and Cd in the streets dust, trafic (heavy, moderete and normal), car parks, school-garden, health and hospital centers in Denizli, Turkey by flame atomic absorption spectrometry (FAAS) after digestion with aqua regia. The average concentration of Mn, Cu, Pb, Ni, Cr and Cd in all samples were found to be 158.0, 147.0, 145.0, 86.2, 75.0 and $<0.1\ \mu$ g g^{1-}, respectively. A good correlation was found between the metal concentrations.

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P018

Effects of red and far-red light on biomass productivity of green microalgae *Chlorella sorokiniana* growing in photobioreactor

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The need to develop and improve sustainable energy resources is of eminent importance due to the finite nature of our fossil fuels [1]. Algae are among the most promising non-food-crop-based biomass feedstocks [2]. Oil producing algae are alternative biofuel feedstock with potential to meet the world's ambitious goal to replace fossil fuels [3]. However, further researches are to be done in order to optimize parameters governing growth and to develop large scale cultivation systems. Light energy is the most important environmental factor that has a positive influence on increasing biomass productivity. But, applying light for long hours yield to Oxidative stress. The aim of this contribution was to study the effect of light quality on biomass production of microalgae *Chlorella sorokiniana* cultivated in tubular photobioreactor. The study shows that biomass production is significantly enhanced when the culture was growth under short photoperiod and received a 1 hour far-red light illumination at the beginning of the dark period. The experimental evidences have established the relationship between the phytochrome system and the control of growth in *C. Sorokiniana.*

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P019

Determination of Anticancer and Modulation Effects of *Fomes fomentarius* (L.) Fr. and *Tricholoma anatolicum* H.H.Dogan & Intini On Breast Cancer Cells

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In recent years the tendency of various natural resources to prevent cancer as well as medical treatments are increasing. One of the sources which are used is fungi. The aim is stopping the growth of cells or preventing resistance developing by also supporting to breast cancer, which is increasing day by day, treatment.

In this study, after investigation of the cytotoxic effects of extracts from *Fomes fomentarius* and *Tricholoma anatolicum* fungi species on the paclitaxel and vincristine resistant MCF-7 cell lines, drug resistance reversing ability of the extracts were determined. Extracts of fungi species were extracted separately by ultrasonication in water, methanol and ethanol. The effects of extracts on paclitaxel and vincristine resistant cells were determined by cytotoxicity tests XTT. Modulation effects of extracts at MCF-7 cells were determined with fluorescence measurements (flow cytometry). Furthermore phenolic compounds were determined by HPLC method. As a result of the cytotoxicity assay of extracts, IC_{50} values for MCF-7/Vinc were between 1.08 and 1.80 mg/mL, IC_{50} values for MCF-7/Pac were found between 1.11 and 2.83 mg/mL. It has been determined that, the *F. fomentarius* methanol and *T. anatolicum* ethanol extracts has the potential effect to become MDR modulator (the agent reversing resistance) for both resistant cells.

The obtained results show that extracts, obtained from mushrooms of our country with an original method, are important studies in terms of benefiting from natural sources in the breast cancer treatment. The findings also provide development of new protocols in courses of chemotherapy in the clinical area and being implemented of new methods in order to prevent drug resistance.

P020-S

Bioactive metabolite of 3,5-dimethyl-1,4-benzoquinone

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3,5-dimethyl-1,4-benzoquinone (DMBQ) is one of the naturally occurring quinones that is found especially in insects as a defensive compound. In the present study, DMBQ was biotransformed by *Corynespora cassicola* micro-fungus. The transformation metabolite was initially screened by TLC and GC/MS, then further characterized by NMR (^{14}C and ^{1}H NMR) spectroscopic techniques. The metabolite identified as '3,5-dimethyl 1,4-benzendiol'. Also both of the substrate and metabolite were evaluated for their *in vitro* anticandidal and antioxidant properties. The results were compared with the positive controls.

P021-S

The use of animal waste materials for production of biologically active substances

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Animal waste materials – by-products of the food industry, are today used either as a raw material for animal food production or as fertilizers. However, the majority is disposed of in rendering plants. Annually, the food industry in the Czech Republic produces about 5,000 tons of these wastes. The aim of this work is to find a new ways of utilization the animal by-products that remain after poultry processing – chicken breast cartilage, skeleton, tendons and feathers. These wastes may serve as an inexpensive material for the manufacture of some biologically active substances or for biomass cultivation.

To obtain chondroitin sulfate and hyaluronan from cartilage two approaches were performed: enzymatic hydrolysis with papain and combination of enzymatic and microwave hydrolysis which helped to disrupt the animal materials. The cartilage waste was also used as a carbon and nitrogen source for cultivating of fungi *Gibberella fujikuroi* and production of gibberel-

lins was tested.

The new keratin-degrading bacteria, which are capable of feather degradation, were enriched from poultry waste. The isolates were identified by MALDI-TOF MS using Biotyper software. In selected isolates the keratinolytic activity and hydrolysis of fine feathers and shaft parts was determined. Medium after cultivation was analyzed for the presence of amino acids.

The work was realized thanks to financial support of TAČR BIORAF project no. TE01020080.

P022

Bovine Parvovirus: Preparation of Antigen and Its Diagnostic Significance

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Parvovirus infection of cattle causes lesions of the digestive and respiratory system in calves, as well as violations of reproductive function in cows and heifers, which clinically manifested as fetal mortality, abortion, endometritis and ovarian hypofunction.

ELISA test system for the serological diagnosis of parvovirus infection in cattle and determination the level of postvaccinal antibodies was established. The diagnosticum is based on the strain ≪ Parvo-32459 ≫, belonging to serotype 1, which is actively circulate among livestock population in Europe, North America and Russia.

To produce the specific antigen parvovirus reproduction was performed under stationary conditions on a monolayer of primary trypsinized culture of cow embryo kidney cells at 37°C within 4-5 days. The cytopathogenic effect of parvovirus in the tissue culture characterized by granularity, rounding and cell destruction. Considering that the concentration of the virus bound to the cells is much higher than that of the extracellular virus, the cell mass, precipitated by centrifugation, was disrupted by sonication and released from cellular debris. The supernatant containing parvovirus was inactivated using 5 % solution of 1,2-aminoethyl azeridin at a final concentration of 0.1 %. Subsequent purification of the antigen was carried out by ultracentrifugation through saccharose layer. In developing the immunoenzyme test system the main parameters of the test, such as working dilution factors of antigen, control sera and conjugate, the antigen sorption conditions and exposure of sera positive-negative threshold were standardized.

The ELISA was tested at laboratory and field conditions in comparison with the methods of comparable focus, and was recognized as sensitive, specific and reproduci-

ble test. The test system is intended for the detection of antibodies to parvovirus in the cattle serum and determination the titer of antibodies in infected, recovered and vaccinated animals. Diagnostic efficiency of suggested test system in comparison with the reference physiological methods is shown in high sensitivity – 96.07 % and specificity – 93.82 %.

P023

An improved fluorescence-based method for a real-time detection and quantification of Polyhydroxyalcanoates production.

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Polyhydroxyalcanoates (PHA) are a large class of polyesters naturally formed as storage compounds by a diversity of gram-positive and gram-negative bacteria under unbalanced growth conditions. PHA are synthesized from renewable carbon sources, namely sugars or fatty acids, and accumulated in variable quantities in cytosolic lipid bodies [1]. Due to their mechanical properties similar to those of petrochemical polymers, complete biodegradability and eco-friendliness, PHA have a great potential in the bioplastics fields involving biodegradable packaging and coating materials [1,2]. As the raw materials cost and downstream processing makes PHA expensive in comparison with petroleum products, most of the actual researches are focused on cost-effective solutions to optimize PHA production yield in different wild-type or genetically modified bacteria strains.

In this context, we have developed a rapid and sensitive method to monitor the PHA biosynthesis efficiency throughout the fermentation course. This method uses the direct inclusion in the culture medium of the lipophilic fluorescent dye Nile red (0.4 % v/v), to bind with the PHA granules inside the bacteria cells. Fluorescence is detection by the combination of microscopic and spectrometric techniques allowing qualitative and quantitative evaluation of the PHA content of the culture.

This method was applied for the rapid screening of six potential PHA-accumulating bacteria isolated from a marine collection under a two-stage fermentation strategy with nitrogen limited conditions. In the same time, the first phase growth medium composed of agro-industrial by-products and the second phase medium C/N ratio yielded the highest fluorescence intensities were then investigated. Under the optimized conditions, batch cultivations in a 4L laboratory scale bioreactor gave PHA productivity up to 1.79 $g.L^{-1}$ with PHA content of 78 % of the cell dry weight (CDW). The identification of the polymer as PHA was confirmed by GS-MS and thermo-chemical analysis (DSC, TGA).

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P024

Cellooligosaccharides for research and practice

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Cellooligosaccharides, or β-1,4-linked glucooligosaccharides, are the basic building units of cellulose. As such, they often have been used as model substrates in studies concerning the catalytic properties and mechanism of action of enzymes involved in degradation of cellulose [1]. Besides that, cellobiose is used as the starting material for the production of different compounds by bioconversion [2,3] or as an alternative carbon source in specialized fermentations of probiotic bacteria [4]. The production of cellobiose by conventional methods, such as acid hydrolysis or acetolysis of cellulose have been hampered by high cost of used chemicals and negative effects to the environment. We have developed a method combining enzymatic and acid hydrolysis of cellulose in order to produce cellobiose and higher cellooligosaccharides DP 2-7 in quantities enabling their commercionalization.

For specification of our products and commercial information, please see our catalogue at
http://www.chem.sk/products/.

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P025-S

Determination of light distribution profile and microalgae cells flow pattern in column photobioreactors

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The slow development of microalgal biotechnology is mainly due to difficulties in designing large-scale photobioreactors (PBR) where light energy is efficiently utilized. Due to the light gradient inside the reactor, and depending on the mixing properties, cells are subjected to light/dark cycles where the light period is characterized by a light gradient. These light/dark cycles will constitute the cells' light history; they determine productivity and biomass yield on light energy.

In order to know the cells' light history it is necessary to analyse the flow patterns inside the PBR. These are closely related with the pattern of movements to which the cells are subjected within the PBR and thus allow establishing a correlation with light regime inside the PBR.

In this work this was achieved by a new approach, combining optical fiber technology and a very simple particle tracking methodology, applied to three column bioreactors: a bubble column and two different types of airlift.

The use of optical fiber technology, improved by our group [1], provides information about quantitative (photosynthetic photon flux density) and qualitative (spectral intensity distribution) aspects of light patterns.

For flow pattern visualization, a particle tracking system based on alginate spheres with incorporated riboflavin was used. The riboflavin-loaded alginate particle was placed in the liquid phase and illuminated at 90 degrees to the camera by two fluorescent black lights (Genesis F20T9/BLB) in order to make the riboflavin glow. Particle flow was followed by sets of images grabbed with a Canon EOS 600D photo camera.

The combination of these techniques allowed obtaining a full light characterization, a clear flow pattern image, as well as particle velocity and circulation time in three different PBRs at different values of superficial gas velocity.

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P026-S

Citric acid production by Yarrowia lipolytica under increased air pressure

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Oxygen mass transfer rate (OTR) from air to liquid phase has been a serious handicap to the productivity of several biological processes, particularly for bioprocesses based in aerobic cultures. OTR improvement can be achieved by the increase of total air pressure in bioreactors with microbial cultures, due to the increase of oxygen solubility in the medium [1].

Yarrowia lipolytica, strictly aerobic yeast, is known for the ability of producing several high value compounds such as enzymes, aroma and organic acids [2]. Citric acid is produced under limited nitrogen conditions but the production can be influenced by other conditions, like pH and oxygen availability. High levels of dissolved oxygen tension between 50 % and 80 % have been reported as required for efficient citric acid production [3].

In this work air pressure increase (up to 4 bar) was applied as a way of OTR enhancement in the production of citric acid by *Yarrowia lipolytica* W29 from crude glycerol, a byproduct from the biodiesel production. Preliminary results indicated that *Y. lipolytica* batch growth was not inhibited by pressure and the production of citric acid was slightly accelerated by the bioreactor pressurization.

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P027-S

Development of immunochemical method for the detection of new synthetic cannabinoids

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In recent years there has been rapid development of new synthetic drugs (NSD). Among these, synthetic cannabinoids belong to the fast growing substances. Cannabinoids are a class of diverse chemical

compounds that act on cannabinoid receptors on cells that repress neurotransmitter release in the brain. Efforts made to circumvent the legislation, because these substances are not on the list of banned substances. The danger lies in the lack of information about their pharmacokinetic properties, interaction with alcohol or other drugs compounds. These compounds can cause not only an overdose, but also psychotic episodes of hallucinations, aggression, impulses and homicidal tendencies. Consequently, there is growing public interest in development of versatile detection methods enabling to effectively intervene in this field.

Due to the serious health risks associated with the use of drugs, it is desirable to develop tools that allow simple and rapid analysis. The traditional methods for analysis of synthetic cannabinoids are liquid and gas chromatography. These techniques are expensive, time consuming (pre-treatment procedures), do not allow the rapid analysis of a large number of samples and usually require specialized instrumentation. Immunochemical methods, especially ELISA (Enzyme-Linked-ImmunoSorbent-Assay) represent a suitable alternative overcoming the problems mentioned above. Therefore immunochemical methods are increasingly considered as alternative methods for analysis.

The aim of this study was to develop the immunochemical method for the detection of selected synthetic cannabinoids. The competitive format of indirect ELISA was being developed using polyclonal antibodies and conjugate of synthetic cannabinoids with bovine serum albumin. ELISA was optimised and the analytical parameters were obtained from the calibration curve (values of IC50 1.2 ± 0.2 ng.ml^{-1}).

Acknowledgement: This work was supported by the Grant of Ministry of the Interior of Czech Republic (no. VG20122015075) and Specific University Research No. MSMT 20/2014.

P028

Extraction protocol for hyaluronic acid UDP precursors determination in Streptococcus zooepidemicus

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Hyaluronic acid (HA) is a linear biopolymer composed of repeating units of alternating glucuronic acid and N-acetylglucosamine molecules. HA is found in various tissues of vertebrates, where it plays various biological functions. The quality of HA is determined by its molecular weight (MW), with high MW HA having applications in the biomedical industries, while low MW HA is used in the cosmetic industry. Several studies have proven that MW of HA produced by Streptococcus zooepidemicus (SEZ) is affected by culture parameters, e.g. temperature and aeration. Although changed culture conditions affect the physicochemical environment of the HA synthase, a

more likely explanation is that MW is affected by the availability of activated sugar precursors (UDP-GlcNAc and UDP-GlcA). Therefore, further improvement in the yield and MW of HA could be attained by metabolic engineering of SEZ to balance the biosynthetic pathway of HA supplying precursor sugar molecules.

Besides analytical techniques, sampling and sample preparation are regarded as the most important steps in intracellular metabolite analysis, especially when studying encapsulated streptococci. To achieve a valuable metabolomics data, an ideal sample preparation protocol should not only stop the metabolomics activity of the system instantly (quenching), but also enable the separation of the organism from the medium prior to metabolite extraction.

In our study, we have employed several extraction protocols of which direct quenching and extraction in hot ethanol was found out to be the optimal one. This technique achieves the highest measured UDP sugars concentration of all methods compared. The concentration of UDP-glucuronic acid was thirty times higher contrary to the method using quick centrifugation. In addition to that, quick centrifugation was not applicable for engineered non-mucoid strains with low HA production. It was probably due to rapid changes in quantity of intracellular metabolites. Conclusively, our results can be further used for investigating the relationship between HA synthesis rate and MW vs. intracellular precursors concentration.

P029

Cloning and functional expression of PqqE from *Methylobacterium extorquens* AM1

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Pyrroloquinoline quinone (PQQ) is an aromatic, tricyclic o-quinone that serves as a cofactor for a number of prokaryotic dehydrogenases. It has been reported recently that PQQ is a novel metabolic modulator involved in mitochondriogenesis and mitochondrial metabolic function; it also functions as a cardioprotectant [1]. It is therefore in growing interest on the application of PQQ as the supplement for human health improvement.

Methylobacterium extorquens AM1 is an aerobic facultative methylotroph which secretes PQQ into the culture medium. Genes encoding the components of PQQ biosynthetic pathway in this bacterium are *pqqABCDE* and *pqqFG*. To elucidate the molecular mechanism of PQQ biosynthesis, we are focusing on PqqE which is believed to catalyze the first reaction of the pathway. PqqE belongs to the radical S-adenosyl-l-methionine (SAM) super-

family, in which most, if not all, enzymes are very sensitive to dissolved oxygen and are rapidly inactivated under aerobic conditions; *e.g.*, PqqE from a facultative anaerobe *Klebsiella pneumoniae* could only be expressed and purified under strictly anaerobic conditions [2]. We here report that PqqE from *M. extorquens* AM1 is markedly oxygen-insensitive; it was efficiently expressed in *Escherichia coli* Rosetta 2 (DE3) cells grown aerobically and purified under atmospheric conditions. The purified PqqE was found to be functional as it showed reductive SAM cleavage activity.

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P030-S

Continuous production of n-butanol from glycerol using *Clostridium pasteurianum* (DSM525) cells immobilized on corn stover.

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Biobutanol has attracted considerable attention in recent years, due to several advantages, as higher energy content and stability, in comparison to ethanol [1]. The research is focused on the utilization of one of the biodiesel byproducts, glycerol, for butanol production, which, together with biodiesel production, could be a successful example of biorefinery utilization of oil crops.

Corn stover was used for cell immobilization in a packed-bed bioreactor. For comparison, bioreactors with suspended culture were also run. The long term productions have been followed using flow cytometry analysis end microscopy observation. Flow cytometry (FC) analysis has been performed in combination with the fluorescent stains 5-Carboxyfluorescin diacetate (CFDA) and Propidium iodide (PI). The FC combined with fluorescent probes has been proved to be a valuable tool for vitality determination [2].

In the continuous production with suspended cultures, an oscillatory behaviour, not correlated with sporulation, has been observed. The production has been performed at different dilution rates, and obtaining a maximum yield of butanol of 8 g/l.

The continuous production with immobilized cells has been carried out at several flow rates and reached, at the steady-state, a butanol production of 10 g/l. Production at dilution rate above the maximum growth rate has been performed showing the efficiency of the immobilization and FC results showed a predominant wash out of dead cells to the waste.

Based on our results, continuous production of butanol with the cells of *C. pasteurianum* DSM 525 immobilized on corn stover is an efficient method, which guarantees high productivity and higher robustness of the process compared with free cells productions.

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P031

Bark beetles' bacterial symbionts: a natural resource of enzymes with biotechnological potential

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Bark beetles reproduce in the inner bark of several tree species. It is well known that bark beetles establish symbiotic associations with microorganisms, which enable host (tree) colonization and utilization, e.g. thanks to the ability of these microbes to hydrolase the wood [1].

Lignocellulosic biomass is a renewable resource that can be used to produce energy through the obtaining of bioethanol, following the conversion of the lignocellulose in sugars and further fermentation of these sugars [2].

Since cellulose, xylan (hemycelluloses) and lignin are the main wood compounds, glucanases, hemicellulases and lignin-modifying enzymes play an essential role in the hydrolysis of lignocellulosic biomass. Microorganisms able to hydrolyze wood are potential reservoirs of this kind of glycosyl hydrolases/oxidative enzymes encoding genes.

In this work we have isolated and identified bark beetles' bacterial symbionts

and qualitatively and quantitatively analysed their ability to produce lignocellulolytic enzymes to study their biotechnological potential for lignocellulose hydrolysis. These are the first steps to select efficient genes which can be molecular engineered and used for industrial production of bioethanol from lignocellulose.

Research was funded by the European Social Fund and the state budget of the Czech Republic (CZ.1.07/2.3.00/30.0003).

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P032

Lanthanides: Toxic and stimulatory effects on microalgae.

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Due to the rapid industrial development, wastes containing various metals are, directly or indirectly, discharged into the environment, resulting in serious environmental pollution. Amongst (non-essential) metals, lanthanides (Ln^{3+}) - also known as rare earth elements - have become indispensable in a number of critical technologies and their accumulation in biospheres has risen as a consequence. Their biological effect is not well understood. High concentrations of Ln^{3+} are toxic to plants and animals, but at low concentrations they may produce beneficial effects, increasing quantity and quality of crops. Lanthanide's effect on algae is particularly poorly understood. *Desmodesmus* spp. are common microalgae of great abundance amongst freshwater plankton. Our first objective was to test five Ln^{3+} against the wild-type strain of *D. quadricauda* and to produce (by induced-cell mutagenesis) and test a series of mutants of this alga. Our second objective was to test on metal-deprived experiments if Ln^{3+} alleviates deficiency-symptoms by replacing essential elements as suggested previously. Results: 1) We have confirmed that non-essential Ln^{3+} produce biological effects on algae and have demonstrated stimulatory and toxic effects on growth at lower and higher concentrations, respectively. 2) We were able to modify the tolerance to Ln^{3+} on our mutants. 3) We proved that Ln^{3+} may have a metal-substitution role only on certain physiological processes under (certain) metal-deficient conditions. Besides a relatively low toxicity, it results difficult to predict the real impact of Ln^{3+} on a community level and further studies are needed.

Study supported by The Ministry of

Education, Youth and Sports, Czech Republic, project Centre for Algal Biotechnologies (Algatech), reg. no. CZ.1.05/2.1.00/03.0110 and project Algain, reg. no.CZ.1.07/2.3.00/30.0059. It was also supported by the Andalusian Government and the Ministry of Education of Spain (FPU grant AP2009-2770).

P033-S

Cyanobacterial-catalyzed reduction of β–oxoalkylphosphonates

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The asymmetric reduction of ketones is one of the most important and practical reactions for producing non-racemic chiral alcohols, which can be transformed into industrially important chemicals. One of the most efficient ways to produce chiral alcohols of high purity is biocatalytical reduction of ketones. This strategy based on biological methods focuses on non-photosynthetic and heterotrophic microorganisms or their isolated enzymes[1]. However, phototrophic prokaryotes such as cyanobacteria have also been identified as a source of reductive activities[2]. Biocatalysis is an effective and in many cases preferable alternative to the standard synthesis of optically active isomers of fine chemicals, including phosphonates of define structure and absolute configuration [3]. Hydroxyphosphonates are a class of organophosphorus compounds with potential biological activity [4].

Morphologically different strains of cyanobacteria were used as a novel source of reductive activities towards β-oxoalkylphosphonates. Screening of cyanobacteria shown that only filamentous strains of *Arthrospira maxima* CCALA 027, *Nodularia sphaerocarpa* CCALA 114 and heterocystous photoheterotrophic cyanobacterium *Nostoc cf-muscorum* CCALA 129 are efficient biocatalysts in reduction of chosen substrate to the corresponding β- hydroxyphosphonates of high enantiomeric purity (Figure 1. R = $-CH_3$; $-C_2H_5$; $-C_6H_5$). In addition, several efforts have been undertaken to optimize the bioconversion conditions to obtain higher degree of conversion of the substrate. The effect of cultivation medium, light source, light intensity and light cycle (day/ night) on the effectiveness of the biotransformation process was examined.

Optical purity of the products was determined by means of ^{31}P NMR spectroscopy with the addition of quinine as a chiral discriminator.

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P034-S

Development of immunochemical method for the methandienone detection

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Anabolic androgenic steroids (AAS) are widely used compounds in sport today. AAS are synthetic derivatives of the male hormone testosterone. Their using is illegal and prohibited without medical supervision. Methandienone, sometimes known as Dianabol is one of the most commonly abused anabolic steroid. Methandienone accelerates the growth of weight primarily in muscle and increases physical fitness and stamina. Top sportsmen or common sportsmen use methandienone as a doping in various forms of tablets, injections or food supplements where steroids are illegally added. Due to the serious health risks associated with the use of anabolic steroids, it is desirable to develop tools that allow rapid and simple analysis for their detection. Traditional methods for analysis of steroids (LC/MS and GC/MS) are highly sensitive and reliable. These methods involve multiple steps during sample preparation and analysis, require expensive equipment and skilled analysts. Immunochemical methods represent a suitable alternative overcoming the problems mentioned above, especially Enzyme Linked ImmunoSorbent Assay and imunnochromatographic test (ICT).

The aim of this work was to develop immunochemical method (ICT) for the detection of methandienone in food supplements. For this purpose, conjugates of methandienone were synthesized in position C3 and C17 of the steroid skeleton. These compounds were conjugated with bovine serum albumin (BSA) and ovalbumin. Conjugates with BSA were used as an immunogen to obtain polyclonal rabbit antibodies, and conjugates with ovalbumin were used as the immobilized antigen. So far, suitable conditions were chosen. Membrane, reaction buffer and appropriate combinations of immunoreagents were selected.

Acknowledgment: This work was supported by the Grant of Ministry of the Interior of the Czech Republic (no. MV0 VG20112015045) and Specific research MSMT No. 20/2014.

P035

Possibilities of pH adjustment using electrodialysis with bipolar membranes presented on the example of acidification of rosehip wine

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Properties of plant materials vary depending on climatic conditions relative to location of cultivation and between years. The ratio of sweet and sour taste is significantly affected by these factors. Coldness and less sunlight cause higher acidity and less sugar content and vice versa. This variability may significantly affect taste of the product and technological process itself. However, the goal of most producers is to have standard quality product without major fluctuations in its properties. [1,2]

Nowadays this problem is solved by mixing raw materials from multiple sources or by addition of acids and bases. But in many cases this is not desirable from technological, legislative or commercial perspective. [3]

Possible solution is to use the technology of electrodialysis with bipolar membranes (EDBM). By suitable arrangement of bipolar, anion-exchange and cation-exchange membranes this technology enables to adjust pH in the order of units with precision of 0.05 without adding any chemicals. [3]

The aim of our study was to verify the EDBM technology with use of our membranes on rosehip wine and to assess sensorically differences between samples.

For testing of this technology rosehip wine was produced by traditional method of fermentation with final pH 3.58. The wine was subsequently acidified by EDBM technology of 0.1, 0.2 and 0.3 pH units. All four samples were subjected to tasting preferential test. The assessment revealed that wines acidulated by 0.1 and 0.2 pH units evince softer, lighter and fresher taste. Wine acidified by 0.3 pH units has been assessed as less distinctive with greater bitterness and astringency, the wine was disharmonious.

The technology has been verified in the beverage area, but its application is possible in other segments where it is necessary to adjust the pH and is not appropriate or desirable to add acids or bases.

Acknowledgement: The results were obtained within framework of the project number CZ.1.05/2.1.00/03.0084 entitled ”Membrane Innovation Centre”.

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P036

Optimization of *Agrobacterium tumefaciens*-mediated genetic transformation in *Dunaliella salina*

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Dunaliella salina, an eukaryotic halotolerant unicellular green alga without a rigid cell wall, can tolerate salt concentration varying from 0.5 % to 35 %. These features of *D. salina* make it an ideal transgenic bioreactorhost for the production of antibodies, oral vaccine, and commercially valuable polypeptides. To produce high level of heterologous proteins from *D. salina*, need to establish the optimum stable gene transfer system [1-2].

In the present study *Dunaliella salina*, Isolated from Qom salt lake used for establishment of *Agrobacterium tumefaciens*-mediated genetic transformation. Plasmid pCAMBIA3301 containing bar and β-glucoronidase gene (GUS gene) have transferred to *Dunaliella salina* by *A. tumefaciens*. Co-cultivation at 0.3 M NaCl allowed growth of both *D. salina* and *A. tumefaciens*.

Cells resistant to 20 μg/ml basta (phosphinothricin) were selected and growth of *Agrobacterium* was completely eliminated by using cefotaxime (500 μg/ml) / potassium clavulanate(300 μg/ml), [3]. The concentration of sodium chloride was gradually increased to 1.0M for better growth of *D. salina. Agrobacterium* was unable to survive in the growth medium used for *Dunaliella*.. Molecular analysis , including PCR, colony PCR showed that expression cassette successfully transformed and integrated in *Dunaliella salina* genome. Histochemical GUS assay showed that β-glucoronidase gene has been transcribed and express in transgenic *Dunaliella salina*.

Our results prove *A.tumefaciens*-mediated transformation of the unicellular microalgae *D. salina. Agrobacterium tumefaciens*-mediated transgene integration along with the outdoor cultivation methods used for *D. salina* may permit the commercial production of high level secondary metabolites and foreign recombinant proteins.

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P037

Study of the antimicrobial effect of *Leuconostoc mesenteroides ssp. mesenteroides* isolated from raw goat's milk in Bejaia, Algeria

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To select strains leuconostocs endowed with antimicrobial activity, test of spot is performed for the 25 leuconostocs strains isolated of raw goat milk against three pathogenic strains (*S. aureus, Pseudomonas, E. coli*). Only 15 strains showed zones of inhibition greater than 15mm and up to 35mm. The acidification kinetics of this 15 strains is determined in the reconstituted skim milk goat at 10 % . Only six strains showed pH 5, after only 6- 10 hrs incubation and 4.2 $\pm$ 0.1 after 24hrs. Three strains were identified genetically as strains of *Ln.mesenteroides* ssp. *mesenteroides* are selected for study of the antimicrobial effect against bigger number of pathogenic strains (8 strains) and against fungal strains (*Candida albicans, Aspergillus flavus*).

The results obtained by the test spot showed that the three strains exert an antagonistic effect with respect to all the strains tested included fungal strains. The origin of the antimicrobial activity of *Ln. mesenteroides* is determined by the test wells of the culture supernatant of 18hrs (10^8UFC/ml) directly and after concentration 4 times by rotavapor . The results show that for all the strains tested , a significant improvement in the antimicrobial activity is obtained after concentration of the supernatants .

Treatment of supernatants by proteases (trypsin , chymotrypsin and papain) , and the neutralization of the pH and after heating at 100°C or 120°C /10min showed that the inhibitory activity of the three leuconostocs strains is not only due to pH but also probably an antimicrobial substance of protein nature and thermo-stable. These results' suggesting these strains not only used like ferments, but also as bio-conservators.

P038

Nostoc flagelliforme: artificial-cultivation and functions

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Nostoc flagelliforme is an edible terrestrial cyanobacterium with great economic value which is distributed on arid or semi-arid area. It is the dominant nitrogen-fixing organism in such areas and considered as special biological resource in extreme environments. Increased market demands have resulted in the excessive exploitation of *N. flagelliforme*, which,

in turn, causes the endangered status of this species and the deterioration of the environment. The Chinese government has strictly prohibited the picking and trading of wild *N. flagelliforme* since July 2000. Artificial culture of *N. flagelliforme* is the promising solution to meet the market demand and conserve the endangered resource. However, as a result of the biological characteristic of *N. flagelliforme*, the alga grows extremely slow so that it is hard to achieve the mass culture of the species. Through years of study, the methods for artificial cultivation of *N. flagelliforme* have been successfully established and optimized in our lab [1], and mass cultivation including closed and open culture has been carried out. The bioactivities of *N. flagelliforme* including anti-virus, anti-tumor, and anti-oxidant properties have been widely investigated and related products have been developed. Moreover, the production of valuable compounds such as biologically active polysaccharides and natural blue pigment phycocyanin from *N. flagelliforme* has been realized, and the according extraction and purification process has been established [2,3].

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P039-S

Characterization of red yeast biomass by Raman spectroscopy

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Yeasts are ubiquitous microorganisms, occuring in soil, fresh and marine water, animals, on plants and also in foods. Yeast biomass, mainly derived from *S.cerevisiae*, represents the largest bulk production of any single-celled microorganism throughout the world. There are also some other yeast with specific uses, which may be grown on a range of substrates unavailable to *S.cerevisiae*. This work is focused on controlled production of biomass and some interesting lipid-soluble metabolites by carotenogenic yeasts. Pigmented yeasts are used as feed and food colorants and, come of them, also as single cell oil

producers. Except carotenoids they are able to form also sterols and other provitamins and biologically active compounds. These yeasts are able to utilize diverse carbon sources including waste substrates.

In presented work metabolic and production activity of several red yeast strains of the genus *Rhodotorula, Sporobolomyces* and *Cystofilobasidium* were analyzed. Yeasts were cultivated on glucose medium with different C/N ratio to switch lipid and/or pigment production. Lipid production was detected by fluorescence microscopy and enzymatically, while pigment production was measuered by HPLC/PDA/MS. Simultaneously, production of targeted metabolites was detected by Raman spectroscopy, which can be utilized for fast and accurate lipids (fatty acids) and carotenoids estimation as the intensity ratios of specific, selected Raman bands (β-carotene C–C stretching 1,157 cm^{-1}, β-carotene C=C stretching 1,525 cm^{-1}, lipid CH_2 scissoring 1,445 cm^{-1}, lipid C=C stretching 1,656 cm^{-1}). Further, Raman spectroscopy has been combined with optical tweezers and microfluidic system for automatic detection/sorting of yeast cells. Since no major sample preparation is required, this analysis can be done *in situ* to establish whether biomass contains the desired composition of the substances of interest.

Acknowledgements: This work was supported by projects "Centre for Materials Research at FCH BUT"No. CZ.1.05/2.1.00/01.0012/ERDF, ALISI No. CZ.1.05/2.1.00/01.0017/ERDF and P205/11/1687 of the Grant Agency of CR.

P040-S

Decreasing the production costs of *Schizochytrium limacinum* biomass

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Schizochytrium limacinum is a marine thraustochytrid able to accumulate in its biomass a considerable amount of lipids, a significant part (40-50 %) of which is docosahexaenoic acid (DHA). Biomass rich in DHA is a suitable feed additive and it can be used in the pharmaceutical industry too. In addition, *S. limacinum* can effectively utilize various sources of carbon and energy (glucose, glycerol), which allows its effective heterotrophic cultivation in bioreactors.

The goal of this work was to evaluate the use of cheap by-products of agriculture and food industry, in order to decrease the production costs of *Schizochytrium limacinum* biomass. To achieve this goal fed-batch fermentation of *Schizochytrium limacinum* were carried out in laboratory scale bioreactor. The medium consisted of saline solutions, glycerol (90 $g.L^{-1}$), yeast extract and ammonium was fed during the cultivation as a source of nitrogen. The fermentation resulted in 40,4 $g.L^{-1}$ dry biomass (4.49 $g.L^{-1}.day^{-1}$ biomass producti-

vity) with lipid content 20,0 % DCW (w/w) and DHA content 48,5 % TFA (w/w).

The new medium was cheaper as compared to the one mentioned in the literature by 80 %.

P041

The probiotic properties of autochthonous Streptococcus thermophilus isolated from traditional Algerian foods

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Lactic acid bacteria (LAB) are widely used in fermentative food processes. *Lactobacillus delbrueckii* and *Streptococcus thermophilus* have been traditionally used as starters for milk fermentation in yoghurt production. *Streptococcus thermophilus* contributes to fast lactic acid production in yoghurt and also to flavor properties.

S. thermophilus, a well known dairy starter has recently been reported to exhibit potential probiotic effects. For their use as potential probiotic, autochthonous *S. thermophilus* strains need to be screened for their capacity to tolerate upper gastrointestinal tract conditions during transit by in vitro studies. A study was undertaken to investigate the resistance to biological barriers and the probiotic value of our autochthonous thermopiles lactic acid bacteria.

Results showed that strains were able to survive at low pH (pH2.5) with a good bile salt hydrolase activity, cell surface hydrophobicity and sensitivity to most of the clinically important antibiotics. In simulated gastrointestinal juice, the strains showed a good viable count, also, these isolates showed good adherence to different epithelial cells.

The tested strains were found in vitro possess desirable probiotic properties and they are good candidates for their application as probiotic starter in the food industry.

P042

Determination of Volatile Compounds of Endophytic Fungus *Diaporthe* sp. from *Stryphnodendron adstringens* (Mart.) Coville

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Endophytic fungi are an important and potential resource of natural bioactive compounds widely used in pharmaceutical,

food and agricultural industries. Many bioactive natural compounds have been successfully discovered from the endophytic fungi [1]. *Stryphnodendron adstringens* Leguminosae, is a medicinal plant distributed in central Brazil and has been widely used for its anti-inflammatory, antiulcer and cicatrizing properties [2]. In the present study the endophytic fungi were isolated from the bark of *S. adstringens* and identified by using molecular methods. All fungi recovered were cultured in appropriate solid media and the volatile terpene derivatives determined by using headspace solid-phase micro-extraction SP-ME/GC-MS techniques. After the screening process, β-himachalene (41 %), β-camigrene (12 %), cuparene (10 %), α-patchoulene (7 %), and α-barbatene (3 %) were identified as major volatiles of the *Diaporthe* sp. microfungus.

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P043-S

Production of Lipids and Starch in *Parachlorella kessleri*

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The green microalga *Parachlorella kessleri* is known to use starch as a primary energy storage compound under optimal growth conditions and neutral lipids as secondary energy storage under nutrient stress conditions [1, 2]. These properties of *P. kessleri* can favour its large-scale cultivation as a feedstock for the biofuels industry. Recent research has demonstrated that exposure of microalgae to heavy-ion beam radiation can produce mutants with the capacity for enhanced lipid accumulation [3,4].

In this work, reduction in nutrient availability was used as an inexpensive method to promote starch and lipid production in seven *P. kessleri* mutants that were exposed to heavy-ion beam radiation. The mu-

tants, and a wild type, were cultivated in a laboratory-scale photobioreactor under a constant light intensity of 1200 μE m $^{-2}$ s $^{-1}$ and were aerated using a mixture of air and CO_2 (2 %, v/v). The cultivation of all strains consisted of two stages: accumulation of biomass through growth in a full mineral medium and a phase of nutrient starvation after three fold dilution of the cultures with H_2O.

Within four days of cultivation, a dry weight of 12 g.L^{-1} was produced by mutant strain 2-8, while the maximum biomass achieved by the wild type was 10.5 g.L^{-1}. The transition between cultivation in a full mineral medium and nutrient starvation had little effect on the starch content of the strains, which remained relatively constant throughout the experiment. In contrast to this, the level of neutral lipids changed dramatically under nutrient depletion conditions, increasing almost ten fold in mutant strains Pk 2 and Pk 4. Based on these results we concluded that these two mutants can be of potential interest for the large-scale production of algal lipids as a feed stock for the biofuels industry.

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P044-S

In vitro biocompatibility testing of biodegradable alloys for orthopaedic applications

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Degradable orthopaedic implants are used as temporary fixations e.g. in case of serious fractures. Gradual degradation eliminates a need of reoperation, which means saving costs and reducing risk of surgical complications. So far, polymers with insufficient mechanical properties are used for this purpose. Therefore, metallic materials, which exhibit more suitable mecha-

nical features for orthopaedic applications, are extensively developed and studied. Attention is drawn to magnesium-, zinc- and iron-based alloys. Apart from mechanical requirements and appropriate corrosion rates, biocompatibility must also be guaranteed.

We tested biocompatibility of newly developed Mg-, Zn- and Fe-based alloys. Cytotoxicity, genotoxicity and mutagenicity of extracts of alloys were evaluated. Cytotoxicity was tested according to ISO 10993-5 standard on mouse connective tissue cell line (L929) and human osteosarcoma cell line (U-2 OS) using microculture tetrazolium assay (WST-1). For genotoxicity testing, the comet assay was used using the same cell lines as for the cytotoxicity testing. The bacterial Ames test was employed for mutagenicity testing.

The extracts of tested alloys showed neither genotoxicity nor mutagenicity. Cytotoxicity was strongly dependent on concentration of extracted alloying elements in combination with actual pH of extracts and the decrease of metabolic activity was higher than 30 % for several samples. This value is suggested as a cut-off between non-toxic and toxic response by the ISO standard; however, at the same time, the ISO standard declares that the results of *in vitro* assays are only informative and should not lead to immediate material rejection from the testing process. In addition, such high concentrations of alloying elements are probably not achievable in human body after implantation, since there could be differences between *in vitro* and *in vivo* corrosion behaviour. We conclude that the alloys should be further studied and modified to obtain the materials that will meet the biocompatibility criteria.

Financial support from specific university research (MSMT No 20/2014) and the Technology Agency of the CR (project no. TE01020390).

P045-S

Rhamnolipid production by *Pseudomonas aeruginosa* NRRL B59-188

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Polycyclic aromatic hydrocarbons (PAHs) are major recalcitrant components of oil contaminations. These compounds are produced by industrial activities such as oil processing and storage. Biological degradation of PAHs is very problematic due to their low solubility in water and strong sorption to soil particles. Their water solubility is the key factor for bioremediation. Nowadays, chemical surfactants are used in biodegradations to enhance the solubility and desorption of hydrophobic compounds, but application of biosurfactants has many advantages, they are lower toxic and better biodegradable. Thus there is an increasing interest in possible use of biosurfactants in the bioremediation. Biosurfactants are amphiphilic compounds with varied structure produced by many bacterial strains. Rhamnolipids, which are from glycolipid family, are the best studied biosurfactants. They are comprised of

one or two molecules of rhamnose linked to one or two β-hydroxy-fatty acid. The biosynthesis of rhamnolipids is mostly studied in genus *Pseudomonas*.

The aim of this project is to obtain information about possibilities in rhamnolipid overproduction by bacteria *Pseudomonas aeruginosa* NRRL B59-188, which was isolated from oil contaminated soil. The influence of culture medium composition (especially source of nitrogen, phosphorus and trace elements) and of cultivation conditions on rhamnolipid production was investigated. After selection of the most suitable medium in microcultivation and Erlenmeyer flasks experiments, bioreactor experiments were performed.

P046

Metabolic profiling reveals differences of *Gluconacetobacter xylinus* metabolism under static and agitated culture

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Two ways can be used to synthesize bacterial cellulose (BC), static and agitated culture[1]. Lots of researches[2] revealed that the production, mechanical properties and micro-structure of BC could be significantly influenced by the culture methods. While, up to now, there are limited researches focusing on revealing the differences inside microorganisms in these two culture methods. The elucidation on cellular response would assist in understanding the possible reasons of phenotypic differences in static and agitated culture.

In this work, *Gluconacetobacter xylinus* (CGMCC 2955) were grown under both static and agitated culture conditions. A metabolic profiling approach was used to profile intra-cellular metabolites changes. A total of 79 intracellular metabolites were identified and quantified using GC-MS. Potential biomarkers were found by the principal component analysis as well as partial least squares. Trehalose, phosphate, alanine, glutamic acid, proline, valine, threonine and gluconic acid were mainly responsible for the discrimination among samples in static and agitated culture. Further analysis by mapping measured metabolites' relative contents onto the metabolic network revealed that the glycolytic pathway was activated under high rotational speeds due to the relative higher level of dissolved oxygen. For example, the glucose consumption increased by 75.8 % compared with that from static culture. In addition, the synthesis of many branch metabolites were also activated, which was believed to be responsible for the low yield of BC. For instance, the increase of liquid shear stress from agitated culture induced the accumulation of metabolites that protected cells from damaging, including trehalso, proline and glutamic acid. Relative contents of the three metabolites increased by 18.7-fold, 3.82-fold and 15.8-fold on 2-day, respectively, compared with that from static culture. As cells adapted to environmental changes on 3-day, the levels of the

three metabolites decreased to 18.2 %, 18.1 % and 6.76 %, respectively, compared with that on 2-day.

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P047

Collection of Microscopic Fungi of the Institute of Soil Biology (CMF ISB)

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The Collection of Microscopic Fungi of the Institute of Soil Biology of the Biology Centre AS CR, v. v. i. (CMF ISB) was established in 1980. In 1993, the CMF ISB was included in the Federation of Czech and Slovak Collections of Microorganisms (FCCM). The presenting author was established as current curator of the CMF ISB in December 2013. The strains are maintained in tubes on slope agar media in refrigerator at 4 °C. For long-term storage of spore-forming strains several other techniques (alginate pellets, glycerol or water stocks, etc.) are being implemented.

CMF ISB has been focused on microscopic, predominantly filamentous fungi. Nowadays, the CMF ISB consists of around 2000 strains of micromycetes isolated mainly from soils of the Czech Republic, Slovakia, Germany, Russia, USA, and Macedonia, then isolated from air, litter, caves (Czech Republic, Slovakia, Romania, Spain, France), intestine and excrements of soil invertebrates, vermicomposts, etc. Deposited strains have been used for the purpose of teaching (University of South Bohemia), basic research (comparative studies, phylogenetic analyses, interactions with soil invertebrates), applied research for pharmaceutical or food industry, as well as agriculture. The CMF ISB harbours some specific isolates which are not deposited in any of the other culture collections worldwide. Hence, fungi from CMF ISB can serve as a source of unknown metabolites for biotechnology. More effective strains of fungi with known potential might also be presented.

P048-S

Brassinosteroids and ecdysteroids as the tool for photosynthesis-efficacy improvement in higher plants

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In higher plants, both brassinosteroids (BRs) and ecdysteroids (ES) are naturally occurring. Whereas the role of BRs is already well analysed – at hormonal level affecting plant growth and development, tolerance to environmental stress or pathogen infection, the significance of ES for plants is not fully clear yet.

Our experiments show that at effector concentration the ES (and also BR) are able to increase the yield of RuBisCO-mediated CO_2 fixation into organic matter between 10–15 % in *Tetragonia tetragonioides* (New Zealand spinach) [1]. We also demonstrated that exogenously-applied ES can increase the net photosynthetic rate in *Tetragonia* [2], and proved it also in other species, *e.g.* spinach (*Spinacia oleracea* L.) and maize (*Zea mays* L.).

Recently we have found that supplementing of ES in effector concentration to isolated spinach chloroplasts increases oxygen production by approx. 10–15 % in plants previously sprayed with BRs [3]. The binding of these steroids to the involved proteins was studied by surface plasmon resonance and by steroid ligand docking.

Research was supported by grant P501/11/1650 of the Czech Science Foundation.

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P049

Succinic acid production from ammonium succinate by electrodialysis with bipolar membrane

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Succinic acid is used in chemical industry for some polyesters and alkyd resins production. Succinic acid can be produced biologically from CO_2 in the air. It is one of the candidate compounds for chemical industry for partial replacement of oil product with biological origin, called bio succinic acid. Pilot production of this compound is already running at companies ARD, BioAmber, DSM & Roquette, Myriant, Purac and others. Electrodialysis with bipolar membrane (EDBM) is one of usual production steps splitting succinate into the succinic acid and corresponding base. Description of EDBM application on this feed is the objective of this work.

Two feeds with difference concentrations and forms of ammonium succinate were treated by EDBM at pilot scale. Three module types were used: 2 compartment module with cation exchange and bipolar membrane, 2 compartment module with anion exchange and bipolar membrane and 3 compartment module with cation exchange, anion exchange and bipolar membrane. EDBM products were succinic acid at 75, 85 and 95 % conversion. By product was ammonium solution. Product quality, by product composition, EDBM capacity, media consumption, water consumption, energy consumption were estimated and compared to recommend best products and EDBM operation conditions for both feeds.

P050-S

Comparison of acid and alkali wheat straw pretreatment for biofuels production

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Wheat straw was pretreated using two different approaches (alkali and acid pretreatment). The conditions of pretreatments were optimized to achieve the highest fermentable sugars in subsequent enzymatic hydrolysis. At the alkalic optimum (80°C, 39 min, 0.18 g NaOH and 0.06 g lime per g of raw biomass), 85 % conversion of cellulose to glucose were achieved after 48 hours of enzymatic hydrolysis and 12.85 g/l of ethanol could be produced by *Saccharomyces cerevisiae* using this supplemented hydrolysate as cultivation medium. For acidic optimum (180°C, 30 min, 0.18 g Na_2SO_3 per g of raw biomass + 1 % H_2SO_4), conversion and ethanol concentration were 49 % and 4.94 g/l, respectively. Alkali pretreatment by combination of NaOH and lime is preferable for wheat straw in comparison with acid pretreatment.

P051-S

Biotransformation of *O,O*-dimethyl-4-oxoazetidin-2-yl-phosphonate using *Penicillium minioluteum*

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Biotransformation is substrate transformation to desired product by using suitable type of biocatalysts. The biocatalysts allow carrying out the reaction even 10^{12} times faster in comparison with non-catalyzed processes. This is currently, a convenient, alternative tool with crucial potential for the development sustainable technologies for the production of chemicals and drugs [1].

Aminophosphonic acids and their derivatives are an important class of organophosphorus compounds. They are analogues of amino acids gained by replacement of carboxylic group by phosphonic or phosphinic moiety. Phosphonates have got wide range of promising biological activities and variety of applications in industry. Bioapplications of such compounds implies in different enzymes inhibition. Phosphonates derivatives also act as antibiotics, plant growth regulators, herbicides and peptide mimetics [2].

Biosynthesis of chiral, organophosphorous compounds is still not fully explored. Main inspiration for presented work were described biocatalytic methods, which allowed obtaining optically pure derivatives of aminophosphonates (with aliphatic side chains) *via* stereoselective biooxidation. Synthesis of acidic aminophosphonates is a subject of presented work. To obtain optically pure enantiomers of phosphonic analogue of aspartic acid via stereoselective biohydrolysis of *O,O*-dimethyl-4-oxoazetidin-2-ylphosphonate - whole cells of *P. minioluteum* were used as a biocatalyst.

Such approach resulted in production of desired product with moderate yield and satisfactory optical purity. Elaborated procedure is promising for further scalling up the process.

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P052-S

Functional analysis of regulation protein Spo0A from biobutanol producer *Clostridium pasteurianum* NRRL B-598

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Clostridium pasteurianum NRRL B-598 is strictly anaerobic, spore-forming, solvent-producing bacterium. Main possible biotechnology use of this strain is in production of biobutanol or hydrogen, the most interesting products of its fermentation. It's not clear yet, whether sporulation of *Clostridium* species is related to solvent production and what endogenous factor is main activator of endospore forming. Function of Spo0A, the central regulation protein of sporulation at *Clostridium* and *Bacillus* species, was investigated in several Clostridia. Here, we present a functional analysis of Spo0A protein at *Clostridium pasteurianum* NRRL B-598. For this purpose, a knock-out mutant strain with Spo0A regulation protein gene deletion was prepared by ClosTron system [1]. Impacts of mutation were investigated in cultivation experiments when we compared wild-type strain and mutant strain.

Financial support from specific university research (MSMT No 20/2014) and TACR BIORAF (TE01020080).

Acknowledgements: We would like to thank to Nigel P. Minton and John T. Heap for use of ClosTron system which was used for construction of pMTL007S-E2::CpaB598-spo0A-407a plasmid.

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P053

Applicability of Blood RNA Quality Biomarkers for EDTA Tubes in High-Throughput qPCR Gene Expression Experiments Using BioMark System from Fluidigm

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It is increasingly recognized that preanalytical factors, if not properly identified

and controlled, can have an effect on sample quality and, consequently, on the quality of molecular analysis. A simple factor as a selection of the type of container for sample processing can make an important difference in the obtained results and can have a significant impact on the stability and levels of mRNA measured in biomarker studies. It has been described previously that dysregulation of some genes in K_2EDTA collection tubes can occur because of the absence of any stabilizer of gene expression in the collection tube [1,2]. So, preferably and if possible, blood should be collected to special collection tubes, for example PAXgene® Blood RNA Tubes, that offer the immediate stabilization of blood RNA [1]. However since recently, there is a solution for controlling the stability of samples already collected to frequently used K_2EDTA collection tubes, which is a panel of quality RNA biomarkers validated within SPIDIA consortium [3,4]. Here, we propose one possibility how to apply them using high-throughput qPCR instrument BioMark from Fluidigm and multivariate analysis tools as principal component analysis and Kohonen's self-organizing map.

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P054

Mycoremediation of metal-polluted soils: towards understanding the biology of metals in ectomycorrhizal fungi

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Mycorrhizal fungi, in their mutualistic associations with plants roots, benefit the hosts in a number of ways of which the most important is enhancing soil nutrient mobilization and uptake. Consequently, mycorrhizal fungi are receiving much attention as useful symbionts stimulating growth of plants in nutrient-deficient or contaminated soils. There is evidence that hosting of different mycorrhizal types is of

functional importance in phytoremediation of heavy metal-polluted soils in which the metal tolerant mycobionts may play two different roles: detoxification of excess metals through such mechanisms as extracellular immobilization, exclusion, and by cellular uptake of metals that helps the phytostabilization of disturbed sites; scavenging of metals and their supply to the host that increases root-to-shoot metal ratio and promotes the phytoextraction of soil metals. Recent studies thus focused on the molecular basis of the biology of heavy metals in metal-tolerant and accumulating species with the aim of rating their host-protection/stimulation capacity. In this presentation we will focus on ectomycorrhizal (EM) fungi, the conspicuous and important fungal elements associated with woody plants, including fast growing poplars, aspens and willows, in pristine as well as metal disturbed environments. We will emphasize principally on the most recent developments about the import, efflux, compartmentalization and cytoplasmic sequestration by peptidaceous ligands from different classes of heavy metals in EM fungi and about the different extent each of these mechanisms contributes in the handling of cellular metals, particularly Cd, Zn and Ag, in different species or ecotypes. The specific examples will involve members of the genera *Hebeloma, Russula* and *Amanita*. We will then discuss how we may apply this knowledge for upgrading phytoremediation of metal polluted soils. *Authors' work supported by the Czech Science Foundation (P504/11/0484).*

P055

Purification and characterization of stable in organic solvents protease from new thermophilic actinomycete isolate

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In the present research a stable in organic solvent protease from a new thermophilic actinomycete isolate was purified and characterised. The approximate molecular mass of 25 kDa was determined by SDS-PAGE and SEC. The purified by ultrafiltration and SEC protease showed maximum activity at 70°C and exhibited broad pH optimum (5.0-12.0). After treatment with 5mM EDTA and β-mercaptoethanol the enzyme fully remained active. The protease showed increased activity and stability in the presence of 20, 40 and 60 % (v/v) organic solvents such as DMSO, DMF, acetone, ethanol, iso-propanol and toluene when incubated for 1h at 30 °C. The proteolytic activity was significantly enhanced in presence of Mn^{2+} and remained more than 90 % active in the presence of 5, 10 and 15 mM Pb^{2+}, Zn^{2+}, K^{+}, Fe^{2+}, Co^{2+}, Cd^{2+}, Mg^{2+}, Ca^{2+}, Fe^{3+} ions for 1h at 30 °C. The kinetic constants were also determined.

P056

Transient plant transformation mediated by *Agrobacterium tumefaciens*: principle, methods and applications

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Agrobacterium tumefaciens is a natural "genetic engineer" globally adapted as an unprecedented tool for genetic engineering of plants. Since the mechanistic elucidation of virulent T-DNA transfer from this soil born plant pathogenic bacterium into plant genome and the development of the first disarmed Ti-plasmid devoid of tumor promoting and opine-synthesis genes more than 30 years have passed [1]. During this period, reliable protocols for *Agrobacterium* mediated stable transformation of many model and crop plant species have been established. Although stable plant transformation is a golden standard for basic and applied plant research, the development of such engineered plants is profoundly time consuming and, in addition, does not allow scalability for high-throughput assays. Therefore, considerable efforts were directed towards establishment of *Agrobacterium* mediated transient transformation protocols in order to facilitate rapid transgene employment and high-throughput assays including functional genomics screens [2-6].

Herein we describe the principle and methods of transient *Agrobacterium* mediated plant transformation and highlight state of the art applications:

1) Rapid molecular cell biology characterisation of plant cell structures based on *Agrobacterium* mediated transient gene expression in *Arabidopsis* cotyledons and leaves of *Nicotiana benthamiana*. As a proof of principle, confocal microscopy images of cotyledon and leaf cells transformed with constructs encoding for endoplasmic reticulum fluorescent reporter or fluorescently tagged cytoskeletal markers are provided. In addition, biochemical evidence further underlying transformation success is also included.

2) Effector genomics of *Phytophthora infestans*, a functional genomics screen which, based on *Agrobacterium* mediated transient transformation of *Solanum* sp., enables in planta identification of potato disease resistance and *P. infestans* avirulence genes. In this case, we highlight high-throughput format of both, in planta transient expression of P. infestans avirulence gene candidates and reconstitution of candidate R – AVR interaction in *N. benthamiana* leaves.

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P057

Characterization of activated sludge by image analysis methods

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We propose automatic image analysis procedure for fast evaluation of characteristics of activated sludge. Independently we analysed several samples of activated sludge from wastewater plant treatment. Digital images of activated sludge flocs were taken by microscope acquisition system and proceed using an automatic image analysis. Morphological parameters of flocs, like form factor, fractal dimension, aspect ratio etc., were determined by software together with flocs composition. Calculated morphological parameters and ratios of filamentous microorganisms and extracellular polymeric substance (EPS) with respect to the total activated sludge area was compared with standard laboratory method (e.g. determination of sedimentation, dry mass) in order to find basic relationships. Low values of settleability indicated occurrence of the small flocs and low ratio of the filamentous organisms. Morphological parameters such as form factor and fractal dimension showed low standard deviation that could indicate very close relationship between them and settleability. Automatic image analysis method can provide reliable support for fast recognition of undesirable events in activated sludge as sludge bulking or foaming.

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P058-S

The production of exopolysacharids via microalga *Dictyosphaerium chlorelloides* and the production of polyunsaturated fatty acids via algae *Vischeria helvetica* and *Eustigmatos vischeri*

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Microalgae are known for their capability to produce commercially interesting biomolecules. Exopolysaccharides act mainly in algae as protective agents. They are typically produced under a certain stressful conditions. Therefore, the design of the cultivation procedure has a major influence on the economic viability of the production. In our work, we manage to attain synergistic influence of stressful factors resulting in a significant speed-up of the production of exopolysaccharides. Polysaccharides can be used in many human areas, e.g. in pharmacology, food industry, and biotechnology.

Furthermore, we study algae containing polyunsaturated fatty acids. Some microalgae have the ability to accumulate polyunsaturated fatty acids for the purpose of increased membrane fluidity, which is necessary, e.g. in case of environmental temperature drop. In this area, we managed to attain a higher productivity of biomass via mixotrophic cultivation with glycerol in comparison to autotrophic cultivation.

The work was realized thanks to financial support of TAČR BIORAF project no. TE01020080.

P059-S

Organic light for organic production – a novel small-scale OLED-based photobioreactor

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Microalgae represent a promising raw material for various industries due to their wide range of valuable ingredients. By photosynthetic processes carbon dioxid is fixed under the influence of light and converted into products like proteins, fats, oils or pigments. Despite the great potential of

algae research only 150 of the estimated 400,000 algae strains are used for industrial applications.

State of the art of microalge cultivation is the illumination with inorganic semiconductor elements known as light emitting diodes (LEDs). However, this technology has some disadvantages like an inhomogeneous ligth distribution (point light source), a strong self-heating and thus the need to integrate cooling elements. Because of these limitations no screening system for the small scale cultivation under defined conditions could be developed so far.

We present the first small scale cultivation system for phototrophic microorganisms with a working volume of 10 milliliters equipped with organic semiconductors as lighting source and optical sensor systems for process monitoring. Organic semiconductors are composed of conjugated molecules or polymers which are capable of intra-molecular charge transfer. These light emitting layers can be produced in almost any shape and are up to 200 nm thin. Thus, it is possible to integrate these polymers in different substrates such as metal, glass or foils. OLED light technology is characterized by a very homogenous light distribution (area light source) and low self-heating. The flexibility and geometry of this next generation light source allows the design of new small scale screening photobioreactor systems.

P060-S

Antioxidant properties in extracts of microalgal biomass produced biotechnologically under heterotrophic culture conditions

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The aging of human skin is influenced by several factors, including genetics, metabolic processes, hormonal changes and environmental exposure. In particular, UV exposure of the skin is substantial and leads to an increase in hydrogen peroxides as well as other reactive oxygen species (ROS), and to a decrease in antioxidant enzymes [1]. Generally, the skin contains antioxidant barriers that can make ROS ineffective, but if UV exposure exceeds a certain dose, those defences will be challenged. As a result, free radical damage to DNA, lipids and proteins occurs [2], usually resultings in skin damage [1]. As microalgae are frequently marketed as antioxidants, reduction in ROS may be possible through the application of cosmetics containing microalgal extracts.

The aim of this work was to enhance the antioxidant properties of microalgal biomass through a specific process strategy, and to test the antioxidant potential of microalgal extracts in physiological tests. Green and red microalgal species were cul-

tured under heterotrophic conditions, biomass was separated, disrupted mechanically, and extracts were prepared using different solvent compositions. Their antioxidant properties were evaluated in both the commercially available Oxygen Radical Absorbance Capacity (ORAC, Cell Biolabs, Inc.) assay and a proprietary assay developed with human skin cells. The latter assay is based on the production of fluorescent dichlorofluorescein (DCF) by the reaction of dichlorohydrofluorescein (DCFH) with intracellular ROS. Intracellular ROS generation is enhanced by peroxide treatment of skin cells and antioxidants in the assay scavenge ROS, leading to a reduction in DCF production, and therefore reduced fluorescence [3]. The microalgal extracts generally showed an antioxidant effect.

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P061

Growth and carbohydrate production by *Dictyosphaerium Chlorellales* as affected by temperature and Irradiance

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A green microalgae *Dictyosphaerium Chlorellales* (CCALA 330), producing the extracellular polysaccharides (EPS) in large amount was grown in cross gradient apparatus of light and temperature to optimize the suitable condition of light and temperature for the maximum production of EPS. Growth rate, division time, EPS as well as intracellular polysaccharide (IPS) production were studied at different condition of temperature and light. Descriptive statistics, two-way ANOVA, Tukey honest significant difference test and redundancy analysis (RDA) were performed to analyze the data. Growth rate and the production of EPS and IPS affected significantly by temperature, irradiance and combination of temperature × irradiance. The maximum growth rate was observed at temperatures around 19.2°C and relatively low irradiances in range of 2.6 – 11 W m^{-2}. The maximum EPS production was observed

at temperatures around 25.7°C and irradiance around 10-11 Wm^{-2}. The maximum IPS production was observed at temperatures around 19.2°C , as maximum growth rate but at irradiance around 11 W m^{-2}. Growth and carbohydrate production of *D. chlorellales* algae were also checked in diluted as well as in P and S limited medium and the result showed that the maximum growth occurred in normal medium while the maximum EPS was produced in diluted medium.

P062-S

Effect of natural substances on *Candida parapsilosis* and *Trichosporon cutaneum* biofilm

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Biofilms can contaminate medical instruments and industrial devices. Because microorganisms in biofilm are up to 1000 times more resistant than planktonic cells the prevention or eradication of biofilm is extremely difficult. The use of antibiotics in high concentrations is not a solution considering microorganisms begin to develop a multiresistance. Opportunistic pathogenic yeasts *Candida parapsilosis* and *Trichosporon cutaneum* are able to form a biofilm on different surfaces. We investigated the use of natural substances for the prevention or eradication of these biofilm infections. The selected natural substances were baicalein, chitosan and usnic acid in comparison with antibiotics amphotericin B and fluconazole. The yeasts were cultivated in 96-wells polystyrene microtiter plates. For the quantification of the biofilms the colonized areas were measured using the automatic microscope CellaVista (Roche, Switzerland). Baicalein and chitosan used in minimum inhibitory concentrations were able to decrease the colonized areas in wells more than 50 %.

P063

An improved fluorescence-based method for a real-time detection and quantification of Polyhydroxyalcanoates production.

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Polyhydroxyalcanoates (PHA) are a large class of polyesters naturally formed as storage compounds by a diversity of gram-positive and gram-negative bacteria under unbalanced growth conditions. PHA are synthesized from renewable carbon sources, namely sugars or fatty acids, and

accumulated in variable quantities in cytosolic lipid bodies [1]. Due to their mechanical properties similar to those of petrochemical polymers, as well as complete biodegradability and eco-friendliness, PHA have a great potential in the bioplastics fields involving biodegradable packaging and coating materials [1,2]. PHA are not economically competitive so far, in comparison with petroleum products, most of the actual researches are focused on cost-effective solutions to optimize PHA production yield in different wild-type or genetically modified bacteria strains.

In this context, we have developed a rapid and sensitive method to monitor the PHA biosynthesis efficiency throughout the fermentation course. This method uses the direct inclusion in the culture medium of the lipophilic fluorescent dye Nile red (0.4 % v/v), to bind with the PHA granules inside the bacteria cells. Fluorescence is detected by the combination of microscopic and spectrometric techniques allowing qualitative and quantitative evaluation of the PHA content of the culture.

This method was applied for the rapid screening of six potential PHA-accumulating bacteria isolated from a marine collection under a two-stage production strategy with nitrogen limited conditions. In the same time, we investigated the combination of specific cultivation media to yield maximal fluorescence intensities. The first phase growth medium composed of agro-industrial by-products and the second phase medium was selected to provide an adapted C/N ratio. Under the optimized conditions, batch cultivations in a 4L laboratory scale bioreactor gave PHA productivity up to 1.79 $g.L^{-1}$ with PHA content of 78 % of the cell dry weight (CDW). The identification of the polymer as PHA was confirmed by GC-MS and thermo-chemical analysis (DSC, TGA).

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P064-S

Screening for novel fungi producing ligninolytic, cellulolytic and xylanolytic activities

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A screening for new fungi was carried out in this work, in order to enlarge the patrimony of microbes for waste conversion into high added value eco-friendly and cost-effective bio-products.

Particularly, different fungal species (*Aspergillus sp., Pleurotus sp.* and *Lentinus sp.*) belonging to DEBB collection were in-

vestigated for ligninolytic, cellulolytic and xylanolytic activities production.

Screening on solid medium for ligninolytic activity was performed in the presence of the colored indicator Guaiacol, whilst the cellulolytic and xylanolytic activities were detected by Congo Red assay in the presence of carboxymethylcellulose and xylan, respectively, as sole carbon source. Starting from 32 fungal strains, the screening led to the selection of 6 strains producing ligninolytic activity, 3 strains producing cellulolytic activity and 6 strains producing both cellulolytic and xylanolytic activities. The selected strains were grown in liquid media for the quantitative estimation of enzymes production. Time-courses of cellulase and xylanase activities production were evaluated in the presence of cellulose microcrystalline 1 % as sole carbon source, whilst ligninolytic enzymes production was measured in the presence of glucose, as carbon source, and $CuSO_4$, as inducer.

3 strains belonging to the species *Lentinus sp.* and *Aspergillus sp.* were selected as the best cellulase activity producers (0.25 U/mL). The maximum value of xylanase activity was produced by an *Aspergillus* strain, up to 19 U/mL, whilst the highest laccase production (12 U/mL) was achieved with a *Pleurotus sp.* strain.

This research was supported by a Marie Curie International Research Staff Exchange Scheme Fellowship within the 7th European Community Framework Programme: "Improvement of technologies and tools, e.g. biosystems and biocatalysts, for waste conversion to develop an assortment of high added value eco-friendly and cost-effective bio-products" BIOASSORT (318931).

P065

Novel Approaches of Producing Biofuels from Microalgae: A Recent Review

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The current world energy crisis and global warming have yielded an ever desperate search for sustainable green energy sources. Microalgae present a potential biochemical/bioenergy source of both renewable and sustainable qualities. Compared to agricultural products and by-products such as sugar cane, soybean, rapeseed and oil palm , microalgae allow the direct generation of desired products like biodiesel, biomass and bioethanol at higher yields and in a shorter timeframe. Utilizing microalgae

has the advantages of increased bioenergy production per unit land area, reduced land area needed, minimization of competition with food crops, zero net carbon emission and reuse of by-products . The biomass remained after lipid extraction is rich in starch and proteins, hence suitable for animal feedstock or fertilizers. Hence, numerous efforts have been put into the commercialization of microalgae-derived biofuel by both government and private bodies. This paper serves to review efficient microalgae culture media and conditions, as well as recent novel techniques for high quality biofuel production, which include acid/base transesterification of microalgal lipids into biodiesel, plus thermochemical and biochemical conversion of microalgal biomass into useful biofuels.

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P066

Characterisation of methanol-free production of *Candida antarctica* lipase B by *Pichia pastoris*

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Optimum growth conditions for the production of a recombinant protein in the widely used *Pichia* system differ according to the target molecule (protein) and promoter used [1]. The relationship between specific product formation rate and specific growth rate has to be characterised empirically for each strain as a rational basis for the development of a production process. Secretion of *Candida antarctica* lipase B (CALB) by a *Pichia pastoris* strain was studied, where the enzyme was built under the control of an synthetic AOX1 promoter variant [2], using glycerol as a sole carbon source. Product formation was only repressed by excess glycerol during batch cultivation. During glycerol-limited fedbatch cultivations, CALB was secreted over a range of specific growth rates from 0.02 to 0.12 h^{-1}. Formation of the heterologous enzyme was time and μ-dependent. Stable (and reproducible) rates of product formation were determined during fedbatch processes at specific growth rates $\leq 0.04\ h^{-1}$, which correspond to ≤ 25 % of μ_{max}. The fedbatch process, conducted at a specific growth rate of 0.02 h^{-1}, and with glycerol as the sole

and limiting carbon source, resulted in 4.2 g l^{-1} (83'000 U l^{-1}) of active enzyme in the supernatant after 68 h of production and a biomass concentration of about 100 (g CDW) l^{-1}. An inducible/repressable promoter thus allows enhanced productivities of heterologous protein (e.g. CALB) to be achieved with *P. pastoris*, without methanol. This offers new applications for the *Pichia* system under circumstances where the use of methanol (and the conventional AOX1 promoter) may be problematic. The authors acknowledge strains obtained from TU Graz and VTU technology, Grambach, Austria.

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P067-S

De novo designed short antimicrobial lipopeptides: biophysical and biological properties

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The group of antimicrobial peptides have been known since early 80s of 20^{th} century. These compounds provide a wide spectrum of biological activities and, in comparison with classical antibiotics, alternative mechanisms of action and could become good candidates for new therapeutic agents.

We designed three peptides composed of six amino acids selected on the basis of statistical data from databases [1]. The sequence was built according to relative frequency of occurrence of residues [2] to form helical conformation. All of these peptides were synthesized with C-terminal amidation. To investigate the importance of increased hydrophobicity at the N-terminus of peptide, all of them were conjugated with palmitic or lithocholic acid. Biological activities were tested on both grampositive and gramnegative bacteria, fungi and mammalian cells [3]. The effect of acylation was demonstrated by rapid increase of biological activities. The unmodified peptides revealed no activity, modified peptides were highly active against grampositive bacteria, especially against strains of *Staphylococcus aureus* or *Bacillus subtilis*,

also they were highly toxic against mammalian liver and kidney cells, but not haemolytic. We found that effect of these lipopeptides is complex, including both interaction with cell envelopes and intracellular targets, what was confirmed by biochemical tests and transmission electron microscopy.

Acknowledgement: Research was supported by the specific university research MSMT no. 21/2014.

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P068

Production of carotenoids and other lipidic compounds by red yeasts cultivated on some lignocellulose waste substrates

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Carotenogenic yeasts are a diverse group of unrelated organisms (mostly *Basidiomycota*), that can be found in soil, fresh and marine water, on plants, commonly associated with animals and can be also found frequently in foods. Due to its ubiquitous and world-wide occurrence, these yeasts have been able to assimilate various carbon sources (glucose, xylose, cellobiose, sucrose, glycerol, etc.). Therefore, several agro-industrial waste materials including lignocellulose can be used as cheap nutrition sources.

Presented work is focused on growth, metabolic and production activity of several non-conventional red yeast strains of the genus *Rhodotorula, Sporobolomyces* and *Cystofilobasidium* cultivated on some wastes as cheese whey, bio-diesel derived waste glycerol, pre-treated whey straw, pine hydrolyzates and others. The main

aim of the current investigation was to assess the potentialities of red yeasts to transform these waste substrates to high-value products as carotenoids (about 1-3 mg/g CDW), ergosterol (2-4 mg/g CDW), coenzyme Q (0.5-1 mg/g CDW), lipids (11–21 % of dry mass) and fatty acids as well as enriched red yeast biomass. Biotechnological production of above mentioned metabolites produced on individual wastes will be compared between individual analyzed strains. Cellular and molecular changes of red yeast cells cultivated under nutrition depletion will be discussed too. Production properties of red yeasts will be studied also during scale-up process in 5-L laboratory fermentor. The yields of about 30 g per liter of biomass enriched by 30-50 mg of total carotenoids and about 50 mg of ergosterol were obtained by the most producing strains.

Such biomass, which is efficiently enriched for provitamins A, D and CoQ could serve as an additional natural source of significant nutrition factors in feed and food industry.

Acknowledgements: This work was supported by project "Centre for Materials Research at FCH BUT" No. CZ.1.05/2.1.00/01.0012/ERDF.

P069-S

The membrane proteome of *Arabidopsis thaliana* treated with isoxaben

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Isoxaben is an industrially used herbicide specific for dicotyledons. It is used for grains and ornamental lawns treatment [1]. It inhibits glucose incorporation to the cell wall inhibiting of cellulose synthase [2]. Previous studies were focused on its mRNA analysis [2,3], the proteomic data trying to clarify defence cellular responses are presented here.

Cellulose synthase is an integral plasma membrane protein (IMP). Therefore, the choice of analytical method is limited; we can not use two-dimensional electrophoresis, because IMPs precipitate at their pI. In 2011, the method for the analysis of IMPs, based on the fractionation of IMPs using reversed-phase chromatography with stepwise elution by 2-propanol, was published [4]. The fractions are digested by trypsin in solution and analyzed by LC-MS/MS.

We isolated microsomal fractions of suspension cell culture of *Arabidopsis thaliana*. The microsomal fractions were isolated for five days every 24 hours after the isoxaben addition. Using the described method, we identified 347 unique proteins in whole experiment; 31 of them were cell wall proteins and 51 IMPs with at

least one transmembrane domain. Cellulose synthase was identified in the control sample, but not in the samples treated with isoxaben; this confirms the assumption that isoxaben causes the decomposition of this enzyme. Isoxaben also influences other proteins in plasma membrane. Some IMPs were detected in higher amounts, such as H^+-ATPase isoforms and β-1,3-glucosidase, both of wich are closely related to the cell wall metabolism. Our results could contribute to the clarification of isoxaben influence and offer a new perspective on the analysis of cell wall proteome.

Financal support from specific university research (MSMT No 20/2014 and MSMT No 21/2014).

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P070-S

Contribution of Alimentary Selenium in the Defense of Organism against Oxidative Stress

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A nutritionally well-balanced diet is crucial for good health and proper function of the organism. This includes not only the main macronutrients, but also micronutrients like vitamins and minerals which also play an essential role. Selenium (Se) is one of these. This trace element once was thought to be toxic; however, later studies have shown that small amounts of selenium are necessary for the body. Selenium is present in some antioxidant enzymes and enzymes that affect function of thyroid gland hormones. The selenium content in food is highly dependent on the amount of selenium in the soil in which plants are grown or animals are raised. The Czech Republic is one of the countries with a rather low concentration of selenium in the soil. Different strategies have been followed in order to supply the population with sufficient selenium: use of Se- enriched fertilizers and supplementation of farm animals with selenium.

This study is concerned with the effect

of the rape seeds, planted in Se-enriched soil as a feed for animals. Oilseed rape (*Brassica napus L.*) naturally accumulates selenium and extracted meal from the seeds can be used in animal diets, where it can replace imported soybean meal. The males of Wistar-Kyoto rats were used in this experiment. The activities of selected antioxidative enzymes (glutathione reductase, glutathione S-transferase, glutathione peroxidase, thioredoxin reductase, and catalase) were measured in plasma, liver extracts, and erythrocyte lysates of rats which were fed with the diet containing different portions of oilseed meal. Content of selenium and other essential trace elements (as zinc and copper) in liver and kidney of the rats under Se-enriched diet was determined by atomic absorption spectrometry.

This work was supported by GACR 13-04580S, and by specific university research (MSMT No. 20/2014).

P071-S

MALDI-TOF mass spectrometry as a tool for identification of new species of the genus *Cronobacter*

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Bacteria of genus *Cronobacter* are gram-negative, facultative anaerobic rods belonging to *Enterobacteriaceae* family. They are known as opportunistic pathogens causing severe infections of neonates and immunodeficient individuals such as meningitis, necrotizing enterocolitis or sepsis. These diseases are accompanied by a high mortality level. Fast and accurate identification of these pathogens can precede consequences of their infections.

The *Cronobacter* genus was created in 2008 but its taxonomy is still changing. Recent changes have been carried out last year when three new species (*C. helveticus, C. pulveris* and *C. zurichensis*) were created. At this time *Cronobacter* genus is divided into ten species and three subspecies. Some species within the genus were associated with neonate infections and have a higher risk of virulence. For this reason, attention is focused on the precise species and subspecies identification. Actual identification methods are relatively precise, but slow. In contrast, MALDI-TOF mass spectrometry is rapid and simply method and has the potential to be handier tool for bacterial identification.

The aim of this study was to find differences between new species and other representatives of the genus *Cronobacter* using MALDI-TOF mass spectrometry. Creating MSP spectra and extension of database of reference mass spectra can help to distinguish individual species. Comparison of mass spectra showed interesting differences between new species and other members of *Cronobacter* genus.

This work was supported by the Czech Grant Agency (project no. 13-23509S) and Specific University Research (MSMT No. 20/2014).

P072-S

Determination of yogurt culture using qPCR

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Molecular methods are being increasingly applied to detect, to quantify and to study microbial populations in food. The objective of our work was to develop a PCR-based method for quantitative determination of the two species of lactic acid bacteria in yoghurt and compare this method with classical plate count methods. Four pairs of primers were designed for each of the characteristic species of yoghurt starter culture. For *S. thermophilus*, primers targeting the genes encoding amidase and peptidoglycan hydrolase were designed. The presence of *N*-acetylmuramidase and amidase were monitored for *L. delbrueckii* subsp. *bulgaricus*. Primers were tested on positive and negative controls, PCR assays were optimized. As specific probes, we selected primers targeting N-acetylmuramidase (*Ldb_0224*) for *L. delbrueckii* subsp. *bulgaricus* detection. None of designed primers were specific for *S. thermophilus*; therefore, a set of primers from literature was applied. Real-time PCR assays were developed for the absolute quantification of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* in broth, yoghurt-like mediums and commercial yoghurts. Cultures quantification in broth obtained by qPCR differed significantly from classical bacterial counts. For example, enumeration for *S. thermophilus* using plating methods was 5.4×10^6 CFU.ml^{-1} contrary to 5.0×10^9 CFU.ml^{-1} obtained using qPCR. Higher cell number obtained by qPCR could be affected by the presence of intact DNA coming from dead cells. In contrast, the results of qPCR in yoghurt-like medium and in commercial yoghurt were lower than results obtained by classical bacterial enumeration. This may indicate that DNA extraction from yogurts was insufficient or qPCR assays were influenced by inhibitors.

P073

Evaluation of Endophytic and Rhizospheric Microflora of Transgenic Plants Prepared for Phytoremediation of Polychlorinated Biphenyls

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Since polychlorinated biphenyls (PCBs) pose a serious problem as widely spread contaminants, there is the need to remediate these contaminated sites. A possible choice for remediation can be the use

of microorganisms, plants and their consortia. To improve the phyto- and rhizoremediation abilities we designed transgenic plants of *Nicotiana tabacum* containing either (i) bacterial bphC gene encoding for 2,3-dihydroxybiphenyl-1,2-dioxygenase cleaving the aromatic ring of dihydroxybiphenyl or (ii) bacterial *bphC* and yeast *HisCUP* genes, where *HisCUP* genetic element encodes for metallothionein able to bind heavy metal ions. The aim of this work is to study the diversity of cultivable and not cultivable (viable but not cultivable, VBNC) rhizospheric and endophytic bacteria. To do that, plants were first cultivated in the soil contaminated by polychlorinated biphenyls (from Lhenice dumpsite). The results so far indicate that the rhizospheric bacterial community of transgenic *bphC* plants has not been changed. Cultivatable endophytic bacteria have been isolated, and among these *Pseudomonas, Rhodococcus, Arthrobacter, Microbacterium* and *Leifsonia* have been identified. The metagenomic endophytic DNA from transgenic plants is under investigation. Further, the phytoremediation abilities were evaluated for transgenic *bphC* plants. The results showed higher decrease of 2,3-dihydroxybiphenyl in the sterile liquid media when cultivated with transgenic plants.

Acknowledgement: This work was supported by grants MSMT KONTAKT LH12087 and GACR P501/12/P521. We acknowledge Jakub Ridl and Miluse Hroudova (IMG AS CR) for pyrosequencing.

P074-S

mi-WELT – A science dialogue for biotechnology

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Besides the visible, tangible world, there is one that cannot be seen at a first glance - the world of microorganisms.The mi-WELT project is an activity founded by the Swiss National Science Foundation (SNSF), and is aimed primarily at children from 7 to 11 years of age, focussing on discovering this hidden world of microbes both in everyday life and in the glass city, i.e. a man-made research laboratory [1, 2].

The overall project focusses on building an effective dialogue based on everyday experiences and driven by natural curiosity and the excitement of a voyage of discovery. The pedagogic concept is based on using graphic arts and creative activities to trigger and enhance the attractiveness of science to children. A child-oriented presentation of text and graphics can also be an asset to adults from groups that lack a strong affinity for education and science as well as to scientists, making clear what is substantial in their work. The combination of workshops, teaching aids and internet presence is designed to provide an insight into microbial behaviour and habitats, and in such a way explain the scientific basis for the use of microbial systems in manu-

facturing. In addition, mi-WELT is used to elucidate, in an unbiased way, the differences between biotechnology and genetic engineering.

Scientists from the Bioprocess Technology Section at the ZHAW carry out research in the field of microbial physiology. Through mi-WELT, they will breakdown complex information into graspable chunks and redevelop sophisticated experiments with the use of conventional articles/items so that they can be reproduced outside of the laboratory. The concept of a children's day, and therefore child-orientated experiments will be presented.

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P075

Survival of free and microencapsulated *Lactobacillus plantarum* G1 in an *in vitro* simulated system in presence of different beverages

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In recent years, people are increasingly interested in foods which offer a balanced diet and promote human health. This is why the demand on functional foods is growing steadily. Many recent studies are focusing on the beneficial effect of probiotics, which are one of the main components of functional foods as well as improving their capacity to survive during passage through digestive tract to arrive viable and in sufficient amounts to colon [1–3].

Therefore, the conception of an efficient, low-cost system for the delivery of probiotic bacteria is highly attracting food science researchers. In the current paper, microencapsulation within sodium alginate is tested for its capacity to improve *Lb. plantarum* G1 viability in an *in vitro* simulated gastrointestinal system designed in the laboratory. Incubation was conducted in presence of three beverages that are commonly and highly consumed by Algerian population and worldwide, they include black coffee, green tea and orange juice.

The results indicated that introducing probiotics simultaneously with one of the studied beverages resulted in a decrease in probiotic viability, however, microencapsulation within alginate beads improved its viability even at the end of the gastrointestinal simulated transit, suggesting that probiotics, when encapsulated can reach the lower intestine. The percent of cell viability for black coffee, green tea and orange juice were 59, 59 and 73 %, respectively.

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P076-S

Enhancement of biomass and lipid production by trace concentrations of iron nanoparticles

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Research of biofuels production has received wide attention because of current environmental problems, including global warming, accumulation of greenhouse gases, and limited amount of non-renewable resources. The most discussed potential source of lipids (oil) for biofuel production are oleaginous green algae which present promising alternative to traditional vegetable oil. The yield of oil which can be obtained is 200 times higher than from plants. Higher photosynthetic efficiency allows green algae to grow much faster than plants. They consume high amount of CO_2 and can be cultivated in variable climates, in marine or brackish water. Under favourable condition, harvesting is possible during the whole year. However, currently economic aspects significantly limit industrial production in practise. Therefore, numbers of studies are engaged in research of optimal culture condition that would lead to technical and economic improvement of microbial lipid production.

The aim of our research was to investigate an effect of nanoscale zero valent iron particles (nano Fe) on growth and fatty acid content of two green algae *Trachydiscus minutus* and *Parachlorella kessleri*, known producers of lipids in higher amount. The influence of concentration of iron ions in culture medium on growth and lipid production has been described. Nano-scale enables to nanoparticles interaction with cell surface. Hypothesis that nanoparticles could be more favourable source of the trace element then bulk analogues has been reported. Kadar et al. (2012) found that green algae cultivated at lower concentration of nano Fe then conventionally used amount Fe-EDTA sustained growth rates [1]. Our research confirmed positive effect of nano Fe on growth and lipid content of studied microorganisms. Further, our study showed that nano Fe amended medium also caused changes of fatty acids profile.

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P077-S

The parameters of oxygen demand and minimum aeration in a bioreactor to avoid oxygen limitation during substrate oxidation

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Biochemical and biotechnological aspects of minimum aeration conditions during application of bioleaching *Acidithiobacillus ferrooxidans* cultures were studied. Cell growth of autotrophic bacteria in bioleaching processes can be limited by carbon dioxide and oxygen. However, substrate oxidation in these bioprocesses may often be a result of the activity of non-growing cells that are not assimilating CO_2. Therefore, oxygen limitation can impact bioleaching processes more severely as compared to CO_2 limitation. The used procedures may represent a universal approach to find minimum aeration conditions in various organism cultures to maintain substrate oxidation without oxygen limitation.

The Michaelis constants for oxygen in *A. ferrooxidans* cultures were 1.07 and 0.71 μmol O_2/l for ferrous iron and elemental sulfur oxidation, respectively. This low but significant difference ($P < 0.05$) indicated a lower demand for oxygen in the case of elemental sulfur oxidation. The critical oxygen concentration (C_{crit}), below which oxygen limitation occurred, was 6.25 and 3.13 μmol O_2/l for ferrous iron and elemental sulfur oxidation, respectively. The k_La required to maintain oxygen-unlimited substrate oxidation was determined under aeration at the critical oxygen concentration using a steady-state method. *A. ferrooxidans* cultures used in the experiments reached maximum respiration rates that were comparable with values published in the literature. The k_La values for ferrous iron and elemental sulfur were 7.70 and 4.88 h^{-1}, respectively (the difference was highly significant, $P < 0.01$). The gassing-out technique confirmed the above values. The rates of iron and sulfur oxidation were also determined at C_{crit}. It confirmed that both iron and sulfur oxidations were not limited by oxygen. Although the results were obtained under experimental conditions and laboratory-scale, these k_La values may be valid as minimum aeration criteria for pilot- and commercial-scale bioreactor conditions employed in biotechnology.

Supported by the Masaryk University Program, project no. MUNI/A/0981/2013.

P078-S

A model of anaerobic dissimilatory iron reduction in the biomining extremophile

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As the extraction of rare metals from low-grade ores and wastes by traditional methods is neither economically nor environmentally favourable, bacterial metal extraction appears to be the most appropriate way. Using a (bio)process with subsequent extraction ensures metal recovery with low cost and environmental contamination. Efficiency of biomining fundamentally depends on proper conditions, in particular on the oxygen level. In the case of oxygen absence, biological reduction of ferric iron may occur in dump and heap leaching operations. Ferric iron is the main oxidant of metal sulfides and decrease in its level negatively affects the efficiency of metal extraction. In addition, the anaerobic process can contribute to environmental acidification due to elemental sulfur biooxidation by ferric iron to sulfuric acid. Anaerobic expression profiles during dissimilatory ferric iron reduction coupled to sulfur metabolism in model biomining chemolithoautotrophic acidophile *Acidithiobacillus ferrooxidans* were investigated. Real-time analysis of the process at the transcription level was performed in growing and non-growing cells, which was controlled by carbon dioxide supply. During anaerobic ferric iron reduction under non-growing conditions, the upregulation of *rus* and *hip* genes involved in iron and sulfur aerobic metabolism, respectively, was detected. Other genes were mostly expressed at the same level or downregulated. However, under growing conditions, most genes were slightly upregulated or expressed at the same level. Surprisingly, *cyc2*, a gene involved in iron oxidation, and iron regulatory gene *regB* were both overexpressed. A recent model of dissimilatory ferric iron reduction coupled to sulfur oxidation in *At. ferrooxidans* [1] was extended to include the obtained transcriptional profiles and previous kinetic and proteomic data [2].

Supported by grant no. 14-27075P from the Czech Science Foundation.

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P079

Novel Stably Transfected Gene Reporter Cell Line for Assessment of Aryl Hydrocarbon Receptor Ligands

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The (Aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor belonging to the basic helix-loop-helix/Per-AhR-Arnt-Sim (bHLH/PAS) superfamily of proteins. Ahr ligands are halogenated aromatic hydrocarbons (polychlorinated dibenzodioxins, dibenzofurans and biphenyls) and polycyclic aromatic hydrocarbons (3-methylcholanthrene, benzo(a)pyrene, benzanthracenes and benzoflavones).

We constructed novel stably transfected gene reporter cell line AZ-AHR, allowing measurement of aryl hydrocarbon receptor (AhR) transcriptional activity. Human hepatoma HepG2 cells were transfected with a construct containing several AhR binding sites upstream of luciferase reporter gene. We prepared 12 clones and we characterized the best five in responsiveness to 2,3,7,8-tetrachlorodibenzodioxin TCDD. Dose-response analyses were performed for various AhR ligands, including TCDD, 3-methylcholanthrene, indirubin, resveratrol, omeprazole, and SP600125. Induction of luciferase was time-dependent, and treatment for 6 h with 5 nM TCDD was sufficient to evaluate AhR transcriptional activity in 96-well plate format (8-24 fold induction) and to detect TCDD in a sample in picomolar concentrations. Cell line remained fully responsive to AhR ligands over 15 passages and 30 days in culture without significant alterations.

Overall, we have developed novel human luciferase reporter cell line AZ-AHR for monitoring AhR and detect toxic AhR ligands. The sensitivity of the assay allows high throughput format (96-well plate) and evaluation of luciferase activity as soon as after 6 h of incubation.

P080

CCACB - Culture Collection of Actinomycetes of the Institute of Soil Biology České Budějovice, Czech Republic

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The CCACB was founded in the Institute of Soil Biology, Biology Centre AS CR, v. v. i. in 2007. The CCACB serves as

a depository for cultures of soil actinomycetes, used mainly for screening of strains producing important secondary metabolites. Moreover, the CCACB isolates (1500) can be used for research, industrial applications, education, and general scientific interest. Most of the cultures belong to the family Streptomycetaceae – genus Streptomyces. Members of this genus are recognized as the producers of many bioactive metabolites that are useful to humans in medicine, such as antibacterials, antifungals, antivirals, antithrombotics, immunomodifiers, anti-tumor drugs and enzyme inhibitors; and in agriculture, including insecticides, herbicides, fungicides and growth promoting substances for plants and animals. The major activity of the CCACB is preserving actinomycetes which were isolated from soil and sediments of some unique habitats worldwide (caves, bat guano, glacial forefield, dead bees, Miocene and town sediments, spoil of brown coal colliery substrates etc.). Cultures are preserved mainly as glycerol conserves (-80°C) or freeze-dried. CCACB offers strains of actinomycetes in catalogue of cultures (www.actinomycetes.cz). A new dynamic electronic version of the catalogue will be presented in the end of the year 2014.

P081

Sulfides from biogas scrubbing as electron donors for denitrification

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Sulfur in any form is especially undesirable component of agriculture wastes, industrial organic wastes, sludges and wastewaters that are treated in anaerobic fermentors for biogas production. The sulfur compounds are reduced under anaerobic conditions to sulfides. Hydrogensulfide coming to biogas causes serious problems of corrosion and flue gas composition. Hydrogensulfide and sulfides can be biooxidized in an oxic or anoxic environment by different sulfur bacteria. [1]

This study was focused on an influence of sulfides and nitrates on the course and effectiveness of autotrophic denitrification with mixed culture of activated sludge. Its results confirmed that denitrification rate is gradually decreased and eventually inhibited, when sulfide concentration exceeded 200 $mg.L^{-1}$. [2]

Hydrogensulfide from biogas was removed by scrubbing with nitrified wastewater from wastewater treatment plant mixed with liquid phase of digestate from biogas plant (10 % v/v) for higher alkalinity and

content of microelements. Washing liquid was recycled to the top of lab-scale packed scrubber with counter-flow of biogas.

The washing liquid with sulfides was pumped to an external anoxic bioreactor with suspended biomass of activated sludge. After start up period of 10 days it was operated at sulfides volumetric loading rate 27 $g.m^{-3}.h^{-1}$ with an average efficiency of 98 %. The stable process of efficient sulfide removal with nitrified wastewater needs molar ratio S/N from 0.65 to 1.05 and sulfides concentration up to 60 $mg.l^{-1}$.

The results proved that activated sludge can be used as a useful source of technical culture for inoculation of desulfurizing bioreactor and a combination of biological removal of hydrogensulfide from biogas and denitrification can be a suitable method of biogas upgrading.

Acknowledgements

This research was supported by the TA-CR – project TA01020798.

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P082-S

Enzymatic synthesis of bioactive compounds: glucosylation of tyrosol

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2-(4-hydroxyphenyl)ethyl β-D-glucopyranoside (salidroside, CAS 10338-51-9) has wide range of proven biological activities as antioxidant [1,2], anti-cancer [3], anti-viral [4], anti-inflammatory [5], anti-diabetic [6] effects and also neuroprotective [7], cardioprotective [1,8], and hepatoprotective [9] properties. In traditional medicine the main source of this compound is the plant genus *Rhodiola*, which contains about 90 species (including 16 species endemic in China) and many species were historically used as an adaptogen in Russia, northern Europe, and in China [10].

We present successful synthesis of salidroside by transglucosylation using cellobiose as glucosyl donor, tyrosol as acceptor and Novozyme 188 as a robust biocatalyst with β-glucosidase activity. The optimum concentrations of tyrosol and cellobiose were 0.22 M and 0.45 M, respectively, and maximal yield (11.9 %) was achieved after two hours of incubation. The effect of pH on the catalyst activity was investigated in the range of pH 3-8, using acetate and phosphate buffers. The maximum conversions were observed at pH 3 and 4, which

are however similar to the conversion of reaction running in water without buffering. The reaction product - salidroside was purified by two chromatography steps: glucose and cellobiose were removed on column of Amberlite XAD-4 (20-50 mesh) and the resting mixture of tyrosol and salidroside was separated by flash chromatography on silica gel using gradient of methanol in chloroform for elution. Purity of the product was determined by HPLC method according to Mao et al. [11] and its structure was confirmed by NMR.

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P083-S

Influence of cultivation condition on production and secretion of fusion protein trypsinogen-EGFP by *Pichia pastoris* yeast

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Because of its high productivity and ability to secrete mature and high quality proteins, the methylotrophic yeast *Pichia pastoris* has been widely used as a host for recombinant protein production in last two decades. However, the quality as well as the quantity of the synthesised protein is strongly affected by the cultivation condition.

In this work, we focused on studying the influence of cultivation condition (pH, specific growth rate, medium feeding rate) on production and secretion of fusion protein porcine trypsinogen-EGFP cloned under control of methanol inducible alcoholoxidase promoter of the *Pichia pastoris* yeast. The fluorescent tag EGFP (enhanced green fluorescent protein) was used in order to facilitate the detection of trypsinogen production and secretion by flow cytometry measurements and fluorescent microscopy monitoring.

Firstly, EGFP was isolated and exploited as a standard for analysis (SDS-PAGE, Western blot) of the fusion protein production and protocol for simultaneous determination of cell viability and intracellular amount of EGFP with the use of flow cytometry was developed. In batch the maximum specific growth rates of the host strain on glycerol (μ_{max} = (0.210 $\pm$ 0.024) h^{-1}) and methanol (μ_{max} = (0.076 $\pm$ 0.005) h^{-1}) were determinated and fed-batch cultivations with constant or exponential feeding rate were performed at two pH values (5.0 and 5.9). Although the growth of biomass was negligibly influenced by pH, 1.7 higher concentration of fusion protein was achieved at pH 5.9. The highest extracellular trypsinogen concentation (82 $mg.l^{-1}$) was reached at pH 5.9 in fed-batch cultivation with methanol exponential feeding rate maintaining the specific growth rate at 0.013 h^{-1}.

P084-S

Heterologous expression of three laccases from different source of origin in yeasts *Saccharomyces cerevisiae* and their use for environmental application

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Laccases are "eco-friendly" oxidoreductases with a wide range of biotechnological applications [1]. In order to easily and cheaply produce laccases for synthetic dye removal from wastewaters of textile factories, three laccase genes were cloned into the S. cerevisiae expression vectors under the different constitutive promoters. The genes originated from *Myceliophthora thermophila* [2], *Trametes versicolor* [3], and *Trametes trogii* [4]. Functional expression of laccases in S. cerevisiae was detected as an ability to convert ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) to a green product. The level of expression was further optimized, and the composition and pH of the expression media were found as crucial for maximum expression and secretion of laccases. All three recombinant laccases were produced as secreted proteins due to their native N-terminal signal sequences, and thus they were easily isolated from the medium by ion-exchange and gel chromatography. The specific activity differed among the three enzymes, as well as their other parameters. The highest specific activity was found for the laccase from *Trametes trogii*, which moreover, was the only laccase showing the ability of dye decolorization.

This work was supported by TA CR grant TA0101 1461.

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P085-S

Characterization of endophytic bacteria from selenium hyperaccumulators

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The goal of this research is study of the symbiotic synergism between plants and microbes resulting in more effective bio/phytoremediation processes. We focus on the cultivable and also not cultivable

(viable but not cultivable, VBNC) endophytic and rhizospheric bacterial communities from plants hyperaccumulating selenium (hyperaccumulators, HA).

The aim of this work is to compare the bacterial diversity of selenium hyperaccumulators (*Stanleya pinnata* and *Astragalus bisulcatus* from Pine Ridge Natural Area, Fort Collins, Colorado, USA) and nonhyperaccumulators to see how the bacterial community differs and whether accumulation of selenium in plants can be facilitated by certain bacteria. Endophytic microorganisms were isolated from plant roots, stems and leaves and characterized by analysis of part of the 16S rRNA gene and MS MALDI-TOF Biotyper. Among others, the bacterial isolates were *Bacillus, Pseudomonas, Pantoea, Paenibacillus, Staphylococcus, Variovorax*. Isolates were tested for the ability to reduce selenite into elemental selenium, nitrite into nitrogen and to produce siderophores. The metagenomic DNA of endophytes was isolated in order to determine the bacterial community (VBNC bacteria) by T-RFLP analysis and will be continued by pyrosequencing.

Acknowledgement: This work was supported by MSMT KONTAKT AMVIS LH12087.

P086-S

Surface Modification of Biodegradable Poly(L-lactic acid) by Argon Plasma: Focus on Cytocompatibility for Fibroblasts and Keratinocytes

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Poly(L-lactic acid) [PLLA] is a biodegradable polymer of growing importance for applications in tissue engineering. This work deals with biocompatibility of PLLA tuned by Ar plasma treatment (3 W) in order to enhance its wettability, to support cell attachment and growth. The plasma treatment of PLLA led to strong decrease of water contact angle, dependently on time elapsed after the plasma treatment. We found that Ar irradiation of PLLA led to dramatic changes in its surface morphology, roughness and elemental chemical composition. Different physico-chemical properties of modified PLLA were studied in relation to adhesion, proliferation and metabolic activity of mouse embryonic fibroblasts (NIH 3T3) and human keratinocytes (HaCaT) *in vitro*. Plasma treatment of PLLA significantly improved adhesion and

proliferation of both cell lines when compared to pristine PLLA. The cell density on the modified PLLA foils was distinctively higher than on the untreated PLLA control and even higher than on tissue culture Petri dish. Change in expression of adhesion molecules, talin 1 and vinculin were also examined in both cell lines. The amount of these proteins found in cells cultivated on modified PLLA matrices was lower than the amount of talin 1 and vinculin in cells grown on untreated PLLA.

The authors gratefully acknowledge the financial support from the GACR project No. P108/12/G108.

P087-S

New cyanide hydratases and their use in continuous cyanide degradation

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Cyanide hydratases (CHTs) exist exclusively in filamentous fungi and they are specific for inorganic cyanide as substrate. In our previous experiments, it was found that CHT from *Aspergillus niger* K10 (CHTAn) exhibited a very high cyanide-transforming activity and, in addition, also an ability to transform nitriles [1].

The aim of this work was to isolate and characterize new cyanide hydratases, which may have distinct and possibly better biochemical properties compared to CHTAn. A homologue of CHT was selected in filamentous fungus *Penicillium chrysogenum* Wisconsin 54-1255 (CHTPc) to be biochemically characterized. To our knowledge, no CHTs have been yet isolated from this fungal genus. As the purification of CHTs from natural sources is difficult because of specific conditions of induction and low production, the enzyme was expressed in *E. coli* cells. The activity of enzyme CHTPc towards HCN was approximately 480 U/mg of protein at optimum conditions (45°C, pH 7). Its operational range was pH 5.5-9.5.

Stability of CHT activity in the cells expressing these two enzymes was studied using a continuous stirred membrane bioreactor. These experiments showed that whole *E. coli* cells expressing cyanide hydratase are usable for a long-term conversion of KCN. In the future, this could be used in industrial biotechnology for the removal of KCN from waste water, which are monitored as one of the world leading source of pollution.

As this approach is promising, this work is also currently focused on the heterologous production, purification and characterization of further new CHTs originating from *Botryotinia fuckeliana, Glomerella graminicola, Magnaporthe oryzae* and *Pyrenophora teres*.

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purification, characterization and use in continuous cyanide degradation. *Process Biochemistry* **49**, 445-450 (2013).

P088-S

Optimization of alcoholic fermentation with immobilized cells of *Saccharomyces cerevisiaen*

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The use of biofuels as an alternative to fossil fuels has expanded in the last few decades. Ethanol, which is both environmentally friendly and renewable energy source, has been considered as one of the best alternatives, leading to a dramatic increase in its production capacity. Hence, any improvement in the ethanol production technology is of huge economic importance [1]. Fermentation of media with high initial sugar concentration has been suggested as an effective way for increasing the ethanol yield. However, high content of sugar in media cause an inhibition of yeast cells due to the high osmotic pressure and high content of final products. These disadvantages can be decreased by using immobilized yeast cells which is less sensitive to high concentration of inhibitors [2]. The aim of this study was optimization of Na-alginate concentration and glucose and yeast extract content in media for ethanol production with immobilized cells of *Saccharomyces cerevisiae*. Experiments based on Box-Benhken design were carried out in batch mode under anaerobic conditions at the temperature of 30°C and agitation rate of 200 rpm for 48 h. The factor variables and their values are: glucose content (50-200 g/L), yeast extract content (0-4 g/L) and concentration of Na-alginate (20-60 g/L). For optimization of alcoholic fermentation concept of desirability function was used. Achieved model predicts that the maximum ethanol content of 7.21 % (v/v) is produced when the optimal values of Na-alginate concentration and initial content of glucose and yeast extract in media are 22.84 g/L, 196.42 g/L and 3.77 g/L, respectively. To minimize the number of yeast cell "eluted" from alginate beads and residual content of sugar additional two sets of optimization were made. Obtained results can be used for the further techno-economic analysis of the process to select the optimum conditions of fermentation process for industrial application.

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P089

Low-temperature Plasma for Biotechnology

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The low-temperature plasma is ionized gas generated by various types of electrical discharges. This cold mixture of free radicals and charged particles stores its energy in kinetic energy of electrons, and does not increase the temperature of target material objects. There are numerous works describing the biological effects of this plasma, devoted mainly to the killing of bacteria and eukaryotic microorganisms and applied in many areas. In microbiology, it represents an alternative method for decontamination and disinfection; in medicine, it may be used for decontamination of living tissues or thermolabile materials, plasma therapy of chronic wounds or skin infections. Some works describe the plasma-induced selective apoptosis of cancer cells and successful plasma treatment of malignant melanomas. Recently, the rapidly developing research is focused on the application of plasma in biotechnology processes ensuring the safety of food. There are numerous works devoted to processes and prototype devices for the surface cleaning of foodstuffs and packaging materials, preventing its mould overgrow and bacterial putrefaction of the products and microbial infections of consumers. In the treatment, both the killing of microorganisms and other processes influencing the microorganisms, e.g. the retardation of grow of non-lethally treated micromycetal spores, occur.

This presentation reviews the mechanisms of the plasma - cell interactions, the influence of the plasma on microorganisms and the possible application in biotechnology.

P090

Preparation of Mycobacterial Antigens for Serological Diagnosis of Tuberculosis

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Preparation of mycobacterial antigens and their application for the diagnosis of tuberculosis remains an important issue. Objective: preparation of mycobacterial antigens with various molecular weights and investigation of their diagnostic significance. *Mycobacterium bovis* Bovinus-8, *M. avium, M. intracellulare, M. tuberculosis* $H_3$7Rv, *M. bovis* Vallee-88, *M. bovis* BCG-1 were used in this study. Mycobacteria were grown on Löwenstein-Jensen solid and Soton liquid synthetic media. Mycobacteria were filtered through membrane filters (Millipore), concentrated, and followed by dialysis against 0.05 M Tris-HCl buffer, pH 7.2-7.4. The protein fraction of supernatant concentrate and cell lysates were detected by electrophoretic fractionation in the plate 12.5 % polyacrylamide gel. Using the method of electrophoresis, followed by immunoblotting we studied the antigenic structure of the protein fractions of different mycobacterial species in comparison with the vaccine strain BCG-1. To determine the molecular weight of antigens a set of proteins ≪ Broad Range ≫ (Bio-Rad) with molecular mass of 6.5-200 kDa were used. Antibodies to obtained antigens were determined in standard indirect ELISA using anti-bovine IgG-HRP. Serum samples from infected by mycobacterial stains as indicated above and control (non-infected) rabbits were used in ELISA. Antigens with various molecular weights from various mycobacterial strains demonstrated their diagnostic efficacy. The antigens with a molecular weight from 28 and 68 kDa showed different activity. Further studies were carried out with serum samples from cattle of intact and affected by tuberculosis areas. Possibly mycobacterial antigens could be used for the differentiation of post-infectious and post-vaccine antibodies of bovine tuberculosis. The high specificity of obtained mycobacterial antigens was also established.

P091

Probiotic properties of free and encapsulated cells of a bacteriocinogenic *Lactobacillus curvatus* G6 of human origin

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Several studies have shown that the viability of probiotics in the gastrointestinal tract and in fermented products is reduced because of their exposure to adverse environmental factors such as the conditions of the bile and acid of the GIT, heat treatment and long period of preservation. Researchers developed new methods to improve the viability of probiotics such as microencapsulation in food matrices [1]. Microencapsulation technology seems to be more promising for improving the viability since it allows probiotics to attain, under active and more concentrated form, supposed sites of action [2-3].

This study was conducted to confirm the protective effect of microencapsulation of a bacteriocinogenic probiotic lactic acid bacteria *Lb. curvatus* G6 by 2 % sodium alginate gel to maintain its viability during food product processing and storage and against the hostile gastrointestinal tract conditions. The tolerance of *Lb. curvatus* G6 to acidic pH, bile salts and pancreatic enzymes was evaluated. In addition, the effect of cold storage and heat treatment on viability was also tested.

Results showed that the strain is resistant to low pH, bile salts, digestion by pancreatic enzymes, cold storage and heat treatment, particularly when encapsulated in sodium alginate. Microencapsulation improved the viability of *Lb. curvatus* against the acidic conditions, bile salts and against the adverse conditions of simulating intestinal fluid by increasing the survival rate by 8 %, 9.5 % and 18 %, respectively. A significant protection of *Lb. curvatus cells* by microencapsulation was observed, with a rate of increase of 12.35 %, 12.78 % and 21.19 % at 40°C, 50°C and 60°C, respectively. Furthermore, microencapsulation effectively protects *Lb. curvatus* cells when stored for 15 days in cold conditions, with an improvement rate of about 18.07 %. The obtained results confirmed the efficiency of the microencapsulation in the improvement of *Lb. curvatus* G6 survival.

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P092

Barley sourdough and fermented whey – development and application in food industry

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Barley is one of the oldest agricultural crops worldwide and it is one of the economically most important plants. Most famous fermentation of barley is connected with malt processing for beer or whisky production. A use of different fermented products of barley milling products provi-

des another possibility of utilization of barley in human nutrition. In order to provide the barley sourdough, barley flour or bran were fermented using both *Lactobacillus plantarum* and *Lactobacillus sanfranciscensis* and moreover *Propionibacterium freudenreichii* subsp. *freudenreichii* under the different processing conditions.

Whey is a by-product which rises in large quantities during production of cheese. Therefore food technologists still look for new applications of whey. Sour cheese whey was used to produce organic acids (lactic, acetic and propionic acid) by batch fermentation (duration 72 hours). The product of fermentation was treated by membrane filtration to concentrate the protein fraction in the retentate and lactose and organic acids in permeate.

Barley sourdough and non-fermented whey, also fermented whey (retentate or permeate) and barley flour or bran was prepared in prototype equipment in MILCOM, Tabor. The composition of liquid, powdered and granulated fermented products were determined. The content of lactic, acetic and propionic acid was the main parameters in fermented products to use the antimicrobial activity of the acids (inhibition of the growth of usual bread spoilage molds). The content of organic acids, total fibre, beta-glucan and soluble protein was increased during fermentation of barley milling products and whey medium.

The fermented sour cheese whey was available to grow lactic and propionic acid bacteria. Filtrated fermented whey retentate and barley flour and/or barley bran was use to prepare special cereal sourdough and to offer for cereal processing (breadmaking) and also for dairy processing.

Acknowledgement: We gratefully acknowledge project no. QI111B053 *New Food* for financial support to this work.

P093

Screening of enzymatic activities for the depolymerization of hyaluronic acid among yeasts

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Hyaluronan (HA) is a linear high molecular weight polymer of β(1,4)-N-acetyl-D-glucosamine and β(1,3)-D-glucuronic acid. It is the major constituent of extracellular matrix and an important regulator of many biological processes in vertebrates [1]. Due to high rate of HA turnover, a large number of HA fragments of different size naturally occur in the organism. These fragments have wide-ranging biological activities different from that of the high molecular weight polysaccharide [2].

HA fragments are promising substances for application in Pharmacia. They can be produced by the polysaccharide depolymerization with hyaluronate 4-glycanohydrolases – a group of enzymes,

described in mammalians and a single species of *Micromycetes*. On the basis of the screening we report a group of non pathogenic yeasts as a new biotechnological sources of enzymes for HA depolymerization.

Out of 20 yeasts included in the screening, 7 species from both *Ascomycota* and *Basidiomycota* phyla were hyaluronidase positive. The HA depolymerization took place in the range of pH between 3 and 7 depending upon microorganism. The enzymatic activity was detected in both cultural broth and cells extracts. HA depolymerization by the yeast enzymes was proved by SEC-MALS and LC-MS analyses.

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P094

Development of suitable bioreactor for mass propagation of Hairy roots of *Catharanthus roseus*

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Plant hairy roots posses an immense potential of synthesizing pharmaceutically important secondary metabolites. However, it is rather difficult to mass produce the vigorously growing hairy roots in the bioreactor primarily due to their peculiar anatomical features and uneven growth leading to entangled tuft like structures which give rise to high fluid (nutrient) flow resistance and low oxygen transfer in the growing high density root cultures. The mass-scale cultivation especially the scale-up to pilot and industrial reactors has therefore proven to be an engineering challenge. For the hairy root cultures to become a potentially active player in commercial drug (bioactive compound) production market, appropriate bio reactors satisfying the specific nutrient/oxygen transfer requirements need to be developed.

In the present investigation the liquid cultures of *Catharanthus roseus* hairy root clone CP 32 maintained under statistically optimized medium conditions was propagated in three different bioreactor configu-

rations which involved complete hairy root submergence in a bubble column bioreactor, partial soaking & drying of the hairy roots in rotating drum bioreactor and a total separation of the roots from the liquid medium by immobilization of the hairy roots onto a Polyurethane foam support. The biomass and ajmalicine content obtained in the modified bubble column bioreactor equipped with polyurethane foam was 7.69±1.1 g/l and 34±4.3 mg/l respectively which was higher than the shake flask observations (biomass : 5.02±0.46 g/l and ajmalicine : 24.9±1.2 mg/l) after 30 days of cultivation. This bio-reactor demonstrated productivity comparable to shake flask cultivation studies and 1.26 fold and 21 fold higher than the bubble column and rotating drum bioreactor respectively. The present investigation clearly demonstrated the need of development of the suitable Bioreactor design for maintenance of an optimum environment for improved hairy root growth and metabolite production.

P095-S

CCMA: A new contact point for microalgal technologies in Switzerland

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Biotechnology industries have emerging interests in economically viable products from microalgae. Nevertheless, there are only a few advances in microalgal research that have been successfully transferred to industrial processes. To facilitate this technology transfer, at the Zürich University of Applied Sciences (ZHAW, Department of Life Sciences and Facility Management LSFM), four institutes have joined forces to optimally combine their expertise in several applied fields of microalgal technologies such as food, feed, cosmetics and pharma. In 2013, a special interest group, Combined Competencies in Microalgae (CCMA), was launched with the goal of establishing an interface between academic research and industry.

CCMA primarily intends to develop production processes for industry using the combined knowledge of the different institutes. The current core competencies involve cultivation techniques under both photoautrophic [1] and heterotrophic [2]

conditions in advanced facilities, which enables us to provide unique material for further research, analysis and product development. Another goal is to communicate and disseminate the joint competencies of the CCMA to promote a network of mutually interested parties that would enable future collaborations. Therefore, several colloquia [3] have been organised, to which numerous interested practitioners and researchers from academia and industry were invited for networking and discussions on possible new ideas. Another task of the CCMA is to establish and maintain a culture collection of industrially relevant, axenic cultures of microalgae. In addition to providing the cultures, supplementary material (biomass, extracts) and information on optimum cultivation conditions, and their special characteristics including analyses of biomass composition, products/metabolites, and bioactivities in cell-based assays will be acquired.

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P096-S

Water activity and probiotic shelf-life relationship

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Have you ever thought about viability of probiotics in food supplements? How is it possible, that probiotics are able to survive longer than one year or more? The answer could be in a parameter called water activity (w_a) – the most critical parameter in the shelf-life of pharmaceutical products containing probiotics.

Therefore, this work is focused on observation of w_a value and their relating parameters. We measured the relationship between w_a and viability of probiotic bacteria during their shelf-life in two different environments. Secondly, we focused on four different primary packaging materials and we measured w_a of probiotic products in different blistering foils.

The results exhibit significant decrease of shelf-life, when probiotik product w_a was around 0.25. Much better results were gained in experiments with material of low water activity (approximately 0,05 - 0,15) carried out at low w_a of material. Furthermore, the results indicate that the both parameters, permeability of oxygen and permeability of humidity across the primary packaging, have an important influence on the value of w_a during product shelf-life.

Based on the result, we highly recom-

mend selecting appropriate primary packaging materials, which are available on the marked nowadays, and thus raising the shelf-life and quality of probiotic product on the market.

P097

Biotechnological approach for the production of human collagen III with *Pichia pastoris*

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Manufacturing human collagen using recombinant *Pichia pastoris* yields an unprecedented pharma-compliant product of a quality that is not achievable by extraction from animal material. To achieve high productivities several challenges are faced: low titers, no secretion, insufficient posttranslational modifications (e.g. hydroxylation of proline, building of disulfide bridges) due to difficulties in co-expressing assisting enzymes (e.g. prolyl 4-hydroxylase, disulfide isomerase) and the lack of stoichiometric assembly of triple-helixes composed of different (procollagen) α-chains.

Typically, expression systems for recombinant (human) collagen also contained a human hydroxylase. However, this yielded insufficient hydroxylation and therefore unstable collagen molecules. In contrast, a new approach was recently demonstrated in *E. coli*, where viral prolyl 4-hydroxylase (P4H) from *Acanthamoeba polyphaga* was co-expressed [1] and showed an exceptionally high percentage of hydroxylation of the accessible proline residues [2]. *P. pastoris* was therefore doubly transformed with a fragment of procollagen and viral P4H.

In batch culture of transformants (single encoding viral P4H and double with P4H and procollagen) with glycerol, neither specific growth rate nor yield coefficient were reduced. From randomly selected clones cultured in shake flasks or stirred bioreactors, intracellular collagen was detected. Further improvements may be achieved by systematic screening for the best clones and optimisation of both the molecular construction and cultivation/production processes.

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P098

Construction of Recombinant *Lactobacillus rhamnosus* with Tetracycline Resistance

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The suicide plasmid pUC-ldhD-Ter containing homologous sequence from *Lactobacillus rhamnosus* was transformed into *Lactobacillus rhamnosus* JCM 1553. Two recombinant strains have been constructed successfully and named GL-1, GL-2. PCR was applied to clone the gene of tetracycline from the chromosomal DNA of recombinant strains. The results showed that the insertion of tetracycline gene into chromosomal DNA of *Lactobacillus rhamnosus* JCM 1553 didn't affect its L-lactic acid production and cell growth.

P099-S

Possible role of visfatin in metabolic syndrome

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Adipose tissue is highly active in secreting a variety of proteins (adipokines), visfatin is one of them. It was originally identified as a factor produced by visceral adipose tissue. This adipokine is identical to two previously described molecules. The first molecule is pre-B cell colony-enhancing factor (PBEF), a cytokine with immune regulatory action. The second one is nicotinamide phosphoribosyltransferase (Nampt), an enzyme involved in the NAD^+ salvage pathway. Besides, visfatin is involved in regulation carbohydrate and lipid metabolism.

To show biological and physiological function of visfatin, we used the RNA interference (RNAi) mechanism to silence sequence-specific expression of visfatin in murine 3T3-L1 preadipocytes, adipocytes and Fao hepatocytes.

Our results demonstrate that changes in visfatin levels regulate glucose uptake in Fao hepatocytes. In addition, we demonstrate a loss of the Namptase activity and NAD^+ biosynthetic ability of the cell lysa-

tes after decrease of visfatin expression.

2D electrophoresis in combination with mass spectrometry MALDI-TOF showed difference in gene expression under RNAi silencing of visfatin, in particular of proteins involved in glucose metabolism, TAG metabolism, or proteins connected with NAD^+ biosynthesis.

These results provide evidence that visfatin exhibits important autocrine effects on sensitivity of liver cells to insulin action possibly through its effects on NAD+ biosynthesis.

This work was supported by specific university research (MSMT No. 20/2014).

P100-S

Production of the biofungicide by *Streptomyces hygroscopicus* in lab-scale bioreactor

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Many microorganisms are plant pathogens that damage crops in the field and cause post-harvest decays. The occurrence of *Alternaria* sp. on several fruit makes this pathogen as dangerous as other more extensively studied moulds, such as *Aspergillus* spp., *Penicillium* spp., and *Fusarium* spp.[1]. In this study, isolate of Alternaria sp. used as a test microorganism was obtained from apple fruit samples expressing rot symptoms. Biological control using microbial antagonists has emerged as one of the most promising alternatives to reduce pesticide use [2]. Actinomycetes are known to have a great potential for control of plant fungal diseases. The aim of this work was to examine the kinetics of batch biofungicide production in medium containing glucose as a carbon source in lab-scale bioreactor using *Streptomyces hygroscopicus* and defining the optimal fermentation time under specified conditions. In this study, antifungal production by Streptomyces hygroscopicus was carried out in 3-litre bench-scale bioreactor (Biostat® Aplus, Sartorius AG, Germany). Fermentation was carried out at 27°C with aeration rate of 0.5 vvm during 7 days. *In vitro* antifungal activity of the cultivation liquid on *Alternaria* sp. grown on potato dextrose agar was determined every 24 h using wells technique. Additionally, the antifungal activity of cell-free culture filtrate and filtrate treated with high temperature and proteinase K was tested. Obtained results showed that maximal antifungal activity was achieved after 5 days of *Streptomyces hygroscopicus* cultivation under defined conditions. For further optimization of cultivation time techno-economic analyses of process should be included. There was no significant difference between the antifungal activities of complete cultivation liquid, filtrate of cultivation liquid and filtrate treated with proteinase K suggesting that active components are extracellular metabolites with non-protein nature. On the other hand, the filtrate treated with high temperature didn't show any antifungal activity suggesting that active components are thermo unstable.

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P101-S

Starch and lipid accumulation in *Chlorella* species under high light intensity in the laboratory and outdoor mass cultures

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Chlorella serves as a potential source of food and energy because of its high photosynthetic-efficiency. *Chlorella* accumulates starch and lipids under the cultivation of nitrogen- or phosphorous-deficiency. However, such a nutrient limitation negatively affects growth and productivity in *Chlorella* species. Alternatively, we focused on the high light-intensity (600-μmol photons m^{-2} s^{-1}) as an inducer of starch and lipids, and investigated productivity of starch and lipids using eight strains of six *Chlorella* and *Parachlorella* species [1]. The 12:12-h light–dark (LD) cycle conditions elicited more stable growth than the continuous light (LL) conditions, whereas the starch and lipids yields increased in LL conditions. Accumulation of starch and lipids was investigated in the eight strains of six *Chlorella* species. The accumulation was strain-dependent and varied according to the medium and light conditions. Five of the eight strains of the six *Chlorella* species exhibited similar accumulation patterns. These results suggest that Chlorella produces constant yields of materials regardless of the growth conditions under the high light-intensity condition. We also demonstrated the production of lipids through large-scale cultivation of *Parachlorella kessleri* PK4 in a thin layer photobioreactor (TL-PBR) [2], under strong sunlight of the late summer in 2013. During culture for 3 weeks, maximal lipid-productivity was 0.59 g l^{-1} day^{-1}, biomass density was 5.8 g l^{-1} dry weight, and total lipid-content was more than 66 % dry weight. P. kessleri PK4 successfully produced the lipids under lipid-inducing conditions in the TL-PBR.

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P102

Pullulan/Gelatin Microspheres for Chondrogenic Regeneration

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Tissue engineering approach is a promising alternative for repairing degenerated cartilage tissue in which self repair process is difficult. Microencapsulation of cells in hydogels provides a three-dimensional cell delivery system that can be used for cartilage tissue engineering. Polysaccharides such as alginate, chitosan, agarose are among the most widely used bioplymers for cell encapsulation.

We aim to develop a novel injectable microcarrier using gelatin and a microbial exopolysaccharide named pullulan for cartilage regeneration.

Cell encapsulated microspheres were prepared through water-in-oil emulsion and stabilized by crosslinking of gelatin and oxidized pullulan. Crosslinking was achieved with borate by a modified protocol of Balakrishnan and Jayakrishnan. [1] In order to find optimum gelling time for microcapsule formation, different degrees of oxidation of pullulan was tested and various ratios of pullulan:gelatin combinations were studied . Morphology, surface structure, porosity and average diameter of crosslinked microspheres were assessed with microscopic analysis. The viability, proliferation and chondrogenic differentiation of encapsulated mesenchymal stem cells in these hydrogel carriers are being conducted.

We here introduce data to demonstrate that pullulan/gelatin microcarriers may serve as an injectable system for cartilage tissue engineering

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P103

Breaking the chitin armour: preparation and use of natural and recombinant chitin-degrading enzymes

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Modern waste management emphasizes economic and environment friendly exploitation of industrial by-products. Within the FP7 ChiBio project we focus on paving the way to efficient bio-processing and usage of chitin-containing waste from the crustacean industry. One of the important steps in this process is enzymatic degradation of polymeric chitin to the monocomponent sugar N-acetylglucosamine. To achieve this goal we exploit both natural cocktails of chitin-degrading enzymes secreted by *Serratia marcescens* and mixtures of recombinant chitinolytic enzymes produced in *E.coli* for the biodegradation of pre-processed chitin. All major *S. marcescens* chitinolytic enzymes were produced in *E.coli*: chitinases A, B and C, the lytic polysaccharide monooxygenase called CBP21, and the chitobiase. We are currently evaluating the efficiency of the natural chitinolytic *S. marcescens* cocktail alone, the cocktail supplemented with one or more of the recombinant enzymes, and mixes of the recombinant enzymes for their efficacy in degrading chitin from various sources. The composition of the reaction mixtures and other parameters (reaction time, temperature, mixing etc.) are being optimized with the ultimate goal of identifying the most efficient and economical setup for the bio-processing of crustacean chitin.

Supported by FP7 project ChiBio (No. 289284).

P104

Selection of Signal Peptide for Effective Secretion of Recombinant Protein in Eukaryotic Cell

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Selection of signal peptide is the important factor that influences in proteinic secretory production. Then, 11 kinds of signal peptides (sericin, melittin, fibroin, calreticulin, luciferase, chitinase, feline IFNω, porcine interleukin 2, chicken lysozyme, human lysozyme, bovine lysozyme) were used to secrete porcine lysozyme (PLY) to carry out those comparative evaluation. The genes coding hybrid

sequences composed of each signal peptide and PLY, were synthesized artificially and expressed in insect cells (BmN4, Sf-9, *expres*SF+) or in yeast (*Saccharomyces cerevisiae*). The effect of substitution of signal peptide on secretion of recombinant porcine lysozyme (rPLY) was studied. In comparison with the original signal peptide of PLY, 4 kinds of foreign signal peptides (sericin, chicken lysozyme, bovine lysozyme, porcine interleukin 2) enhanced the secretion of rPLY in *expres*SF+ cell, but the other signal peptides did not. In the 11 kinds of signal peptides, there were 15 times as many differences in the productivity of rPLY at the maximum. Then, chicken lysozyme signal peptide (CLSP) was altered to improve the secretion of rPLY in insect cell (*expres*SF+ cell) or in yeast. Addition of arginine residues at the N-terminal region of CLSP was effective for secretion of rPLY in yeast, but not in insect cell. However, the increase of leucine residues in the central core region of CLSP enhanced the secretion of rPLY in both insect cell and yeast. Although they are eukaryotes, the structural requirements of signal peptide have some differences in yeast and insect cell in the N-terminal region, but those of the central core region may be similar over species.

P105

Isolation and identification of new chitinolytic fungi *Petromyces aliaceus*

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It is believed that chitinases play important physiological roles in filamentous fungi since chitin is one of the major cell wall components in these organisms. In this study we present a screening and identification of a new fungal isolate that could be used for production of chitinase. For this purpose soil samples, as well as from death insects were collected and processed for further investigation. Eighteen isolates producing chitinolytic activity on synthetic medium with sole carbon source colloidal chitin were segregated. The one yield highest chitinase activity was identified (by sequencing of the rDNA ITS fragment) as an unreported before chitinolytic strain *Petromyces aliaceus*. Subsequent cultivation in bioreactor yield a chitinase activity adequate to other reported in literature. The producing level is highest between 72 h and 96 h and highest chitinase activity was achieved on 120 h of cultivation.

P106-S

Esterification of 9-(2,3-dihydroxypropyl)adenine by sol-gel immobilized *Pseudomonas fluorescens* lipase

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Different free lipases have been used previously for the esterification of antiviral compound 9-(2,3-dihydroxypropyl)adenine (DHPA) in a polar solvent [1]. Based on this work, the lipase from *Pseudomonas fluorescens* was chosen for its good catalytic properties towards longer chain vinyl esters, and it was immobilized by the sol-gel method using three types of hydrophobic silane precursors: propyl-, octyl- and phenyltrialkoxysilane.

The prepared immobilized biocatalysts were characterized by means of their transesterification and hydrolytic activities towards model substrates, 4-nitrophenol and its esters. The sol-gel lipases were then used for esterification of DHPA with vinyl esters of various chain lengths (C2 to C16) in a nonpolar solvent; the reactions proceeded with up to 90 % conversion of DHPA.

Acknowledgments This study has been financially supported by a research project of the Academy of Sciences of the Czech Republic (Project No. M200551203). The work is a part of a research project of the Institute of Organic Chemistry and Biochemistry AS CR (RVO: 61388963).

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P107

Evolution of fatty acid content during alcoholic fermentation of Carménère wine using GC-FID

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Fatty acids play an important role during wine alcoholic fermentation; however, they have not been studied with detail, specifically their behavior across the fermentation. Previous studies revealed two kinds of fatty acid during the progress the alcoholic fermentation and with different roles.

Medium chain fatty acids ($C_{6:0}$, $C_{8:0}$, $C_{10:0}$ and $C_{12:0}$), they are toxic to the yeast; and unsaturated long chain fatty acids ($C_{14:1}$, $C_{16:1}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$) which are essential constituents of the cell membrane and they are survival factors under stress conditions for the yeast. The presence or not of these substances at different levels of concentration may cause fermentations with abnormal behaviors. There is not enough study about their evolution through fermentation and their behavior.

For that reason, in this work we are studying the content of medium and long fatty acids during alcoholic fermentation of Carménère variety, in normal operational conditions (T=28 °C and initial oxygen saturation by 15 minutes) at laboratory scale. We are measuring each 24 hours and quantifying by gas chromatography with flame detector (GC-FID). For extraction of analytes chloroform-methanol 2:1 (v/v) was used subsequently for derivatization, first was saponified with 1 mL NaOH/MeOH O, 5N and then methylated with 1 mL of BF_3/MeOH 10 % (w/v). The evolution of these has been determined in different varieties of grape must but never before in Carménère or more than three points of fermentation. Preliminarly, concentrations of medium chain fatty acids were determined in a saturated range from 0.12 to 24.89 [mg/l] throughout the fermentation, being the C10:0 which showed the highest concentrations. Additionally, long chain fatty acids were determined in unsaturated range of 0.06 to 14.95 [mg/l], and it was observed for C18:2 the highest concentrations at the start, but they decreased at the end of fermentation.

P108

Immobilized yeast as strategy to control the ethanol level in wine

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Nowadays, low alcohol wines present in the market have been produced by dealcoholization or alcohol elimination using reverse osmosis, vacuum distillation and evaporation technologies. These are effective and legal technologies in Europe since 2009, but the high cost and change of sensory wine quality after the process make them disadvantageous. Dealcoholized wines have about 0.5 % alcoholic degree. However according to the international legislation (OIV) any beverage has to be at least 8.5 – 9.0 % to be called wine.

This work studied the immobilization of yeasts on different supports with the aim to manipulate the fermentation residence time of the yeasts in the must and so achieving the desired alcoholic degree. Natural (grape wastes) and synthetic (calcium alginate pellets) supports were tested for this

purpose. During the fermentation the yeast count, density, sugars and ethanol were monitored. In addition sensory quality parameters were evaluated. A protocol of immobilization yeast was developed and implemented. Normal, coated, and dehydrated encapsulated yeasts were tested. In addition, results of laboratory fermentations showed similar behaviors of fermentations using soluble and immobilized yeasts. Preliminary results showed that it is possible to produce wine with reduced alcohol using yeast immobilized, which was withdrawn at 84 hours of fermentation, reaching about 8 % [v/v]. However, we are now validating these results, and study the strategy to consume the remaining sugar in the must, once separated the immobilized yeast from the medium.

P109-S

Production of (*R*)-mandelic acid by nitrilases from filamentous fungi – comparison of the co-solvent and fed-batch setup

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The enzymes tested in this study fall into the group of arylacetonitrilases, enzymes potentially applicable in the production of optically pure α-substituted carboxylic acids or amides from the corresponding nitriles.

The nitrilases from *Aspergillus niger* (NitAn), *Neurospora crassa* (NitNc), *Nectria haematococca* (NitNh) and *Arthroderma benhamiae* (NitAb) were overexpressed in *E. coli* and identified as arylacetonitrilases [1]. As the whole-cell biocatalysts, they hydrolyzed 100-560 mM (*R,S*)-mandelonitrile enantioselectively. The substrate was added in the batch or fed-batch mode. The batch biotransformations were performed in buffers of pH from 5 – 10 with addition of toluene as a co-solvent. The fed-batch experiments were done in the buffer of pH 10 and consisted of 5 or 12 feeds of 0.33 or 0.67 g of (*R,S*)-

mandelonitrile.

In the batch experiments without toluene, 500 mM substrate was converted to 75-77 % in NitNc and NitAn. NitAb converted about 90 % of the substrate regardless of the co-solvent presence. NitNh was only able to hydrolyze lower mandelonitrile concentrations (250 mM) with good yields.

The addition of toluene (4 or 10 % of the total volume) and an increase in pH (to 9 or 10) improved not only the substrate conversion, but also the enantioselectivity of all tested enzymes - the e.e. was increased from 69 to 94 % in NitAn, from 73 to 96 % in NitNc, from 73 to 97 % in NitNh and from 43 to 73 % in NitAb.

In the fed-batch mode, NitAn showed the highest volumetric productivity (545 g l^{-1} d^{-1}) and catalyst productivity (39.3 g g^{-1} of dry cell weight) in the absence of co-solvent.

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P110-S

Biofuels and nutraceutical compounds from wet biomass of Chlorella

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The great biodiversity and variability in the biochemical composition of the biomass obtained from cultures of microalgae and technological improvements in mass production, have favored the commercial cultivation for the production of high value compounds as bioactive compounds (eg.: PUFAs, carotenoids). Within this context the necessity of enhancing biomass of microalgae and the challenge to produce, extract, identify all compounds that microalgae can offer and intelligently use this type of biomass. This study aimed to develop a new technological route to produce fatty acids to obtain biofuels from wet microalgal biomass in order to obtain high value-added co-products. The wet biomass of *Chlorella* sp. was treated with enzymatic hydrolyzes (Betaglucosidase), to get the sugars in addition to extraction, also direct saponification for the separation of fatty acids was demonstrated with NaOH and KOH. The method for separating unsaponifiable compounds containing carotenoids

was shown. The transesterification of fatty acids from wet biomass was further demonstrated by the characterization of biodiesel. The biomass of *Chlorella* was characterized through various methods, such as analysis of total carbohydrates by methanolysis, FTIR, SEM and TEM morphology, HPLC, GC-MS, CG-FID, TGA and organic chemical analysis for the determination of nitrogen, sulfur and carbon. From 100 grams of biomass was possible to obtain approximately 7 % purified biodiesel, 12-14 % of total lipids (with large amounts of phenolic compounds and PUFAs), 7 % fatty acid, 20 % sugar, 60 % proteins and 2 % unsaponifiable fraction (contain carotenoids). Wet biomass handling processes, like saponification, benefit from the fact that complete dewatering of the algal stream is not required, thereby saving in related drying costs. Finally, a biorefinery concept has been proposed for the use of biomass of microalgae may prove to be a promising source for biofuels production and simultaneously obtain different compounds of high commercial value.

P111

Diagnostic tool for monitoring histone modifications using chromatin immunoprecipitation

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DNA together with histones forms a nucleoprotein structure which wraps around histone octamers and thus forms the basic structure of chromatin. Histone modifications (e.g., acetylation, methylation, and phosphorylation) are predominant part of the mechanisms of gene regulation that requires free access to the DNA of individual regulatory proteins (e.g., transcription factors) and complex systems.

Anti-tumor therapy, for the purpose of influencing chromatin structure is built primarily by inhibition of enzymes involved in the acetylation status of histone proteins. The aim of our study is to develop a commercial version of the kit and diagnostic software that integrates two detection methods ChIP (chromatin immunoprecipitation) and MeDIP (meDNA immunoprecipitation) reflecting the condition before and after treatment with chromatin-modulating drugs.

SureChip and MeDIP protocols based on immunoprecipitation were established upon a several optimization and validation steps. The complexity of the whole set was supplemented by SureSoft diagnostic software, which was developed in Java using NetBeans Platform application.

The final product is optimized metho-

dology based on chromatin immunoprecipitation. The most preferred conditions of the immunoprecipitation were chosen three hour incubation chromatin specific antibody, and thereafter isolation of these complexes by using magnetic beads. Software SureSoft is divided into several panels. They give information about the value of enrichment of histone acetylation and DNA methylation patterns between the observed and actual number of copies of the DNA in the sample. The final output of the diagnostic evaluation is examining the report on the results obtained, the patient's response to treatment with chromatin modulating agents.

The proposed essay is among others specific for hemato-oncological patients or for some subtypes. Intuitive software tool for the analysis and interpretation of data from analyses SureChIP and SureMeDIP measured using qPCR allows the prediction of response to the therapy. This work was supported by grant FR-TI2/509.

P112

Antifungal Coating For Preservation Of Perishable Small Fruits

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Recently the consumer interest towards small red fruits is also due to the recognized human health benefits of these fruits when consumed regularly[1]. Unfortunately, one of the main problems that these products have, is the extreme perishability of the fruit destined for fresh consumption. In view of the growing interest of the market, some researchers are implementing their studies with respect to the issues of quality preservation of small red fruits in post-harvest, with the aim to preserve high organoleptic and nutritional characteristics at the same time.

Optimization techniques for the preservation and management of post-harvest may contribute to the enhancement of these crops and their placement at a lower cost and higher quality on the market. The choice of the most appropriate storage conditions will depend on the destination, quantity and preservation period of product, of course.

The use of suitable coating techniques can minimize the loss of product, preventing alterations by pathogens and time, maintaining the quality characteristics similar to those of the product just harvested.

In this study we exploited the ability of an active coating to slow down the fungal attack on small fruits highly perishable, such as strawberries.

The active coating is a complex obtained by spray-drying inclusion of peony extracts in chitosan [2] and subsequent additivation to polysaccharides gel. Chemical, microbiological and sensory analyses were carried out to assess the antimicrobial effectiveness of this new coating.

The microbiologic tests [3] showed a very high antifungal activity of the edible active coating also at very low concentration of the peony extract. Particularly, antimicrobial activity assays have shown that the combination of natural extracts and chitosan have the ability to inhibit the growth and kill of some potentially pathogenic microorganisms with efficacy comparable to that of toxic antifungal agents conventionally used in therapy.

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P113

Metabolic Engineering of *Saccharomyces cerevisiae* for Accumulating Pyruvic Acid

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Pyruvic acid is an important organic acid, which is an important intermediate metabolite in *S. cerevisiae*. In order to get a high yield of pyruvic acid and increase the productivity, it is necessary to block the metabolism of pyruvic acid. There are three main enzymes which can catalyze pryuvic acid for further metabolic[1]. But its main metabolic pathway is metabolized to acetaldehyde by pyruvate decarboxylase (PDC), and further produce acetaldehyde into ethanol. So we tried to decrease the PDC activity by metabolic engineering to accumulate pyruvic acid. There are three structural genes (*pdc1, pdc5* and *pdc6*) that encode active PDC isoenzymes[2]. *Pdc1* and *pdc5* gene are the main structure genes coding PDC. In this research, we disrupted *pdc1* gene and *pdc5* gene to block the further metabolism of pyruvic acid using

metabolic engineering principles, and thus to accumulate pyruvic acid.

The plasmid TP1K contains a *pdc1* gene disruption cassette (P1K) and the plasmid TP5H contains a *pdc5* gene disruption cassete (P5H). First, P1K was transformed into *S. cerevisiae* Y2 by LiAc/SS carrier DNA/PEG method. Positive transformants were selected by G418 resistance and further confirmed by PCR and southern blot. Hence, we got Y2-1 with *pdc1* gene disruption. Second, P5H was transformed into *S. cerevisiae* Y2-1 by LiAc/SS carrier DNA/PEG method[3]. Positive transformants were selected on YNBG plates with histidine supplement and further confirmed by PCR. Hence, we got Y2-15 with both pdc1 gene and *pdc5* gene disruption. At last, the PDC activity of Y2-15 was about 2 % that of the parent strain and it could accumulate about 24.85g/L of pyruvic acid in 96h, which increased the yield of pyruvic acid by more than 1900 % by shaking flask cultivation as compared with the parental strain Y2.

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P114

Molecular Properties and Antioxidant Activities of Polysaccharide-Protein Complexes from Mycelial Culture of a Medicinal Fungus *Cordyceps sinensis*

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Polysaccharides (PS) from edible and medicinal fungi provide an attractive source of nutraceutical and pharmaceutical products because of their remarkable antitumor, immunomodulatory activities and other medicinal properties. *Cordyceps (Ophiocordyceps) sinensis*, the Chinese caterpillar fungus, is a famous and precious medicinal fungus in China with a wide range of health health-promoting and pharmacological activities such as anticancer, antioxidant, antiaging, and antifatigue. Because natural *C. sinensis* is rare and very expensive, mycelial culture of a *C.sinensis* fungus Cs-HK1 has been established in our lab and applied to liquid or submerged fermentation for production of mycelium biomass and bioactive PS. The exopolysaccharide (EPS) isolated from the liquid medium of Cs-HK1 mycelial culture was composed of polysaccharide-protein (PSP) complexes in a wide molecular weight (MW) range from ∼5 kDa to more than 10,000 kDa. The high-MW fractions of EPS were mainly composed of protein-

free PS and the lower-MW fractions had higher protein contents ranging 20-50 % (w/w). The low-MW fractions with higher protein contents exhibited more significant antioxidant activities.

P115-S

Selenium bioaccumulation and toxicity in *Chlorella sorokiniana* cultures

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The aim of this work was to investigate selenium bioaccumulation and toxicity in green microalga *C. sorokiniana* batch and continuous cultures. Microalga *C. sorokiniana* was cultivated in batch culture with sub-lethal selenate concentration of 40 $mg.L^{-1}$ in order to study the effect of selenate on culture growth, photosynthetic efficiency, cell ultrastructure, protein expression and selenomethionine (SeMet) accumulation.

Exposure of *C. sorokiniana* to selenate decreased culture growth and oxygen evolution rates but had no effect on pigment content. Se toxicity in *C. sorokiniana* cell was confirmed by ultrastructural alterations of chloroplast: fingerprint-like thylakoids, granular stroma, autophagic vacuoles, plastoglobules and overproduction of starch. Based on growth rate inhibition, toxicity of selenate was expressed as EC_50 with a value of 238 μM. Selenoproteins which appeared in the protein isolates of Se-treated cells, but not in Se-free cells, were identified as 53 kDa large subunit of *C. sorokiniana* Rubisco enzyme, suggesting that Se interferes with proteins located in chloroplast and might be incorporated into proteins as Se-amino acids.

Batch culture of *C. sorokiniana* exposed to 40 $mg.L^{-1}$ selenate accumulated up to 140 $mg.kg_DW^{-1}$ of SeMet after 120 h of batch cultivation. Continuous biomass production of *C. sorokiniana* in selenate-added culture medium was feasible by carefully selecting sub-lethal selenate concentrations which allowed both cell viability and high growth rates. Based on productivity and yield on light energy, optimal selenate concentration for long-term continuous cultivation of *C. sorokiniana* was 40 $mg.L^{-1}$. In a 2.2 L glass bioreactor up to 0.246 $mg.L^{-1}day^{-1}$ (335 $mg.kg_DW^{-1}$) of SeMet could be produced in continuous cultivation. Production of Se-enriched biomass as a secondary product of algae farming could in future represent one of the possible factors contributing to cost reduction of microalgae fuel production if Se-enriched microalgae became sufficiently attractive for the health food supplement market.

Acknowledgements
This work was supported by the grant from Agrifood Campus of International Excellence (ceiA3) (Spain) for foreign PhD-students.

P116-R

Recombinant expression profile quantitation in *E. coli* fermentation.

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Quantification of recombinant protein expression during fermentation processes in mid- or large scale is a challenging approach to confirm the efficiency of fermentation process. To get over the issue of comparing fermentation processes employing different cultivation conditions we used two common bioanalytical techniques: high performance liquid chromatography (HPLC) and quantitative SDS-PAGE to establish a quantitative view on fermentation process. We compared the SDS-PAGE and HPLC approach for two different endoproteases (ALV001 and ALV002). The results showed that both methods could be successfully applied to reveal the expression profile in *E. coli* fermentation process and establish the baseline for further optimizations. Acquired data indicate good comparability as well as good repeatability (bellow 5 %) and recovery (93-103 %) for both methods. Differences were obtained for linear range favouring HPLC technique (2-15 mg of cells with average relative standard deviation of 2.8 % across levels). To conclude, it is obvious that also HPLC approach can be used for quantitation of proteins in complex fermentation broth including quantitative solubilisation step.

P117-S

Objectification of methods for biomass growth measuring

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A common issue for the fermentation processes is the determination of the inoculum concentration. During the fermentation, it is very important to observe the growth of biomass. The most common measurements used for biomass growth determination are cell weight (wet, dry), plate count, free sedimentation and optical density. Optical density measurement is rapid and easy to perform. However, the readings are affected by the optics of the system, homogeneity of the sample and dilution. It must be stressed that for the technology transfers it is crucial to run the process within the target ranges. This study provides an approach to justifying the differences in biomass growth measurement, focusing on the bacterial optical density measurement. From the industrial point of view, the advantages of this approach are

knowledge and statistically based discussion with the customer.

P118-S

Crystallization of decarboxylase-like enzyme

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Crystallization of decarboxylase-like protein was used in downstream process as the last and main purification step. Crystallization conditions leading towards best yield and crystals of defined size were optimized. Tuning of parameters such as reverse vs. direct crystallization buffer addition, velocity of buffer addition, speed of stirring, impact of initial protein concentration and temperature will be discussed. Results will be supported by crystal microscope pictures, flow particle image analyses and RP HPLC.

P119-S

Green synthesis of Silver decorated Reduced Graphene Oxide for Antimicrobial and Biosensing Applications

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Silver nanoparticles have been long used in medical diagnosis, drug delivery systems, wound healing and biosensing. Most of the conventional synthesis methods suffer from low yield, high-energy requirements, and a need for difficult and wasteful purifications as well as unstable and forms agglomeration. By growing silver particles onto reduced graphene oxide (RGO), a uniform silver nanoparticles formation was observed on the RGO platform. In order to curb toxicity, a one-pot green synthesis was developed to produce silver loaded RGO by using a mushroom extract, *Ganoderma lucidum* as the reducing agent. Besides simple and inexpensive, this synthetic route will eliminate the need of organic solvents and does not leave any residual by-products. Several characterization techniques such as XPS, TEM, Raman, DLS, XRD and FESEM confirm the successful formation of Ag-RGO composite. The composite was found to show good antimicrobial activities, specifically towards wound bacteria, due to synergistic effects of silver and RGO. This was also due to RGO platform, which was able to load more silver particles, thus increasing the surface area to volume ratio and demonstrating improved antibacterial properties.

In addition, due to the enhanced electrical properties of the composite, it also showed promising application in non-enzymatic H_2O_2 detection. Due to its cost effective synthesis, the composite can be used for fabrication of wound dressings, scaffolds, bandages, medical device as well as for biosensing purposes.

P200-S

Growth profile of gilthead bream in mega flow recirculating aquaculture system

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This study was primarily designed to compare the effect of two exchange rates (ERs; 0.5 and 1.5 m^3 feed /kg) in recirculating aquaculture systems (RAS) on water quality and fish growth profiles on fish stocks (200 indiv/m^3 of 251.4±20.67 g) of gilthead bream, *Sparus aurata*. Water quality, fish growth, specific growth rate and feed conversion ratio under the two ERs, were examined for a period of six months (Nov 2009-April 2010). Inorganic nutrient in RAS, temperature, dissolved oxygen and pH were measured daily. Feeding regime effect was also determined on the fish quality and organoleptic characteristics and thus opt to energy optimization of the system.

Results suggest that the 1.5 m^3 feed /kg positively affect the fish quality and fish growth profile.

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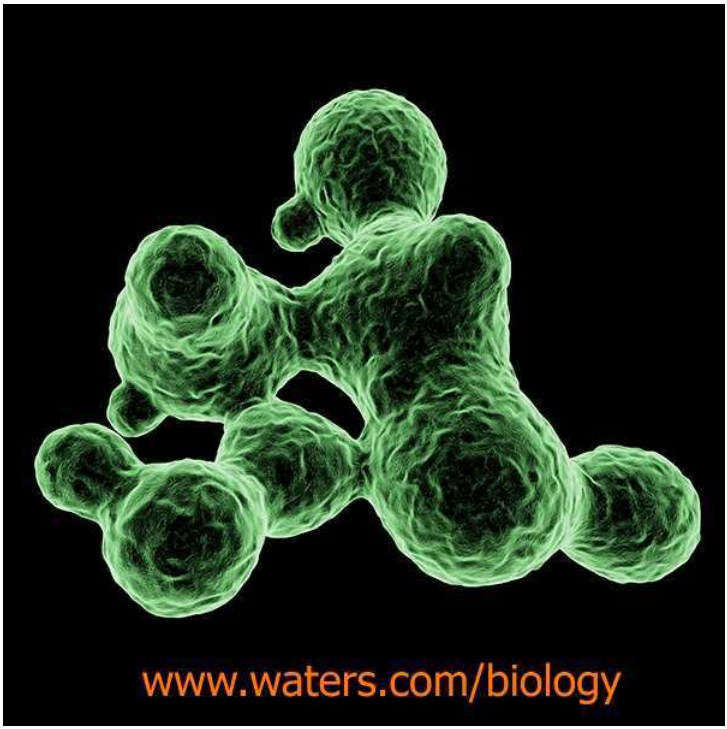
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Pre-Conference Workshop: Biotechnology for controlled remediation of sites contaminated with chlorinated ethenes: Czech-Swiss teamwork joins research

Techtool project: Integrative technology for assessment and enhancement of complete removal of chloroethenes from groundwater

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Chlorinated ethenes (CEs) - tetrachloroethene (PCE) and trichloroethene (TCE), are used as degreasing agents and solvents worldwide. Improper use, storage and waste disposal, together with the high mobility, persistency and toxicity place them among the major groundwater and soil pollutants. Their remediation is complicated due to their characteristic features of long plumes and DNAPL. Enhanced reductive dehalogenation (ERD), supporting a faster sequential microbial reductive transformation of PCE and TCE to the non-toxic end-product ethene, belongs to the most efficient remediation techniques. However, the initially successful process can be halted at *cis*-1,2-dichloroethene and carcinogenic vinyl chloride, and in several cases the reasons for incomplete reduction are unknown.

In the Czech-Swiss **Techtool** consortium, the undesired phenomenon of dechlorination stalling is holistically addressed by linking the knowledge achieved in research laboratories – molecular ecology, microbiology and numerical ecology, with the practical experience and development efforts of the environmental companies. Final goal of the project is to better understand the complex transformation of CEs, thus, finding the optimal remedial conditions for improving the biotransformation capacity of the autochthonous microbial communities. For the purpose, a database is developed as a repository for information on nine contaminated sites throughout the Czech Republic, where ERD technologies are used for remediation of CEs. Multivariate statistical analysis is applied for explaining the interconnectedness of geochemical data and hydrogeological conditions with shifts in the bacterial community structures, presence of dechlorinating bacteria and reductive dehalogenase genes.

The main emphasis is put on the comprehensive interpretation of the multidisciplinary approach outcomes in order to effectively meet the needs of practice, to support companies' competitive advantage, and to move the state of knowledge in a field, contributing to the community well-being – the remediation of environmental damages.

The **TECHTOOL** consortium was supported by the Technology Agency of the Czech Republic, program ALFA – grant TA02020534.

Remediation companies' perspective on the stimulated reductive dehalogenation of chlorinated ethenes: Case studies

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Chlorinated ethenes are, after the petroleum hydrocarbons, the second most frequent pollutant worldwide. Enhanced reductive dehalogenation (ERD) leading to transformation of tetra- and trichloroethene (PCE and TCE) to non-toxic final products ethenes and ethylenes is one of the most used remedial approach. The principle of ERD is application of an appropriate organic substrate (emulsion of vegetable oil, HRC – hydrogen released compounds, molasses, lactate, cheese whey) into the aquifer environment, and creation of anaerobic *in situ* reactor where selective stimulation of biodegrading bacteria takes place (*Dehalococcoides*, *Dehalobacter*, *Anaeromyxobacter*, *Geobacter* etc). Among the anaerobic bacteria known to use PCE and TCE as electron acceptors for supporting their growth, *Dehalococcoides* are the only known bacterial group capable of complete reductive dechlorination of PCE to ethene (perchloroethene > trichloroethene > *cis*-1,2-dichloroethene > vinylchloride > ethene, ethane). At some sites, for yet not determined reasons, a cessation of the transformation occurs resulting into accumulation of cis-DCE or carcinogenic vinylchloride (VC). This phenomenon is called "DCE stall". The incidence of the above phenomenon during realization of remediation leads to significant increase of the remediation costs. In order to comply with the contractual obligations, the remedial companies have to cover the elevated costs from their own resources. Chemical factors (presence of terminal electron acceptors, pH) and hydrogeological conditions (unevenness in groundwater flow) can influence diversity and biodegradation activity of the aquifer bacteria. That's why it is necessary to work with a larger set of data from several contaminated sites in order to better understand the complex transformation of chlorinated ethenes, which is one of the goals of the Techtool project. This presentation demonstrates a case study on three sites (out of total nine sited tested) where cheese whey was used as organic substrate for ERD. The data will be presented in unified form of time-dependant monitoring with corresponding changes of main chemical parameters selected for the three localities. Thus, complete information of each test-site is provided. Outputs from microbial, molecular-genetic and statistical methods related to the three localities will be presented by the other Techtool partners with a referral to the information included in this paper (Brennerova, Tarnawski, Holliger, Mikes, Sakmaryova).

This study was funded by grant No. TA02020534 – **TECHTOOL** of the Technology Agency of the Czech Republic.

The use of nanofibers for production of biomass carriers

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Growth in biofilms is a general ability of microbial populations which are historically used in wastewater treatment. The basic aim of biofilm formation is the fixation of microorganisms at a given place as a means of stabilized living conditions. In a biofilm environment, organisms are partially protected against negative environmental influences. Compared to dispersed growth, biofilms offer many advantages that enable their use in specific biological treatment of industrial wastewater. Main advantages are: i) the increase in the residence time of biomass in the biofilm reactors allowing the concentration of slow-growing microorganisms, and ii) the diffusion barrier of the biofilm which reduces the unfavourable impact of toxicants and suboptimal physico-chemical conditions. The development of the biofilm depends on many factors, from the surface properties to the supply of nutrients and hydrodynamic forces in the bioreactor. Objective of many research projects in the field of wastewater treatment is to technologically improve the biomass carrier. Final requirements are excellent colonization, high cleaning efficiency given the maximum specific surface area, optimal density and ease of production.

Based on these crucial parameters the Technical University of Liberec initiated development of a new types of carrier which is based on the use of polymeric nanofiber materials. This has resulted in a yarn which consists of a carrier fiber supporting a layer of nanofibers with a diameter in the order of hundreds of nanometers. The aim of the contribution is to highlight the possibilities and potential of biofilms in presence of a composite nanofiber carrier.

Priority of the nanotechnology is the high protected specific surface area which promotes the initial adhesion and further development of the microbial population on the surface of the carrier. Additional protection of the population from surrounding adverse effects, enabling an easy supply of nutrients, and supporting the compactness of the biofilm are ensured.

This study was funded by grant No. TA02020534 – TECHTOOL, of the Technology Agency of the Czech Republic.

The results of this project LO1201 were obtained through the financial support of the Ministry of Education, Youth and Sports in the framework of the targeted support of the ”National Programme for Sustainability I” and the OPR&DI project Centre for Nanomaterials, Advanced Technologies and Innovation CZ.1.05/2.1.00/01.0005.

Assessment of bioremediation potential and monitoring of biological reductive dechlorination in sites contaminated with chlorinated ethenes

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Chlorinated ethenes (CEs), such as perchloroethene (PCE) and trichloroethene, are one of the most common classes of groundwater contaminants. In this project, the contaminant biodegradation capacities of two aquifers, presenting both dichloroethene (DCE) and vinyl chloride (VC) accumulation, was carried out. Aquifers are considered nowadays as dynamic ecosystems, showing multiple interactions between the physical, chemical and biotic components. In this sense, an integrative methodology using multivariate statistics and combining together bacterial community structures, detection of dechlorinating bacteria and genes and water geochemical data were used to investigate these aquifers.

Results from multifactorial analysis of data collected from a PCE-contaminated site in Switzerland (25 groundwater samples) showed that manganese reduction (MR) was a key terminal electron accepting process, suggesting a potential competition between MR and DCE degradation to VC. *Dehalococcoides* sp. and VC reductive dehalogenase genes were detected but ethene concentration was below 0.007mg/L. Potential for a complete natural biodegradation of PCE was present in this aquifer. However, DCE reduction will be strongly inhibited under local conditions as long as oxidized manganese resources are present. The second site located in Czech Republic (Velamos) and sampled at 7 different dates (35 groundwater samples) was under active biostimulation process. Multifactorial analysis showed that successive cheese whey injections modified the aquifer habitat that became favourable not only for a complete dechlorination, but also for sulfate reduction (SR) and methanogenesis. DCE and VC accumulated along with the production of ethene, methane and hydrogen sulphide, indicating a competition between CEs dechlorination and SR and methanogenesis. This possibly explained the transitional slower reaction of CEs dechlorination observed during the remediation process.

In conclusion, the used methodology allows evaluation of the bioremediation potential present in contaminated aquifers and monitoring biostimulation processes.

This study was funded by grant No. TA02020534 – **TECHTOOL** of the Technology Agency of the CR, and the Swiss Federal Office for the Environment FOEN.

Removal of chlorinated ethylenes by anaerobic microorganisms

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In this contribution, basic principles of anaerobic microbiology in bioremediation are presented to show what kind of limitations, obstacles, and needs has to be considered. Regard to TECHTOOL, consolidated views on recent outputs and findings are put forward to both scientific, so industrial audience. Fluorescent microbiology seems to be very powerful tool which can be modified to requirements of objective monitoring and process characterisation. Also fermentation playing irreplaceable role in microbial dehalogenation, is grasped in such a way which can be easily applicable in routine bioremediation practice. Principal impact put on technical solution of this applied research proves the essential need to do all microbial experiments under suitable conditions – in the laboratory of anaerobic microbiology that is briefly promoted on an example from the company of EPS.

Each new method developed in order to be applied in technical microbiology has to comply with some requirements. It should be user friendly method generating reliable results with low risks. This method should be also substitutable by another methodical approach in order to get an inspectional view. All approaches which have been developed in this phase of TECTOOL project were approved by their application on real samples.

This work was supported by the Technology Agency of the Czech Republic, program ALFA, **TECHTOOL** project (TA02020534).

Insight into anaerobic organohalide respiring microbial communities associated with PCB-polluted river sediments

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Polychlorinated biphenyls (PCBs) are persistent organic pollutants which have entered the environment both through usage and disposal. Due to their long-time persistance, and although their manufacturing was ceased, PCBs are considered nowadays as a major environmental pollutant at a global scale. Specific bacterial species, including the unique genus *Dehalococcoides* sp., were found to couple the degradation of these chlorinated compounds to energy conservation by a catabo-

lic pathway called organohalide respiration (OHR). This anaerobic process is thought to be a promising technique for the remediation of PCB congeners.

In the present study, anaerobic microcosms were started using PCB-contaminated sediment samples taken from an old efflux channel of the former PCB manufacturer Strazske (Slovak Republic). Goals were to identify active PCB-degrading bacteria as well as environmental variables that influence PCB degradation *in situ*.

Total RNA and DNA were extracted and analysed during continuous cultivation of the microcosms which were able to degrade up to 50% of the congener initial contents. We observed a clear shift in the composition of the bacterial communities with time as well as very different community structures in each single microcosm. In depth analysis of two microcosms using cDNA samples targeting both phylum *Chloroflexi* and class *Dehalococcoidia* revealed very diversified microbial community structures and putative active PCB-degrading members. One sediment-based anaerobic culture approximated the known model for OHR consortium, in which key OHR guild members are composed of strains closely affiliated with *Dehalococcoides*-like or/and DLG organisms. The PCB degradation in the second microcosm was not driven by an OHR guild structured around *Dehalococcoides* sp., thus, indicating the multiple potential degradation pathways involved in the PCB degradation.

This study was funded by grant No. TA02020534 – TECHTOOL of the Technology Agency of the Czech Republic, the Scientific Exchange Program Sciex-NMS from the Swiss Confederation, and by the EPFL-ENAC Faculty.

Nanofibre biomass carriers as a valuable tool for analysis of microbial community at polluted locality

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Chlorinated ethenes (CE) are the second most common soil contaminant worldwide. Hence there is long-term experience with a range of clean-up techniques for such pollutants. One of the most successful methods is enhanced reductive dehalogenation (ERD), which utilises anaerobic microbial degradation of CE, stimulated by organic substrate application in situ. At some sites, however, cis1,2-DCE and cancerous vinyl chloride have been shown to accumulate due to a reduction in ERD efficiency, and the mechanisms are not fully understood. A deeper insight into the key-factors affecting the ERD process, therefore, is highly desirable.

Here, we demonstrate our initial test of nanofibre biomass carrier composed of sample stabilisation for transport to the laboratory, isolation of DNA and PCR amplification. We aim to accelerate, automate

and increase the precision of pre-treatment biomass sampling for DNA isolation by replacing expensive and time consuming groundwater filtration by newly developed trap-samplers that include nanofibre-carriers. The nanofibre carriers have a high specific surface, resulting in higher biomass grow, and hence DNA yield. The first step was to test four ways of stabilization of nanofibre biomass carrier directly in situ. The stabilization procedure is important for successful isolation of DNA. The comparative methods were i) cooling box, ii) RNA later solution, iii) dry ice and iv) liquid nitrogen. DNA was measured on Qubit (Life Technologies) and Tape Station System (Agilent Technologies). All isolated DNA samples were tested using PCR and qPCR method. The DNA yield was comparable for all stabilization procedures. All DNA samples were able to be amplified by PCR and real-time quantitative PCR methods without any with presence of inhibition. The nanofibre biomass carriers are still under testing procedure. We aim to compare the microbial communities in groundwater, soil and the carrier, and the first results are very promising.

This study was supported by grant No. TA02020534 – **TECHTOOL** of the Technology Agency of the Czech Republic. The results of this project LO1201 were obtained through the financial support of the Ministry of Education, Youth and Sports in the framework of the targeted support of the "National Programme for Sustainability I" and the OPR&DI project Centre for Nanomaterials, Advanced Technologies and Innovation CZ.1.05/2.1.00/01.0005.

Program at Glance

	Balling Hall	Hall 01	Hall 02
	We 11/6/14		
	9:00-18:00 - Registration		
9:00		Pre-Conference Workshop: Biotechnology for controlled remediation of sites contaminated with chlorinated ethenes: Czech-Swiss teamwork joins research	Pre-Conference Workshop: International Postgraduate Research Programme Concept Meeting
13:00	Pre-Conference Workshop: How to get manuscript published?		
15:00	**Conference Opening Welcome messages**		
16:30	Plenary lecture		
17:30	Presentation of Main Sponsors		
18:30	**Welcome Party - Gallery**		
	Th 12/6/14		
8:30	Large and Small Molecules for Pharma	Environmental Biotechnology	Biorefinery
10:45		Coffee Break & Poster Session	
11:15	Large and Small Molecules for Pharma	Environmental Biotechnology	Biorefinery
12:30		Lunch & Poster Session	
13:30	Food, Feed and Nutrition	Environmental Biotechnology	Biorefinery
15:15		Coffee Break & Poster Session	
15:45	Food, Feed and Nutrition	Environmental Biotechnology	Biorefinery
17:00	Short Presentations of Highlighted Posters		
19:30	**Gala Dinner - Břevnov Monastery**		
	Fr 13/6/14		
8:30	Large and Small Molecules for Pharma	Microalgae Biotechnology	
10:45		Coffee Break & Poster Session	
11:15	Large and Small Molecules for Pharma	Microalgae Biotechnology	
12:30		Lunch & Poster Session	
13:30	Biomaterials and Biochemicals	Microalgae Biotechnology	
15:15		Coffee Break & Poster Session	
15:45	Biomaterials and Biochemicals	Microalgae Biotechnology	
16:35	Poster Awards		
17:00	**Closing Ceremony**		
18:00		Guided Prague Sightseeing	
	Sa 14/6/14		
8:30	Excursion to Budweiser Budvar with Český Krumlov Sightseeing		

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