Book of Abstracts

The 2nd International Symposium
“VERA JOHANIDES”

Biotechnology in Croatia by 2020

Zagreb, May 10–11, 2013

The Great Hall of the University of Zagreb,
Zagreb, Trg maršala Tita 14

Organisers:
Croatian Academy of Engineering
and
University of Zagreb

In cooperation with:
Croatian Society of Biotechnology
and
Biotechnical Foundation of the Faculty of Food Technology
and Biotechnology

Zagreb, May 2013
Program of the Symposium

8.00 – 9.00 Registration

Co-Chairpersons: Zlatko Kniewald, Peter Raspor, Srđan Novak

9.00 – 9.15 Opening Address
Welcome Remarks
Aleksa Bjeliš, Rector of the University of Zagreb

9.15 – 9.50 Plenary Lecture
Srđan Novak (CRO) and Ivo Friganović (CRO):
How to initiate a new phase of biotechnology development in Croatia?

9.50 – 10.25 Plenary Lecture
Peter Raspor (SLO):
Biotechnology at the door of Horizon 2020

10.25 – 10.40 Coffee Break

Green Biotechnology

Co-Chairpersons: Jagoda Šušković, Milena Mandič

10.40 – 11.05 Thematic Lecture
Slaven Zjalić (CRO):
Aflatoxins fifty years after: A still unsolved challenge

11.05 – 11.20 Domagoj Šimić (CRO):
Green biotechnology in agriculture – food, biofuel and recombinant gene technologies

11.20 – 11.35 Neža Čadež (SLO):
Culture collections for future: case yeasts from genetic to metabolic diversity
11.35 – 11.50 **Boris Kovač (SLO):**
Amylolytic activity inhibition of low falling number flours

11.50 – 12.05 **Matjaž Deželak (SLO):**
Processing of a gluten-free fermented beverages based on malted pseudocereals

12.05 – 12.20 **Borislav Šćulac (CRO):**
Modernisation of production plant and production processes implemented in Zagrebačka pivovara in period from 1995 till 2013

**Red Biotechnology**

*Co-Chairpersons: Dragán Primorac, Višnja Bačun-Družina, Hrvoje Petković*

12.25 – 13.00 Thematic Lecture: **Dragan Primorac (CRO):**
Molecular genetics: The present and the future

13.00 – 13.25 Thematic Lecture **Hrvoje Petković (SLO):**
New approaches in the development of antibiotics – The return of tetracyclines

13.25 – 13.40 **Goran Gajski (CRO):**
Safety assessment and antioxidant activity of sodium copper chlorophyllin

13.40 – 13.55 **Aleš Podgornik (SLO):**
Production of bacteriophages for human applications

13.55 – 14.30 **Coffee and Lunch Break**

**Blue Biotechnology**

*Co-Chairpersons: Jasna Franekić, Dušica Vujaklija*

14.30 – 14.55 Thematic Lecture **Dušica Vujaklija (CRO):**
Molecular study of dominant soil bacteria: Streptomycetes in nature and application to biotechnology
14.55 – 15.10  **Bruno Zelić (CRO):**
Intensification of agro and food industry waste bio-degradation process

15.10 – 15.25  **Maja Borić (SLO):**
Rheology of liquid media influences bacterial physiology

15.25 – 15.40  **Jure Šumi (SLO):**
Green roofs – The future of urban life

**White Biotechnology**

Co-Chairpersons:
Božidar Šantek, Gerhart Braunegg (AUS)

15.45 – 16.10  Thematic Lecture
**Gerhart Braunegg (AUS):**
Sustainable production of polyhydroxyalkanolates

16.10 – 16.25  **Božidar Šantek (CRO):**
Development of integrated bioprocess for ethanol production from sugar beet

16.25 – 16.40  **Želimir Kurtanjek (CRO):**
Systems biology and biotechnology

16.40 – 16.55  **Anita Slavica (CRO):**
Advances in biotechnological processes for lactic acid production

16.55 – 17.10  **Višnja Gaurina Srček (CRO):**
Aquaculture and fish cell technology

17.10 – 17.30  Presentation of the new book “Bioprocess Engineering” by Peter Raspor (SLO), reviewer

17.30 – 18.00  Round Table – Co-Chairpersons' Reports and Discussion

18.00 – 19.30  Get Together Party
SATURDAY, May 11, 2013

Co-Chairpersons: Vladimir Mrša, Blaženka Kos

9.15 – 9.50 Plenary Lecture
Vladimir Mrša (CRO):
Higher education for the requirements of industrial production – The European experience and Croatian challenges

9.50 – 10.00 Martina Tijardović (CRO):
Overview of the past and the look on the future of forest reproductive material production in Croatia

10.00 – 10.10 Ivana Bošnjak (CRO):
Toxicity of bisphenol A (BPA) on urchin embryo gene expression and morphology

10.10 – 10.20 Lucija Nuskern (CRO):
Removal of heavy metals and phosphates from wastewater by bioparticles

10.20 – 10.30 Rok Gaber (SLO):
DNA guided assembly line

10.30 – 10.40 Teuta Murati (CRO):
Endocrine disruptors and animal-free toxicology

10.40 – 10.50 Ana Bielen (CRO):
Scanning for genes and encoding GDS(L) hydrolases in actinobacteria from wide diversity of ecological niches

10.50 – 11.00 Mario Franić (CRO):
Phytoextraction of cadmium using recombinant DNA technology in maize

11.00 – 11.20 Coffee Break

Co-Chairpersons: Damir Ježek, Jasna Beganović

11.20 – 11.30 Jasna Beganović (CRO):
Role of S-layer proteins in probiotic activity of Lactobacillus strains

11.30 – 11.40 Anamarija Štafa (CRO):
Genetic side effects during gene replacement in yeast Saccharomyces cerevisiae
11.40 – 11.50  **Etjen Bizaj (SLO):**
Development and application of *Saccharomyces* interspecies hybrids in wine industry

11.50 – 12.00  **Dušan Goranovič (SLO):**
Regulation of biosynthesis of immunosuppressant FK506 (tacrolimus) by *Strephtomyces tsukubaensis*

12.00 – 12.10  **Mojca Brložnik (SLO):**
Identification of genetic variants responsible for biotechnologically important traits and the design of new generation industrial yeast strains

12.10 – 12.20  **Luka Ausec (SLO):**
Mining bacterial genomes for laccases

12.20 – 12.30  **Bojan Žunar (CRO):**
Genetic transformation of yeast *Dekkera/Brettanomyces bruxellensis*.

12.30 – 12.40  **Antonio Starčević (CRO):**
Mass spectrometry-based clinical proteomics

12.40 – 12.50  **Ana Butorac (CRO):**
Evolution of bacterial populations: The metabolic engineering of *Lactobacillus brevis*

12.50 – 13.00  **Q. D. Nguyen (Hungary):**
Enhancement of efficiency of ethanol production from Jerusalem artichoke

FRIDAY, May 10, 2013

Co-Chairpersons: Zlatko Kniewald, Peter Raspor, Srdan Novak
Welcome Remarks

The Croatian Academy of Engineering was established in 1993 with the aim and vision:

– to be the leading creative and innovative multidisciplinary community of scientists in the field of engineering,

– to contribute with excellence and effectiveness to the development of engineering and biotechnological sciences and to the transfer of technological knowledge important for the welfare and progress of the Croatian economy and the benefit of people,

– to advocate safe and appropriate use of technology, environmental protection and the protection of people against an inappropriate use of technology, to promote professionalism and responsible behavior with regard to high ethical norms.

In 2013, the Croatian Academy of Engineering celebrates its 20 years of acting on the realization of the established aims. This year the Republic of Croatia shall become full member of the European Union and affirm its historical affiliation to the European community of free and democratic nations.

Today a widely accepted notion is that the 20th Century has been a century of physics and electronics, while the 21st Century is a century of biology and its application in various areas of biotechnology. According to all indicators biotechnology is currently economically more important than physics/microelectronics: available funds, number of employees, number of
major discoveries as well as the number of established small and medium enterprises, both in Europe and worldwide. One especially important project for Croatia is the ENPI Horizon 2020 Capacity Building/Mediterranean Environment Programme (H2020 CB/MEP).

Biotechnology in this region has been conceived as early as 1956 by the establishment of the higher educational Study of Biotechnology at the former Technological Faculty of the University of Zagreb, with cooperation and for meeting the exigencies of the pharmaceutical and fermentative industries. Twenty years had passed since first ideas and demands for experts in the field of biotechnology to its international recognition as a high technology, the application of which shall in the future change many areas of human activity. At the 1st European Congress of Biotechnology, which had taken place on September 25, 1978 in Interlaken, Switzerland, the Foundation Document of the European Federation of Biotechnology has been signed. Among the first signatories was Professor Vera Johanides, Ph. D., President of the Association of the Yugoslav Societies of Microbiology and President of the Croatian Society of Microbiology. Since the establishment of the Croatian Academy of Engineering in 1993, Professor Emeritus Vera Johanides has been Honorary Member of the Academy until her demise in 2000 at the age of 83. She was a distinguished scientist who, aside from her devoted work as a pedagogist who raised young experts at graduate and postgraduate studies, also realized an excellent cooperation with the industry in the fields of antibiotics production, fermentation processes and environmental protection. By her patents she greatly contributed also to the intellectual property protection. Therefore the Academy named by Professor Vera Johanides its annual award to the young scientists who are not Academy members.

The annual awards (five at the most) are granted regularly since 2003, and funds are provided by the Croatian industry.

On September 28, 2011, the Academy had organized the 1st International Symposium by which the achievements of Professor Emeritus Vera Johanides have been revived. On that occasion the Academy had set up the memorial bust of this distinguished scientist. In the previous period the Croatian Academy of Engineering, in cooperation with the Croatian Society of Biotechnology, has organized five international conferences in English and published their respective proceedings: Biotechnology and Biomedicine (1999), Biotechnology and Environment (2001), Biotechnology and Food (2003), Biotechnol-
ogy and ImmunoModulatory Drugs (2005) and Biotechnology, Energy, Chemicals and Renewable Raw Materials (2007). Starting from all aforementioned and recognizing exceptional significance of biotechnology, we, the biotechnologists, have reached a conclusion that we will organize our regular meetings biannually, addressing the issues of developmental directions and strategically investments in the fields of application of biotechnology in Croatia as follows:

– primary and secondary food and beverages production – green biotechnology,

– health care and pharmaceutical industry – red biotechnology,

– biotechnology of water resources, marine biotechnology and environmental protection – blue biotechnology

– production of industrial raw materials, chemicals, materials and fuels – white biotechnology.

This International Symposium is directed to:

– strategical directions of the development of Croatia by 2020,

– economy,

– bioinnovation cores and technological centers,

– bioscience and higher education.

Organizational and Scientific Committee:

Prof. Emer. Zlatko Kniewald (Chairman), Prof. Jasna Franekić, Ph. D., Prof. Jasna Kniewald, Ph. D., Prof. Marijan Bošnjak, Ph. D., Prof. Jagoda Šušković, Ph. D., Prof. Drago Šubarić, Ph. D., Prof. Ivica Grbac, Ph. D., Prof. Daslav Hranueli, Ph. D., Prof. Predrag Horvat, Ph. D., Prof. Božidar Šantek, Ph. D., Prof. Milena Mandić, Ph. D., Prof. Damir Ježek, Ph. D., Prof. Vladimir Mrša, Ph. D., Prof. Mirjana Hruškar, Ph. D., Prof. Vesna Zechner-Krpan, Ph. D., Prof. Višnja Gaurina Srček, Ph. D., Prof. Anita Slavica, Ph. D., Prof. Višnja Bačun-Družina, Ph. D., Prof. Peter Raspor, Ph. D. (Slovenia), Prof. Vito Turk, Ph. D. (Slovenia).

Technical support: Melanija Strika prof. soc., Zvonko Zidarić, Vladimir Pavlić
Co-Sponsoring institutions: Croatian Society of Biotechnology, Biotechnical Foundation FTBT, Faculty of Food Technology and Biotechnology University of Zagreb, University of Zagreb, Faculty of Food Technology Osijek, Zagrebačka pivovara Inc., Podravka Inc., Franck Inc., Kraš Inc., Vindija Inc., Lidl Inc.

I would like to thanks to all persons in organization, to speakers and institutions for their participation providing a success of this International Symposium and industry for providing a good social environment during the Symposium.
Srđan Novak is Full Professor at University of Zagreb, Faculty of Food Technology and Biotechnology since 2004 and Head of the Centre for research, development and technology transfer at University of Zagreb since 2010. International experience he received at California Institute of Technology, Pasadena, California as Fulbright Scholarship fellow and at Labatt Institute, London, Ontario, Canada.

At 2012. he received Rector’s Special Award for establishment of Centre for research, development and technology transfer at University of Zagreb.

He participated as researcher on 8 national scientific projects since 1977 and published 37 scientific papers.

How to initiate a new phase of biotechnology development in Croatia?

Srđan Novak,¹ Ivo Friganović²

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We are living in time where three grand technology revolutions are pervading: ICT in a very mature phase, biotechnology with strong commercialization momentum, and nanotechnology which is in pre-commercial phase of development.

Every technology revolution offers undreamt possibilities for development of a number economy sectors. Thus, it is not surprising that all developed countries of today, as well as those that are efficiently considering their progress, made a strategic commitment and concentrated their resources into biotechnology development as a leverage for sustainable economy growth.

Thanks to a visionary action of Vera Johanides Croatia has been among first of European countries to establish university study of biotechnology, a critical mass of researchers and experts in the field has been created on University of Zagreb, in research institute of PLIVA company and some other companies as well (breweries, yeast, alcohol and vinegar production, Immunology institute etc.). This concentration of knowledge and expertise resulted in development of new products and technologies that found application in real production lines. That's why the second half of 20th century is remembered as golden age of Croatian biotechnology. War and privatization took the tribute from this field as well. Point where everyone agree is that Croatia needs a new growth cycle of biotechnology. Who will initiate this cycle? The state, the capital – domestic or foreign, or somebody else? Does Croatia’s accession to EU open some new opportunities for biotechnology? Authors discuss these questions through examples of BICRO-BIOCENTRE project and strategic plans of University of Zagreb for exploitation of structural EU funds.
Kako pokrenuti novu fazu razvoja biotehnologije u Hrvatskoj?

Srdan Novak,1 Ivo Friganović2

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Živimo u vremenu u kojem se međusobno prožimaju tri velike tehnološke revolucije: informacijsko-komunikacijska tehnologija koja je u zreloj fazi, biotehnologija koja uzima značajan komercijalni zamah te nanotehnologija koja je još u početnoj, predkomercijalnoj fazi razvitka.

Svaka tehnološka revolucija pruža neslučene mogućnosti za razvoj mnogih sektora gospodarstva. Stoga ne čudi činjenica da su se danas sve razvijene zemlje bez izuzetka, ali i mnoge druge zemlje koje djelotvorno promišljaju vlastiti razvoj, strateški opredijelile i koncentrirale svoje resurse u razvoj biotehnologije zbog neupitnog potencijala ove moćne tehnološke platforme da djeluje kao generator održivog gospodarskog rasta.

Zahvaljujući vizionarskom djelovanju Vere Johanides u Hrvatskoj je uspostavljen jedan od prvih sveučilišnih studija biotehnologije u Europi, stvorena je kritična masa istraživača i stručnjaka na ovom području kako na Sveučilištu u Zagrebu tako i u Istraživačkom institutu tvrtke PLIVA te drugim tvrtkama (pivovare, tvornice kvasca i alkohola, tvornice octa, Imunološki institut itd.). Ta koncentracija znanja i ekspertiza rezultirala je razvojem novih proizvoda i tehnoloških postupaka koji su našli primjenu u realnoj proizvodnji. Stoga se druga polovica 20. stoljeća pamti kao zlatno doba hrvatske biotehnologije. Rat i privatizacija odnijeli su svoj danak i na tom području. Točka u kojoj se svi danas slažu jest da hrvatskoj biotehnologiji treba novi razvojni ciklus. Tko će taj ciklus pokrenuti? Država, kapital – domaći ili strani, ili pak netko treći? Da li se i za biotehnologiju otvaraju neke nove prilike ulaskom Hrvatske u EU? Autori razmatraju ova pitanja kroz primjere projekta BICRO-BIOCENTAR te strateške planove Sveučilišta u Zagrebu u korištenju strukturnih fondova EU.
Peter Raspor, doctor of biotechnological sciences, professor of industrial microbiology and biotechnology, teaching and researching at University of Ljubljana, Slovenia.

He started as a baker and later he finished his education and graduated in food science and finally in biotechnology. Since 1992 head of the Chair of Biotechnology, Biotechnical Faculty, Food Science and Technology Department, Since 2003 Head of Undergraduate study of biotechnology. He established post diploma studies in biotechnology and lately diploma studies of biotechnology at Biotechnical Faculty. Currently he is running study of microbiology and heading chair of biotechnology, microbiology and food safety. He is also professor in Euroleague for Life Sciences programme “Safety in the Food Chain” (MSc SIFC), as well as regular guest professor at University of Vienna in Nitrition studies with his own course. With his mentorship more than 100 students have finished studies in area of food technology and biotechnology on diploma and 30 at doctoral level. He conducted more than two dozens international and national projects in last 20 years.

Since 1995 he is active with COST, having different functions up to president of TC and currently vice-chairing COST Domain committee for food and agriculture. From 2007–2009 he is President of EFFoST (European Federation of Food Science and Technology) with 80 societies in 21 European countries with about 150 000 members. He was the Secretary General of (FEMS) Federation of European Microbiological societies from 2000 to 2006 dealing regularly with 54 member societies with about 40000 members. He is also involved with other international and national governmental and nongovernmental organizations.
He is a member of many scientific and professional societies and a member of editorial boards or editor in highly respected journals in the field. He published more than 130 scientific publications, more than 30 of book chapters and he edited more than dozen books at the same time organized more than 60 national and international meetings of large importance for food and microbiology and biotechnology filed, he is regular lecturer of international audience. In last years he was invited to at least 70 events in that area. His professional profile is highly respected in the area of food technology and nutrition, industrial microbiology and biotechnology and lately in food safety area.

For his contribution to science and education he received a few awards, like, “100 years of Virology” medal from All-Russian Scientific Council of Virology in 2004 and the highest state Award for academics in Slovenia for achievements in the high education in 2003 and Medal Of Excellence of University of Ljubljana for achievements in teaching and research in 2007, and in 2011 Jesenko award for life achievements given by his Faculty to exceptional results, among others. The echo of his work elected him to prestigious position at different universities and academia’s, like Doctorem Honoris Causa Universitatis de Sancto Stephano, Gödöllő, Hungary in 2002, Doctorem Honoris Causa University of Pecs, Hungary in 2003 and to International Academy of Food Science and Technology IAFoST in 2008. In 2010 he become member of ECPD (European Center for Peace and Development) Academic Council of The University for Peace erected by United Nations.

Professionally he is also involved as auditor for ISO 9000 standards and ISO 22000 in terms of HACCP and food safety management issues for more than 15 years in food and pharmaceutical sector. In last years he also conducted and chaired few commissions performing international auditing of quality management systems at prestigious universities al pedagogical and research level.
Biotechnology at the door of Horizon 2020

Peter Raspor,1 Vito Turk2

1Head of Chair of Biotechnology, Microbiology and Food Safety, Food Sci. and Tech. Dep., Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, 1000 Ljubljana, Slovenia
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The end of the FP7 process has streamed towards the launch of Horizon 2020 in 2014. As we know Biotechnology plays an important role in addressing societal, environmental and economic challenges and it is recognised as a key enabling technology in the transition to a green, low carbon and resource-efficient economy. Biotechnologies for health and particularly for non-health applications have received a considerable attention in the European Union's 7th Framework Programme for Research, Technological Development and Demonstration Activities (FP7). “Food, Agriculture and Fisheries and Biotechnology”, also referred as a European Knowledge-Based Bio-Economy (KBBE), is one of the 10 thematic areas in the FP7 Cooperation programme and, to date, it has supported transnational and international projects with an amount of about 500 Million Euro in different sectors of biotechnology. In mid 2012 the final FP7 calls have been launched. They aimed at bridging the gap between FP7 and the new programme for research and innovation, Horizon 2020. Running from 2014 to 2020, with a proposed budget of €80 Billion Euro, Horizon 2020 will combine research and innovation funding currently provided through FP7, the innovation related activities of the Competitiveness and Innovation Framework Programme (CIP) and the European Institute of Innovation and Technology (EIT). Horizon 2020 offers a coherent package of support with simplified funding rules for the best research that provides major business opportunities. Its three priorities are 1) Excellent Research 2) Industrial Leadership and 3) Societal Challenges. Biotechnology is listed among technologies which are fundamental to our economy (Key Enabling Technologies – KETs) and it will be financed under the Industrial Leadership priority. Biotechnology will be also supported under the third priority, Societal Challenges inside different priority challenges. Horizon 2020 builds on the thematic research from FP7 and reflects the goals of the Europe 2020 strategy. “European bioeconomy challenges: Food security, sustainable agriculture and forestry, marine and maritime and inland water research” are among major concerns shared by European citizens and therefore this area is one among the six priority challenges selected for funding.
Green Biotechnology

Co-Chairpersons: Jagoda Šušković, Milena Mandić
PhD Slaven Zjalić, assist. prof. was born in Zagreb where he finished primary and secondary school education. He graduated from University of Rome La Sapienza in Biology, course Biotechnology, and has earned PhD in Botanical sciences at the Department of Plant Biology University of Rome La Sapienza with the thesis entitled “Use of polysaccharides from basidiomycetes in a control of aflatoxin production by Aspergillus parasiticus Spare”. He started his research work in the Italian National Research Council collaborating on the projects of bioconversion of agricultural by-products by mushrooms and production of mushroom metabolites of industrial interest. He continued his research at the University of Rome La Sapienza in the Laboratory of Plant Pathology and Mycology, studying the correlation between the fungal cell oxidative stress and mycotoxins, mostly aflatoxins, biosynthesis and strategies for control of the presence of mycotoxins in feed and food. He collaborated on several different Italian and EU projects regarding prevention, control and early detection of mycotoxins in food and feed. Since 2010 he is assistant professor in Microbiology and Mycology at the Department of Ecology, Agronomy and Aquaculture, University of Zadar.

**Aflatoxins fifty years after: still unsolved challenge**

Aflatoxins were discovered after the massive death of turkeys in UK more than 50 years ago. These toxins, secondary metabolites of moulds grouped in Aspergillus section Flavi, present a high health hazard due to their cytotoxic, genotoxic immune-suppressing and teratogenic effects on humans
and animals. Therefore the concentration of aflatoxins in food and feed commodities is limited by law in almost all countries. The contamination with aflatoxins can occur on different plant and animal derived substrates (seeds, dried fruits, cheese, dried meat), but seeds, especially the oily ones, are considered the major vehicle of aflatoxins in a food chain. After ingestion aflatoxins can be transmitted along the food chain either unchanged or metabolised in other more or less toxic forms like aflatoxin M₁ in milk. The aflatoxigenic moulds require relatively low water activity for their growth ($a_w \geq 0.7$) and consequently contamination of seeds with aflatoxins can occur in field or during any phase of postharvest processing and storage. The contamination in field occurs only in conditions of high humidity and high temperatures. In last decade, probably due to the climatic changes, the contamination in fields occurs more frequently also in European counties where, in the past, the presence of aflatoxins in food and feed were considered problem of imported raw materials or inadequate storage. Different strategies, either preventive or detoxification, have been applied to control the presence of aflatoxins in food and feed but none of them has completely solved the problem. The research of novel, more environmentally friendly strategies and tools in aflatoxins control is still ongoing.

**Aflatoksini pedeset godina poslije: još neriješen problem**

Aflatoksini su otkriveni prije pedesetak godina nakon pomora pura u Velikoj Britaniji. Ovi sekundarni metaboliti plijesni svrstanih u *Aspergillus* sekcija Flavi predstavljaju visoki rizik za ljudsko zdravlje zbog njihovog citotoksičnog, genotoksičnog, imuno-supresivnog i teratogenog djelovanja na ljudi i životinje. Stoga su zakonodavstva većine zemalja ograničila količinu aflatoksina u hrani, krmivima i sirovinama za hranu i krmiva. Plijesni koje proizvode aflatoksine mogu kontaminirati sировine biljnog i životinjskog podrijetla kao npr. sjemenke, plodove, mlijeko i meso. U hranidbeni lanac, aflatoksini najčešće dospiju putem sjemenki, posebice onih bogatih uljem. Konzumiranjem kontaminirane hrane, aflatoksini mogu ostati neizmijenjeni ili se metabolički transformiraju u druge, više ili manje toksične aflatoksine poput transformacije aflatoksina $B_1$ u $M_1$, koji se izlučuje u mlijeku sisavaca. Plijesni koje proizvode aflatoksine rastu pri relativno niskom aktivitetu vode
(a_w ≥ 0.7), te se kontaminacija može dogoditi u polju tijekom rasta biljke ili u bilo kojoj fazi preradačkog procesa i skladištenja. Za kontaminaciju u polju, potrebni su visoka vlažnost zraka i visoke temperature. U europskim zemljama, gdje se u prošlosti smatralo da je kontaminacija aflatoksinima problem uvezenih sirovina ili lošeg skladištenja, u zadnjih desetak godina sve ječešća kontaminacija u polju, vjerojatno zbog klimatskih promjena. Da bi se izbjegla prisutnost aflatoksina u hrani i krmivima primjenjuju se različite strategije, bilo preventivne bilo detoksifikacijske. Niti jedna, do danas, nije omogućila potpunu kontrolu aflatoksina pa su u tijeku istraživanja novih, za okoliš prihvatljivijih strategija i metoda za suzbijanje prisutnosti aflatoksina u hrani i krmivima.
Domagoj Šimić
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Domagoj Šimić received his MS degree in Biometrics and Plant Breeding from the University of Zagreb, Croatia in 1994, and his PhD in Population Genetics and Plant Breeding from the University of Hohenheim, Stuttgart, Germany in 1999. He has more than 20 years’ experience in maize genetics and breeding, as well as in investigations of specific genotype by environment interactions in breeding programs of maize, soybean, sunflower and wheat in southeast Europe. Currently, he works at the Agricultural Institute Osijek as a senior researcher in maize breeding responsible for long term breeding programs and selection methodology. Since 2007 he served as the head of research program on ionomics in maize grain (seed), leaf, pollen and root focused on biofortification and phytoremediation aspects of metal accumulation. He also runs an active research program on genetic and quantitative trait loci analyses of chlorophyll $a$ fluorescence parameters in maize as early indicators of drought stress. From 2006 to 2009 he has served as the Deputy Director of the Agricultural Institute Osijek. He is co-founder of the Panonian Plant Biotechnology Association (PPBA) in Visegrad, Hungary, 2006, and President of Committee for deliberate release of GMOs, Ministry of Health; Republic of Croatia since 2009.
Green biotechnology in agriculture – food, biofuel and recombinant gene technologies

Domagoj Šimić,1 Krešimir Dvojković,2 Aleksandra Sudarić3

1Agricultural Institute Osijek, Department for Maize Breeding and Genetics
2Agricultural Institute Osijek, Department for Breeding and Genetics of Small Cereal Crops
3Agricultural Institute Osijek, Department for Breeding and Genetics of Industrial Plants

Encompassing a wide range of procedures for changing living organisms according to human purposes in agriculture, green biotechnology has expanded to include new and diverse sciences such as genomics and recombinant gene technologies applied particularly in plant production. At Agricultural Institute Osijek, genomics has been adopted and used as a standard tool during process of developing recent varieties of maize, wheat and soybean, for example. Furthermore, we detected chromosome segments in maize which seem to be associated with biofortification traits in grain to aid increase the concentrations of bioavailable minerals such as iron and zinc. This might be also valuable for further research in food biotechnology. However, green biotechnology in agriculture is much tighter associated with recombinant gene technologies in crop plants. Since recombinant gene technology in food production is viewed controversially in Europe, shifting the paradigm in agriculture to biofuel production seems to be promising for further progress of green biotechnology. Recently, we apply recombinant gene technologies for altering cadmium concentration in maize in order to provide efficient plants for biofuel production advocating safe and appropriate use of technology and environmental protection.
Zelena biotehnologija u poljoprivredi – hrana, biogoriva i rekombinantna DNA tehnologija

Domagoj Šimić,1 Krešimir Dvojković,2 Aleksandra Sudarić3

1Poljoprivredni institut Osijek, Odjel za oplemenjivanje i genetiku kukuruza
2Poljoprivredni institut Osijek, Odjel za oplemenjivanje i genetiku strnog žitarica
3Poljoprivredni institut Osijek, Odjel za oplemenjivanje i genetiku industrijskog bilja

Obuhvaćajući veliki broj postupaka u poljoprivredi, pogotovo u biljnoj proizvodnji pomoću kojih se mijenjaju živi organizmi prema ljudskim potrebama, zelena biotehnologija se dodatno proširila na nove i vrlo različite znanosti kao što su genomika i rekombinantne DNA tehnologije. Na Poljoprivrednom institutu Osijek, genomika je prihvaćena i koristi se kao standardni alat tijekom postupka razvoja recentnih kultivara kukuruza, pšenice i soje, primjerice. Nadalje, detektirali smo kromosomske segmente kod kukuruza koji su u vezi sa biofortifikacijskim svojstvima hrane kako bi se mogla povećati koncentracija biodostupnih minerala kao što su željezo i cink. Ova istraživanja bi mogla imati također značenje i za prehrambenu biotehnologiju. Međutim, zelena biotehnologija u poljoprivredi se najčešće veže s rekombinantnim DNA tehnologijama kod oraničnih biljnih vrsta. Budući da se rekombinantna DNA tehnologija u proizvodnji hrane ponekad čini kontroverzna u Europi, mijenjanjem paradigme u poljoprivredi prema proizvodnji i biogoriva, zelena biotehnologija mogla naglo napredivati. U posljednje vrijeme, koristimo rekombinantne DNA tehnologije za mijenjanje koncentracije kadmija kod kukuruza kako bi se dobile učinkovite biljke za proizvodnju biogoriva zagovarajući prikladnu uporabu tehnologije i zaštitu okoliša.
Neža Čadež, doctor of biotechnological sciences, assistant professor of microbiology, researching, teaching and curating Collection of Industrial Microorganisms at University of Ljubljana, Slovenia. She has been employed as a researcher at Department of Food Science and Technology since 1997. In 2005 she defended her thesis entitled “Polyphasic approach to the taxonomy of the yeast genera Hanseniaspora and Kloeckera” and in 2005 she was awarded by Krka’s award for research. Since 2007 she is lecturing at BSc and MSc study programs of Biotechnology and Microbiology subjects Analytical biotechnology, Industrial microbiology and Microbial Biotechnology. As a researcher she is active in the fields of yeast taxonomy, culture collection and wine microbiology. Her bibliography lists 131 items, of those 17 original scientific papers and 3 chapters in monographs.

Culture collections for future: case yeasts from genetic to metabolic diversity

Neža Čadež and Peter Raspor

Culture Collection of Industrial Microorganisms, Chair of Biotechnology, Microbiology and Food Safety, Department of Food Science and Technology, Biotechnical faculty, University of Ljubljana, Jamnikarjeva 101, 1000 Ljubljana, Slovenia.

Microbial culture collections are a critical component of the infrastructure of biotechnology and life sciences. They serve to document and
support past and present research and preserve microbial biodiversity for tomorrow. The Convention on Biological Diversity and OECD’s Best Practices Guidelines for Biological Resource Centres placed additional demands on culture collections in terms of common operational standards. With it an increasing demands on culture collections for authenticated, reliable biological material and associated information have paralleled the growth of biotechnology.

Slovenian Collection of Industrial Microorganism was established in 1991 and today it holds and maintains 2500 strains of industrially important yeasts, bacteria and filamentous fungi deposited in last 20 years by Slovenian researchers. Strains were identified based on state-of-the-art techniques at the time such as morphology, physiology and recently by molecular techniques. In present, all yeast strains to be deposited in ZIM collection have sequenced their barcoding regions resulting in description of several new taxa of Slovenian origin. Beside genetic diversity of microbial resources, by the year 2020 metabolic diversity will be exploited and will become part of information delivered to the users.
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He graduated in food science in 1989 and started as a researcher in the Chair of Biotechnology, Biotechnical Faculty, Food Science and Technology Department, University of Ljubljana. He finished his PhD thesis in biotechnological sciences in the same department in 1997. In 1997 he started as R&D manager in food industry Mlinotest d.d. He is part time involved in teaching and researching at University of Primorska, Slovenia. His basic research activities are focused on bread biotechnology, exploitation of starch milling by products for the production of microbial biomass, enzyme production studies and studies of microbial biomass enriched foods.

Amylolytic activity inhibition of low Falling Number flours

Yeast-leavened and sourdough breads represents one of the oldest biotechnical applications. A modern baking process may take advantage of biotechnology in its widest sense, from the improvement of cereal grains and starter cultures by recombinant DNA technology, through the use of enzymes or enzymes inhibitors as processing aids. The influence of supplementing pulses extract, containing natural amylase inhibitors on amylolytic activity of wheat flour with low falling number was studied. The amylase activity in flour was indirectly observed with the falling number (FN) method. The research showed a positive correlation between pulses flour supplement and rise of FN of wheat flour. The result of adding bean flour was a smaller volume of the bread, organoleptic characteristics did not change but freshness was improved. Correlations between different concentrations of extract and rheological properties of dough were observed by farinograph.
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Matjaž Deželak, born 1984 in Celje, Slovenia
– In 2009 graduated Biology at University of Ljubljana, Biotehnical Faculty, Department of Biology. From January 2010 till present part-time employed as a postgraduate researcher at University of Ljubljana, Faculty of Medicine, Institute of Biochemistry and as a young researcher at Slovenian Institute of Hop Research and Brewing, Department of Agrochemistry and Brewing. He is postgraduate student of doctoral programs Biosciences and Biomedicine at University of Ljubljana.
– From February to July 2012 a visiting scientist at Technical University Munich, Center of Life and Food Sciences.
– In 2012 awarded with Scholarships for participation at FEBS3+ Meeting and for research cooperation of Slovenian doctoral students in foreign countries. From 1999 till 2009 awarded with Zois scholarship by the Slovene Human Resources Development and Scholarship fund.
– Member of Slovenian Biochemistry Society and of Alpine Association of Slovenia
Processing of a Gluten-free Fermented Beverages Based on Malted Pseudocereals

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The reaction to gluten by celiac patients leads to the malabsorption of several important nutrients such as iron, folic acid, soluble vitamins, together with a low bone mineral density. The only acceptable treatment is the strict life-long elimination of gluten from the diet. However, concerns have been raised over the long term strict dietary habits of celiac patients, as results indicated an unbalanced intake of main nutrients and limited intake of certain essential nutrients. It is assumed that the quality of the gluten-free products available in the market, and food choices, may represent major determinants in the poor nutrient status. Overall, more emphasis should be placed on the nutritionally quality of the gluten-free diet. In addition, studies also suggest beneficial effects of gluten exclusion in adiposity gain and reducing body weight, inflammation, and insulin resistance in non-celiac patients.

When the production of a functional beverage is planned, raw materials and processing steps need to be carefully assessed in order to fulfill the demands of the consumer with regard to taste, aroma, appearance, and nutritional quality. The aim of this study was to prepare a bottom fermented gluten-free beer-like functional beverage based on 100% malted buckwheat and together with a barley beverage as a reference. Final beverages were intensely characterized including sensory analysis, determination of major brewing attributes, metal cations, sugars, amino acids, esters and higher alcohols content.

Results revealed a significant nutritional advantage of buckwheat and quinoa over barley beverage together with a satisfactory sensory acceptance. The amount of amino acids, iron, copper, zinc, manganese, and sugars are significantly higher in beverages from pseudocereals than from barley. In each beverage a specific profile of volatile compounds has been observed. Since customers are looking for innovative products and additional health benefits while consuming food or drinks our findings make attempts to further investigate buckwheat and quinoa for malting and brewing purposes worthwhile.
Born in Karlovac on December 20, 1964. Finished Faculty of Food technology and Biotechnology, University of Zagreb in 1990, Master Degree in Food Technology.

First worked at Karlovačka pivovara until 1996, and then moved to Zagrebačka pivovara. From 1998 has a role of Plant Manager.

Married, has three children.

Modernisation of production plant and production processes implemented in Zagrebačka pivovara in period from 1995 till 2012

Zagrebačka pivovara was founded in year 1892 with initial capacity of 30.000 hl per year and started with production of Ozujsko beer which is nowadays the most sold Croatian beer. It was independent until 1994 when it becomes a member of Interbrew. Today is a member of MolsonCoors.

A large modernization of the brewery happened during sixties and seventies following the growth of beer sales, but in nineties the equipment mostly became obsolete. In period from 1995 till 2012 all together 63.845.000 € was invested in replacement of existing and purchasing of new equipment for beer production but also in environment protection and safety at work. Parallel with new equipment, modern processes for beer production were implemented. High Gravity Brewing with higher original extract in wort and correction of extract after filtration phase was implemented. Wort fermentation
process was modernised with diacetyl measurement and follow up, as an indicator of fermentation end.

Cold maturation process on –1 °C was implemented in order to assure colloidal beer stability.

Capital expenditures and implementation of modern brewing processes resulted with better process control, more reliable and more efficient production, and assurance of constant product quality. Also, they resulted with substantial increase of production capacity, so today brewery has production capacity of 2.000.000 hl/year.

Zagrebačka pivovara is leader in innovations and today it produces 11 beer types. A lot of packaging innovations were implemented e.g. replacement of returnable glass bottles, introduction of branded crates for returnable glass bottles, introduction of PET bottles and one way glass bottles.

Today Zagrebačka pivovara holds leading position on Croatian beer market with market share of 43% and leading brands in core, premium and value segments.

Plans for future are connected with beer and flavour beer innovations and different packaging innovations, in order to maintain and win market share. Already in 2013, brewery invests 10.000.000 € in new embossed Ozujsko bottles and related packaging machines. Modernisation of production equipment will take place in relation with evolution of production processes and aging of current equipment, with goal to assure quality and efficiency, and to assure environmental sustainable development.

Modernizacija proizvodnih pogona i proizvodni postupci primijenjeni u Zagrebačkoj pivovari u periodu 1995 do 2012. godine

Veliki val modernizacije pivovare se dogodio tijekom 60-tih i 70-tih godina prošlog stoljeća prateći porast prodaje piva, no u 90-tima je oprema već postala zastarjela. U periodu od 1995. do 2012. godine je ukupno uloženo 63.845.000 € u zamjenu postojeće i kupovinu nove opreme za proizvodnju piva, ali i u zaštitu okoliša i sigurnosti radnika.

Usporedo sa novom opremom, uvodili su se i moderni proizvodni postupci za proizvodnju piva. Uveden je postupak High Gravity Brewing sa povećanim ekstraktom u osnovnoj sladovini te korekcijom ekstrakta nakon faze filtracije. Moderniziran je i postupak fermentacije sladovine sa praćenjem razgradnje diacetila kao mjerila završetka fermentacije. Primijenjen je i postupak hladne maturacije piva na temperaturi od –1 °C radi osiguranja koloidne stabilnosti piva.

Kapitalna ulaganja i primjena modernih postupaka proizvodnje piva su rezultirali boljom kontrolom procesa, pouzdanijom i efikasnijom proizvodnjom te osiguranjem stalne kvalitete proizvoda. Također su rezultirali značajnim povećanjem proizvodnog kapaciteta danas pivovara raspolaže kapacitetom za proizvodnju od 2.000.000 hl godišnje.

Zagrebačka pivovara je i lider u inovacijama te trenutno proizvodi 11 vrsta piva. Inovacije su zahvatile i pakiranja, u nekoliko navrata su zamijenjene povratne staklene boce, uvedeni su brendirani sanduci za povratne boce, uvedene su PET boce i nepovratne staklene boce.

Danas Zagrebačka pivovara čvrsto drži vodeću poziciju na hrvatskom tržištu piva sa tržišnim udjelom od 43% , te vodećim brandovima u core, value i premium segmentima.

Planovi za budućnost pivovare su vezani uz inovacije piva i piva sa okusima, te različitih pakiranja radi očuvanja i daljnog osvajanja tržišta. Već u 2013. godini, pivovara investira 10.000.000 € u nove “embossed” Ožujsko boce i povezane strojeve za pakiranje. Proizvodna oprema će se nastaviti modernizirati ovisno o razvoju tehnologije i zastarjevanja postojeće opreme, sa ciljem osiguranja kvalitete i efikasne proizvodnje, te sa ciljem ekološki održivog razvoja.
Red Biotechnology

Co-Chairpersons: Dragan Primorac, Višnja Bačun-Družina, Hrvoje Petković
Prof. Dragan Primorac is a pediatrician, forensic expert and geneticist. He serves as adjunct professor at Penn State University and University of New Haven in the United States and as professor at Medical Schools in Split and Osijek in Croatia. Prof. Primorac is a pioneer in the application of DNA analysis for identification of bodies in mass graves and one of the founders of forensic DNA analysis in Croatia. He authored more than one hundred scientific papers and abstracts in clinical medicine, molecular genetics, forensic science, population genetics, genetic legacy of *Homo sapiens sapiens* and education, science and technology policy. His papers have been cited more than 1500 times and currently he is the most cited clinician in Croatia in his ages in the field of biomedicine. Primorac received 21 domestic and international awards including The Young Investigator Award of the American Society for Bone and Mineral Research in 1992, The Michael Geisman Fellowship Award of the Osteogenesis Imperfecta Foundation in 1993, The Life Time Achievement Award by the Henry C. Lee’s Institute of Forensic Science in 2002, The Award of the Italian Region Veneto for Special Achievements in Promoting Science in the EU in 2007, and The University of New Haven’s International Award for Excellence in 2010. He is honorary citizen of five cities in and outside of Croatia. For his contribution to the field of martial arts during August of 2012 he entered Imperium Hall of Fame of Martial Arts.

Prof. Primorac was invited speaker at sixty conferences all around the world and he is the co-founder of International Society of Applied Biological Sciences (ISABS). Several renowned media outlets, both electronic and print, have reported on the results of his work, such as the New York Times, USA
Today, Chicago Tribune, Hartford Courant, JAMA, Lancet, Science, NBC, Channel 8 (Connecticut TV Station).

From 2003 to 2009 he served as Minister of Science, Education and Sports, Republic of Croatia. According to the International Republican Institute survey of October 1, 2007, he was rated as the most successful minister in the Croatian Government with 31% approval rate. Under his leadership, chapters “Science and Research” and “Education and Culture” were the first two chapters that Croatia closed in negotiations with the European Union. As the Minister of Science, Education and Sports, Prof. Primorac launched a series of successful reforms in primary, secondary and tertiary education as well as in science, technology and sports that significantly improved the system. The award for numerous efforts made in the Croatian educational system is the survey of the famous Newsweek (2010) which rated Croatia 22nd in education ahead of 12 countries from the G20 group.

Molecular genetics: the present and the future

The availability of the human genome sequence opened the doors for diverse investigations into the biology of many organisms and human diseases. During the last decade we have witnessed the first translation of some fundamental discoveries from the field of molecular biology into medical tools. A great achievements have been made in order to understand molecular mechanism of different diseases. Future health care will offer us a personalized medicine and improved translation from research to clinical medicine. A central aspect of this is the development of novel molecular technologies to predict, diagnose and treat diseases. Furthermore, personalized medicine aims to provide the tools and knowledge to fight chronic diseases and treat them more effectively than ever before. When coupled with personal pharmacogenetics, personalized medicine is a unique approach that may be well suited for the health challenges we face in the new millennium. Another, very progressive area of molecular genetics is stem cell therapy. Today many physicians believe that stem-cell treatment have the potential to cure many human diseases. At the same time several regenerative medicine institutes are already capable to grow tissues and organs and finally transplant those
laboratory-grown organ into humans. Given the amount of clinically actionable genetic data that will become available in coming years, we will no doubt see genetic information integrated into the design and analysis phases of numerous types of clinical trials.

Technology has continued to advance at a similar pace providing those responsible for managing crime with a need and opportunity to identify and predict new and future applications of science and technology; not just in decreasing and detecting crime but also in predicting how technology will be used by criminals in the future. A new technique, “forensic DNA phenotyping”, allows investigators to predict suspects’ hair and eye color by analyzing stand-alone DNA evidence. This is especially useful in situations where little is known about the alleged offender and the pool of possible culprits is wide. In recent years, molecular genetics has given important insights into the origin of life and its evolution, the emergence of humans, and our intimate relatedness to every other species on Earth. Another new discipline, genetic genealogy involves use of genealogical DNA testing to determine the level of genetic relationship between individuals. Finally, there is no doubt that molecular genetics will play an increasing role in the future and that genomic technologies will change our lives for the better.

**Molekularna genetika: sadašnjost i budućnost**

Sveučilište u Splitu, Medicinski fakultet, Split, Hrvatska; Sveučilište u Osijeku, Medicinski fakultet, Osijek, Hrvatska; Sveučilište Penn State, State College, PA, SAD; Sveučilište New Haven, West Haven, CT, Sjedinjene Američke Države

Sekvencioniranje ljudskog genoma otvorio je širom vrata za niz različitih istraživanja kako u biologiji tako i u razumijevanju molekularne osnove nastanka niza bolesti. Tijekom posljednjeg desetljeća svjedoci smo iznimno brzog prijenosa znanja iz područja bazičnih znanosti prema kliničkoj medicini. Posebno veliki napredak je napravljen u razumijevanju genetske osnove brojnih bolesti. Isto tako, nedvojbeno je da će u budućnosti sustav zdravstvene zaštite sve više biti oslonjen na tzv. personaliziranu medicinu, a središnji aspekt ovog novog koncepta medicine sadržan je u individualiziranim obli-
Cima terapeutskih postupaka, isključivo ovisnim o strukturi genoma pojedinca. U kombinaciji s razvojem farmakogenetike, personalizirana medicina nudi jedinstven pristup dobro prilagođen zdravstvenim izazovima s kojima ćemo se sve više suočavati u budućnosti. Liječenje matičnim stanicama još je jedno iznimno propulzivno područje, a danas mnogi već vjeruju da je u istinu samo pitanje dana kad će se liječenje matičnim stanicama početi rutinski koristiti u svakodnevnoj praksi. Istodobno, nekoliko svjetski priznatih instituta koji se bave tzv. regenerativnom medicinom već postižu signifikantne rezultate u laboratorijskom uzgoju tkiva i organa. Brza i vrlo progresivan napredak uočljiv je u području forenzičke genetike. Nove tehnologije poput tzv. "forenzičke DNA fenotipizacije" u mogućnosti su iz pronadenog biološkog traga predvidjeti boju kose i očiju počinitelja kaznenog djela. Posljednjih godina, molekularna genetika je imala vrlo važnu ulogu u istraživanju podrijetla naroda analizirajući Y kromosom i mtDNA. Zaključno, nedvojbeno je da će molekularna genetika imati veliki utjecaj na razvoj novih dijagnostičkih i terapeutskih postupaka, što će nadamo se se bitno doprinijeti kvaliteti života svakog pojedinca.
Dr. Hrvoje Petković: CEO, Director of research and development. With many years of industrial experience home and abroad, a number of high-impact scientific publications and patents, Hrvoje steers the innovation and research focus of the company.

Hrvoje Petković holds PhD in biotechnological sciences and is currently a Professor of microbial biotechnology at the Biotechnical faculty (University of Ljubljana). Dr. Petković is co-founder and R&D director of biotechnology SME Acies Bio d.o.o. (Ljubljana) and co-founder and head of AGL group at institute IBBTEC at the University Cantabria (UC, Spain), which was co-founded in collaboration with Acies Bio and UC. Dr. Petković graduated in 1990 at Food and biotechnology faculty (University of Zagreb), and continued further with MSc study at the Faculty of Sciences, University of Zagreb. Immediately after graduation, he joined Department of biotechnological development at Krka pharmaceuticals, where he was involved in the development of biotechnological processes for production of secondary metabolites and enzymes of medical and industrial importance. During employment at Krka, he completed PhD at the University of Ljubljana, spending two years at the University of Glasgow and University of Strathclyde and competed his PhD study in 1998. For his contribution during his PhD study he was awarded by the First Krka’s award. In 1998, he was awarded NATO/Royal society fellowship and spent 1 year at the University of Wales (UK). In 2000 Dr. Petković joined Biotica Technology SME (University of Cambridge, UK), where he led strain improvement group. In 2004 he returned to the Biotechnical faculty and in 2006 co-funded together with his research group SME Acies Bio. Since 2005, Dr. Petković has received a number of public grants from national and international (EU) organisations, as well as a number
of industry-funded projects, resulting in a number of EU/US granted patents and published high-impact papers. Currently, he is involved in number of drug-discovery and process development projects with international Biotech and Pharma companies and academic research groups.

New approaches in the development of antibiotics – return of tetracyclines

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Objectives: Tetracyclines (TC) are clinically important broad-spectrum antibiotics with excellent therapeutic index. However, their use is hampered by wide-spread resistance. TCs act on the ribosome. Smaller group of so-called “atypical” TCs has been identified, including a potent broad-spectrum TC, chelocardin (CHD), with unknown mode of action, produced by Amycolatopsis sulphurea. Application: Particularly the fact that CHD displays different mode of action, and shows activity against TC-resistant analogues is of significance, when considering generating novel potentially clinically useful analogues. Results: We have cloned the gene cluster encoding CHD biosynthesis from Amycolatopsis sulphurea, spanning over 20kb of DNA and containing 18 putative genes. Specific structural features of CHD is reflected in the presence of several genes, not observed in typical TC gene clusters, and have been used to generate novel analogues. Conclusions: We have developed versatile tools for genetic manipulation of A. sulphurea and a biosynthetic engineering platform for generation of novel TC analogues. Our platform enables the generation of novel TC scaffolds using a combinatorial biosynthesis approaches which can be further expanded by semi-synthetic efforts, resulting in potent TC analogues of potential clinical value.
Goran Gajski was born on August 31st 1980 in Zagreb where he has completed primary and high school education. He graduated as an engineer of Biology, Molecular Biology in 2006 at Faculty of Science, University of Zagreb. Since 2007 he is employed as a scientific novice in Mutagenesis Unit at the Institute for Medical Research and Occupational Health. From 2007 he is engaged in PhD programme at Faculty of Science, University of Zagreb where he defended his thesis in 2012. Up till now he published 35 scientific papers and 6 book chapters. He participated in several international and domestic congresses and workshops. He is a member of several international and domestic scientific societies and has received several awards for scientific achievement.

Safety assessment and antioxidant activity of sodium copper chlorophyllin.

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Sodium copper chlorophyllin (CHL) is a semi-synthetic mixture of water-soluble sodium copper salts derived from chlorophyll that is widely used as a colour additive in foods, drugs, cosmetics and as a dietary supple-
ment. Present study aimed to investigate the toxicological profile of CHL as well as its free radical scavenging capacity. Toxicological profile of CHL was assessed by measuring its cyto/genotoxic potential in human peripheral blood lymphocytes (HPBLs) exposed to CHL in different concentrations (0.1 to 100 µg/ml) for 4 and 24 h. After the treatment, cytotoxicity and genotoxicity (assessed with the comet assay and micronucleus assays) was measured. Results of cytotoxicity showed that CHL in tested concentrations had no effect on HPBLs viability regardless of exposure time. Additionally, CHL in concentration range tested did not cause DNA damage that could be detected with the comet assay and did not induce statistically significant difference in either CBMN assay parameter compared to untreated control. The antioxidant activity of CHL was determined using the DPPH assay. Results of DPPH assay revealed that CHL is an effective DPPH· scavenger with the concentration-dependent radical scavenging ability. By comparing the antioxidant activity of CHL with some of well-known and powerful antioxidants, such as Trolox, ascorbic acid and butylated hydroxytoluene (BHT) the DPPH· free radical scavenging capacity of CHL was about 1.6 and 1.9 fold lower than that of ascorbic acid and Trolox, respectively, and 4.3 fold higher than that of BHT. Taken together, we have shown that CHL has no impact on cell viability and genome damage in the concentration range tested and therefore it can be considered safe from the aspect of cyto/genotoxicity. Since CHL also displayed radical scavenging ability it could be a perfect candidate for testing as a novel antioxidant.
Procjena sigurnosti i antioksidacijska aktivnost natrijeva bakrenog klorofilina

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Natrijev bakreni klorofilin (CHL) je polu-sintetski preparat dobiven iz prirodnog klorofila topljiv u vodi koji se koristi kao aditiv u hrani, lijekovima i kozmetici te kao dodatak prehrani. Cilj ovog istraživanja je provjeriti sigurnost CHL utvrđivanjem njegovog cito/genotoksičnog potencijala, odnosno ispitivanjem toksikološkog profila, te određivanje antioksidativne aktivnosti CHL pomoću DPPH testa. Cito/genotoksični potencijal CHL ispitivan je na ljudskim limfocitima periferne krvi obrađenim različitim koncentracijama CHL (0.1 do 100 µg/ml) u trajanju od 4 i 24 sata. Rezultati citotoksičnosti pokazali su da CHL nije imao učinak na preživljenje limfocita neovisno o koncentraciji i vremenu izlaganja. Također, pomoću komet testa i mikronukleus testa utvrđeno je da CHL nije genotoksičan za ljudske limfocite. Nadalje, rezultati komet testa su pokazali da CHL u ispitivanim koncentracijama nije uzrokovao oštećenje molekule DNA dok istovremeno nije uočeno statistički značajno povećanje parametara mikronukleus testa u odnosu na kontrolu. Rezultati DPPH testa su pokazali da CHL ima sposobnost hvatanja slobodnih radikala, a taj učinak bio je ovisan o koncentraciji CHL. Uspoređujući antioksidacijsku aktivnost CHL s poznatim i jakim antioksidansima kao što su Trolox, ascorbinska kiselina i butilirani hidroksi-toluen (BHT) uočeno je da je antioksidacijska aktivnost CHL prema DPPH radikalu 1,6 odnosno 1,9 puta slabija u odnosu na ascorbinsku kiselinu odnosno Trolox, te 4,3 puta veća u odnosu na aktivnost BHT. Dobiveni rezultati ukazuju da CHL nije utjecao na stanično preživljenje i oštećenje genoma u ispitivanim koncentracijama te se stoga može smatrati sigurnim sa stanovišta cito/genotoksičnosti. U sklopu ovog istraživanja CHL je pokazao i snažnu mogućnost uklanjanja slobodnih radikala te bi se stoga mogao testirati kao novi antioksidans.
Excessive use of antibiotic has increased incidence of antibiotic resistant pathogenic bacteria that became difficult to control or to treat. Therapeutic potential of bacteriophages has been recognized long time ago, but recently interest in them has been revived. Bacteriophage based products are considered as biological agents by regulatory authorities and have to be characterized with regard to their identity, biological activity, purity and quality when used for humans. Up to date several clinical trials have been conducted according to modern standards in order to obtain solid and reliable data about performance of bacteriophage therapy.

Bacteria of genus \textit{Campylobacter} are foodborne pathogens and number of infections in 2008 is believed to reach 9 mil cases. \textit{Bacteriophages specific for} \textit{C. jejuni} were isolated and characterized in terms of morphology, genomic traits and efficiency. In order to proceed with safety and efficiency tests, fermentation parameters for propagation of bacteriophage PC5 and its host \textit{C. jejuni LBA65} have been optimized. Further, purification protocol based on chromatography has been optimized in order to ensure safe and stable bacteriophage suspension. These bacteriophage formulations are currently being tested for their stability and efficiency in in vivo system.
Blue Biotechnology

Co-Chairpersons: Jasna Franekić, Dušica Vujaklija
I graduated in the field of Microbial Genetics, at the University of Zagreb (FTB). In 1992, I gained PhD from the University of Tokyo. My scientific effort was focused on the model organism, *Streptomyces griseus* and the signaling mechanisms of A-factor that controls cellular differentiation and antibiotic production. During my postdoctoral research at the University of British Columbia (1993–1995), I was studying horizontal gene transfer between distantly related bacteria and tyrosine protein phosphorylation in various *Streptomyces* species. Since 2008 I have been appointed as a Senior Research Associate at the Ruđer Bošković Institute. Currently my research group is investigating the molecular microbiology of antibiotic-producing *Streptomyces*, in particular the model species *S. coelicolor*. We study the functions, interaction and temporal expression of genes important for DNA metabolism and chromosome segregation during reproductive phase. We also investigating an unusual class of lipolytic enzymes present in streptomycetes and other Actinobacteria from various ecological niches. The research findings are published in scientific journals that cover a wide range of topics in molecular microbiology and biochemistry. In addition I am an active member of several professional microbiology and biochemical societies and I am also co-director of the international Summer Schools in Applied Molecular Microbiology (http://www.jic.ac.uk/science/molmicro/summerschool/).
Molecular study of dominant soil bacteria: streptomycetes in nature and application to biotechnology

Streptomyces coelicolor is the soil-inhabiting bacterium with a complex life style and a good model system to study streptomycetes, the best known antibiotic producing microorganisms. The genetic adaptability to a wide range of terrestrial environments is evident in the genome of *S. coelicolor*. The large linear chromosome of this bacterium encodes 7825 ORFs with numerous genes predicted to encode regulatory proteins. This lecture will address two aspects of *Streptomyces* biology. Summary of our current research on DNA metabolism that involves answering the question what is the driving force that has led to the duplication and retention of paralogous *ssb* genes will be presented. In addition, proposed cellular functions of SsbA and SsbB during *Streptomyces* growth and reproductive phase will be described. Secondly, this lecture will focus on genomics and biotechnology in order to show that streptomycetes still represent an excellent source for genome mining and new biotechnological approaches. An interesting subclass of lipolytic enzymes will be presented. This protein family possesses a distinct GDS(L) sequence motif and has multifunctional properties with potential for use in the hydrolysis and synthesis of important ester compounds of biotechnological interest. The sequencing of the genomes of several streptomycete species revealed their great potential for synthesis of a broad spectrum of lipases. Beside that our group has done intensive bioinformatic screening of GDS(L) lipases from *Streptomyces* and related microorganisms (Actinobacteria). Moreover, we are searching for a new Actinobacteria from wide diversity of ecological niches.

Temeljna istraživanja dominantnih zemljišnih bakterija: streptomiceti u prirodi i primjena u biotehnologiji

Bakterija *Streptomyces coelicolor* je prirodni stanovnik tla, ima složeni životni ciklus i predstavlja izvrstan modelni sustav za proučavanje streptomyceta, najpoznatijih mikrobnih proizvođača antibiotika. Genetički potencijal za prilagodavanje širokom spektru ekoloških niša zapisan je u genomu ove bakterije. Njezin veliki linearni kromosom bakterije kodira za 7825 otvorenih
okvira čitanja s mnogobrojnim regulatornim genima. Tijekom ovog predavanja bit će obuhvaćena dva aspekta biologije streptomiceta. Sažet će se rezultati naših istraživanja metabolizma DNA koji daju odgovor na pitanje o evolucijskom pritisku koji je doveo do udvostručavanja i zadržavanja paralognih gena ssb. Osim toga izložit će se stanične funkcije proteina SsbA i SsbB tijekom staničnog rasta i reprodukcije streptomiceta. Drugi dio predavanja će se usredotočiti na genomiku i biotehnologiju da bi se pokazalo kako streptomicetni genomi imaju veliki potencijal za nove biotehnološke pristupe. Prikazat će se zanimljiva grupa lipolitičkih enzima. Ovu familiju proteina karakterizira sačuvan GDS(L) motiv te višestruke funkcije i potencijal za upotrebu u hidrolizi i sintezi važnih esterskih spojeva od biotehnološkog značaja. Sekvencionirani genomi nekoliko streptomicetnih vrsta otkrili su da streptomiceti imaju veliki genomski potencijal za sintezu različitih lipaza. Zato, bioinformatičkim alatima intenzivno tražimo GDS(L) lipaze u bazama u kojima su pohranjeni sekvencionirani genomi streptomiceta i pripadajućih bakterija (aktinobakterija). Štoviše istražujemo i prisustvo aktinobakterija u različitim ekološkim nišama.
Bruno Zelić was born 1973 in Osijek. In 1996 he completed his undergraduate study and in 1999 he completed his graduate study and received his M. Sc. in chemical engineering from the Faculty of Chemical Engineering and Technology, University of Zagreb. From 2000–2002 he was guest researcher at Forschungszentrum Jülich, Institute für Biotechnologie, Germany, where he performed the experimental part for his PhD thesis. In 2003 he received his PhD in chemical engineering at the University of Zagreb. From 2003–2007 he worked as assistant professor, from 2007–2012 he is associate professor, and 2012 he became full professor at the Faculty of Chemical Engineering and Technology, University of Zagreb. In 1994 and 1995 he got Rector's price for the best student work, 1999 he got award for the young researchers and artists of Society of university professors and other scientist, University of Zagreb, 2003 he got award for the young chemical engineers of Croatian society of chemical engineers and technologist and 2007 he got award for the young scientist “Vera Johanides” of Croatian Academy of Engineering. From 2005 he is a member of editorial board of “Chemical and Biochemical Engineering Quarterly” journal. He is a member of European Federation of Chemical Engineering, Croatian Academy of Engineering, Croatian Society of Chemical Engineers and Technologists and The society of university teachers, scholars and other scientists – Zagreb. His research interests are in the field of bioreaction engineering and bioseparation processes. More than 40 scientific and professional publications, 2 patents, and more than 60 oral (plenary, key note, invited) and poster presentations on the international conferences present his scientific work. He was a leader of FP-6 project, two bilateral projects and one national project. From 2009 he is vice dean for education at the Faculty of Chemical Engineering and Technology, University of Zagreb.
Intensification of Agro and Food Industry Waste Biodegradation Process

Marina Tišma,1 Natalija Velić,1 Mario Panjičko,2 Bruno Zelić3

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Environmental compatibility is the most important property of agro and food industry waste. Renewable waste materials from different sources, such as agriculture, forestry, industries and residential, are convertible to useful energy forms like biogas and bioethanol. The biogas production using anaerobic digestion offers significant advantages over other forms of waste treatment processes such as high-energy efficiency and process simplicity.

Solid-state fermentation could be a suitable technology for the production of value-added products by utilization of the above mentioned renewable waste materials, which makes it also economically feasible. So far, this technology was used for production of enzymes, organic acids, mushrooms, flavor and aroma compounds, pigments, polysaccharides, hormones, human food and animal feed. Different type of bioreactors have been developed and successfully used for solid-state fermentation of broad range of substrates and in production of value-added products.

In this work solid-state fermentation will be demonstrated on couple of examples developed in our group such as lab-scale and pilot-scale biogas production using brewer’s spent grain as a substrate, cultivation of T. versicolor on sugar beet waste and cultivation of P. chrysosporium on sawdust from three wood species (beech, cerris and oak). Selected examples are clear demonstration of environmentally friendly and economic technologies used for efficient treatment of agro and food industry wastes.
Intenzifikacija procesa biorazgradnje poljoprivrednog otpada i otpada iz prehrambene industrije

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Kompatibilnost s okolišem jedno je od najvažnijih svojstava poljoprivrednog otpada i otpada prehrambene industrije. Obnovljivi otpadni materijali iz različitih izvora, poljoprivrede, šumarstva, prehrambene industrije i domaćinstava, su lako razgradivi i mogu se uspješno konvertirati u različite oblike energenata kao što su primjerice bioplin ili bioethanol. Poznato je da proizvodnja bioplina anaerobnom razgradnjom biološki razgradivog otpada rezultira brojnim prednostima, u odnosu na druge procese obrade ovog otpada, kao što su primjerice visoka energetska učinkovitost i jednostavnost provedbe procesa.

Fermentacija na čvrstoj podlozi pogodna je tehnologija u proizvodnji visoko vrijednih produkata, a kao supstrat su u dosadašnjim istraživanjima korišteni različiti poljoprivredni proizvodi i njihovi ostatci. Ekonomski opravdanost fermentacije na čvrstoj podlozi pokazana je u proizvodnji enzima, organskih kiselina, gljiva, mirisa i aroma, pigmenata, polisaharida, hormona, hrane i krmiva. Različite vrste bioreaktora su razvijene i uspješno primijenjene pri provedbi fermentacije na čvrstoj podlozi širokog spektra supstrata u proizvodnji visoko vrijednih produkata.

U ovom radu biti će pokazano nekoliko primjera provedbe fermentacije na čvrstoj podlozi kao što su proizvodnja bioplina iz pivskog tropa u laboratorijskom i poluindustrijskom bioreaktoru, uzgoj T. versicolor na repinim rezancima te uzgoj P. chrysosporium na piljevini tri vrste drveta (bukva, cer, hrast). Odabrani ogledni primjeri, razvijeni u našoj grupi, jasno će prikazati po okoliš prihvatljive i ekonomski opravdane tehnologije učinkovite obrade poljoprivrednog otpada i otpada iz prehrambene industrije.
Maja Borič was born in Ljubljana, Slovenia, and graduated in Microbiology from the University of Ljubljana. As an undergraduate student she participated in the research of genetic diversity of *Escherichia coli* isolates at the Chair of Molecular Genetics. After graduation in 2009, she continued her research work as a young researcher at the Biotechnical Faculty of University of Ljubljana. Under mentorship of prof. dr. David Stopar she focused on bacterial ecophysiology with emphasis on marine bacterium *Vibrio ruber*. In 2013 she defended her PhD thesis entitled “The effect of viscosity on ecophysiology of *Vibrio ruber*”. During work as a young researcher she was the first author of two published original scientific papers and presented her work at several conferences. Her research interests include microbial physiology, metabolic activity, secondary metabolism, cell-cell communication, bacterial biofilms and effects of environmental factors on bacteria. She hopes to combine them with the field of applied microbiology.

**Rheology of liquid media influences bacterial physiology**

*Maja Borič, Tjaša Danevčič, David Stopar*

Chair of Microbiology, Department of Food Science and Technology,
Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia

Viscosity of activated sludge in water treatment processes is an important characteristic that influences pumping, hydrodynamics, mass transfer rates and sludge-water separation. Rheological behaviour of activated sludge is in-
between water and bacterial biofilms. Since planktonic and biofilm cells differ significantly in their physiology, we studied if the metabolic activity of bacteria changes with increasing viscosity. The viscosity of growth media was increased with hydroxyethyl cellulose in the range from 0.8 to 26.5 mPas.

Studied bacteria differed in their response to increased viscosity. No major impact of viscosity on *B. subtilis* growth rates and catabolic activity was observed, resulting in constant growth efficiency. On the other hand, *E. coli* reduced its growth rate and metabolic activity at the highest viscosity. However, its growth efficiency was improved at 2.4 mPas and remained approximately the same in media with higher viscosity. The activity of pentose phosphate pathway was increasing with viscosity, while EMP glycolysis and flow to citric acid cycle were down-regulated. In sharp contrast, *V. harveyi* was not able to maintain unchanged growth rate at 26.5 mPas despite a significant increase in its metabolic activity. Growth efficiency of *V. harveyi* dropped dramatically with increasing viscosity, reaching 5-fold lower efficiency at 26.5 mPas. In addition, carbon flow through central metabolic pathways was changed.

Noteworthy differences in bacterial physiologic response to viscosities in the intermediate range were observed. These results indicate that bacteria used in water treatment could also depend on the viscosity of activated sludge, which might alter purification efficiency.
Jure Šumi, has for the past 15 years been involved in international market & business development holding senior positions in well-established multinational companies.

Currently he holds position of European Marketing Director in company Knauf Insulation where he is leading strategic marketing and new product development. He is leading a team of marketing & product development specialists and has been driving new strategic product development with focus on new market trends.

He has been actively involved in horticulture business development since arrival to the company. In recent years he has spent big portion of his time researching global horticulture and landscaping market and finding future trends and opportunities. Recently he has taken over Business development of company’s “Green Solution” division focusing on green aspects of Urban Landscape.

Green roofs – future of urban life

Saša Bavec, Jure Šumi
Knauf Insulation, d.o.o., Škofja Loka,
Škofja Loka, Slovenia

Knauf Insulation is one of the World’s biggest producers of mineral wool insulation. Beside our standard insulation products Knauf Insulation produces also products that are used in horticulture. In the last years we have noticed
that out there is a huge market of so called “green roofs” without proper solutions. Green roofs are used to the big extend in Western Europe, USA and Asia, to improve storm water management, improve insulation properties of the buildings, to reduce so called heat island effects in the cities and to improve air quality. Therefore we have developed a special product that can be used as a substrate replacing soil in green roofs, with special focus on being light, easy to handle and having high water absorption and retention capacities. The initial test results show, that we have really a good product that exactly match the needs of green roofs.
White Biotechnology

Co-Chairpersons: Božidar Šantek, Gerhart Braunegg (AUS)
Gerhart Braunegg
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Prof. Gerhart Braunegg holds tenure in “Applied Microbiology and Biochemical Engineering” from TU Graz.

Invited professor at: École Polytechnique, Montréal, CDN; University of Czestochowa, PL; University of Metallurgy, Leoben, AT; Joanneum University of Applied Sciences, Graz, AT.

Prof. Braunegg was summoned into the UNIDO Working Group on Biodegradable Plastics and Polymers with consultations in 3rd world countries worldwide.

As a consultant for industry he was working (among others) for
- ANDRITZ AG (Graz, AT): Development of bioreactors in lab- and pilot plant scale;
- ASTROE (Graz, AT): Development of optimised baker’s yeast production;
- FLEISCHMAN'S YEAST (Montréal, CDN): optimisation of yeast production;
- PHB-ISA (Serrano, SP, BRA): PHA Production process;
- SCA PAPER (Ortmann, AT): Hygenisation for recycled tissue paper plant.
- BASF (Ludwigshafen, DE): continuous production of Polyhydroxyalkanoates

Academic courses: General Microbiology; Environmental Technology; Biochemical Engineering; Plant Cell Technology; Technical Mycology.
Special expertise in Fermentation technology, White Biotechnology; Process design and optimisation for biopolymer production; cultivation of microalgae.

Prof. Braunegg was the co-ordinator of 2 EU-funded projects (acronyms: WHEYPOL, ANIMPOL) and partner in a number of national projects.

**Sustainable production of polyhydroxyalkanoates**

*Gerhart Braunegg,¹ Martin Koller²*

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The environmental necessity to constrict the utilization of fossil resources for the production of polymers by switching to alternatives is generally undisputed and underlined by recent discussions about packaging materials from mineral resources. Polyhydroxyalkanoates (PHAs) are biodegradable polyesters that are stored intracellular in granules when growth of the producing bacteria is limited by essential nutritional compounds like the nitrogen or phosphate source of the growth and production medium. Under such conditions the PHA content in the cells can increase to more than 80% of the cell dry weight formed, and the quality of the polyesters stored can be influenced by feeding precursors for synthesis of copolyesters or terpolyesters. A drawback for this development is the fact that in most cases production costs for PHAs are still higher than costs for conventional resins. Biotechnological polymer production occurs in aerobic processes, therefore only about 50% of the main carbon sources, and even a lower percentage of the precursors used for production of co-polyesters end up in the products wanted. To overcome this problem, cheap carbon and nitrogen sources for microbial growth and PHA synthesis are needed to lower the production costs.

Selection of the appropriate waste stream mainly depends on the global region where PHA production is intended; required facilities should be integrated into existing production lines, where the waste streams accrue. In Europe, huge amounts of surplus whey are available in dairies, providing enormous amounts of lactose. Additionally, the production of biodiesel in-
CREASES AND GENERATES GLYCEROL, ITS MAJOR BY-PRODUCT. FOR EFFICIENT CULTIVATION OF SELECTED MICROBIAL PHA PRODUCTION STRAINS, DIFFERENT WASTE STREAMS ARE POTENTIAL NITROGEN SOURCES REQUIRED FOR MICROBIAL GROWTH; E.G.: MEAT & BONE MEAL (MBM) FROM SLAUGHTERING RESIDUES SHOW EXCELLENT IMPACT ON THE MICROBIAL CULTIVATIONS. IN MANY AREAS OF THE WORLD, MOLASSES, STARCH, ALCOHOLS, WASTE LIPIDS, AND LIGNOCELLULOSES ARE AVAILABLE. THE OCCURRENCE OF WASTE LIPIDS IS VERSATILE: WASTE COOKING OIL, DIFFERENT PLANT OILS, OR LIPIDS EXTRACTED FROM MBM ARE AVAILABLE, BEING APPLIED FOR PHA BIOSYNTHESIS EITHER DIRECTLY OR AFTER CHEMICAL CONVERSION TO FATTY ACID ESTERS AND GLYCEROL.

INTEGRATION OF PHA PRODUCTION INTO A SUGAR CANE MILL IS REALIZED IN BRAZIL, WHERE SUCROSE IS CONVERTED TO BIOETHANOL AND TO PHA. HERE, THE REQUIRED ENERGY FOR ETHANOL AND POLYMER PRODUCTION IS GENERATED BY BURNING BAGASSE, AND A WASTE FROM THE ETHANOL PRODUCTION CAN ACT AS PHA EXTRACTION SOLVENTS.

A NEW CARBON SOURCE OF INTEREST MIGHT BE THE UTILIZATION OF OILS PRODUCED BY MICRO ALGAE*, BECAUSE AT LEAST FOR SCL-PHAS PHA YIELDS FROM PLANT OIL (AROUND Y_{PHA/OIL} = 0.6 – 0.7 g/g) ARE MUCH HIGHER THAN YIELDS FROM GLUCOSE (AROUND Y_{PHA/GLUCOSE} = 0.3 – 0.4 g/g).

ADDITIONALLY TO THE QUESTION OF SUITABLE CARBON SOURCES THE PRODUCTION MODE MIGHT BE DECISIVE FOR EFFECTIVE AND CHEAP PHA PRODUCTION. IN THIS CONTEXT EXPERIMENTAL RESULT OF CONTINUOUS PHA PRODUCTION IN A 5 STEP STIRRED TANK CASCADE WILL BE SHOWN AND DISCUSSED.

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He was born in Ludbreg, Croatia on May 27th, 1966. He graduated at Faculty of Food Technology and Biotechnology, University of Zagreb in 1990. In June 15th 1991, he started to work as a research assistant in the Department of Biochemical Engineering of the Faculty of Food Technology and Biotechnology, University of Zagreb. On November 18th 1994 he defend his M. Sc. Thesis and on December 13th 1996 PhD Thesis, respectively. He was appointed for Assistant Professor in the Department of Biochemical Engineering of the Faculty of Food Technology and Biotechnology, University of Zagreb on May 13th 1998.

From February 1st 2001 until December 1st 2002 he was guest researcher at Technische Fakultät Universität Bielefeld (Germany) as a member of Prof. E. Flaschel group.

He was appointed for Associate Professor in the Department of Biochemical Engineering of the Faculty of Food Technology and Biotechnology, University of Zagreb on December 1st 2003 and for Full Professor at the same Department on December 16th 2008, respectively.

For Head of Laboratory of Biochemical Engineering, Industrial Microbiology and Malting and Brewing Technology at Faculty of Food Technology and Biotechnology, University of Zagreb he was appointed in 2004. He was participated or leaded 5 national and 2 international scientific projects. He received State Award of Science Republic of Croatia in 2004 and until now he published 28 a1, 6 a2 and 3 a3 scientific papers. He is also co-author of university teaching book “Biochemical Engineering” that was published in 2009.
Development of integrated bioprocess for ethanol production from sugar beet

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Ethanol production (as a biofuel) from renewable raw materials is still more expensive than gasoline production from fossil oil. Therefore, integration of different technological stages into one single stage could resulted in a more cost-effective and energy saving bioprocess. In this study, different ethanol production stages from sugar beet were analyzed in order to improve bioprocess energy efficiency and sustainability. On the basis of bioprocess energy analysis research plan was established that includes ethanol production from the raw sugar beet juice and raw sugar beet cossettes. For ethanol production from the raw sugar beet juice in the stirred tank bioreactor batch and fed batch cultivation techniques were used. In horizontal rotating tubular bioreactor (HRTB) batch ethanol production from raw sugar beet cossettes was studied by different combinations of bioreactor operating parameters. In both cases, *Saccharomyces cerevisiae* was used as a production microorganism. During batch ethanol production from the raw sugar beet juice, bioprocess efficiency was 78.8 % and in the fed batch process 93.4 %, respectively. At the same time, ethanol production in the HRTB from the raw sugar beet cossettes with inoculum of 16.7 % V/m (raw sugar beet cossettes) and interval HRTB rotation (3 min / 10 min⁻¹) resulted in the highest bioprocess efficiency of 78.7 %. On the basis of experimental data it is clear that both intermediates of sugar beet processing can be successfully used for ethanol production. However, operating conditions of HRTB have significant impact on the bioprocess performance and therefore they have to be optimized in order to achieve the highest bioprocess efficiency.
Razvoj integriranog bioprocesa proizvodnje etanola iz šećerne repe

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Proizvodnja etanola (kao biogoriva) iz obnovljivih sirovina je još uvijek skuplja u odnosu na proizvodnju goriva iz fosilnih izvora. Zbog toga integracija različitih tehnoških faza u jednu fazu može rezultirati u ekonomski i energetski učinkovitijem bioprocesu proizvodnje etanola. U ovom istraživanju proučavane su različite tehnološke faze proizvodnje etanola iz šećerne repe s ciljem da se poboljša energetka učinkovitost i održivost bioprocesa. Na osnovi energetske analize bioprocesa formiran je plan istraživanja koji uključuje proizvodnju etanola na sirovom soku i sirovim rezancima šećerne repe. Za proizvodnju etanola na sirovom soku šećerne repe u bioreaktoru s mješalom primijenjeni su ovi načini vođenja bioprocesa: šaršni i šaršni proces s pritokom supstrata. Kod proizvodnje etanola na sirovim rezancima šećerne repe u horizontalnom rotirajućem cijevnom bioreaktoru (HRCB) primijenjena je šaršna tehnika vođenja bioprocesa. U oba istraživanja kao radni mikroorganizam korišten je kvasac Saccharomyces cerevisiae. U šaršnom procesu proizvodnje etanola na sirovom soku šećerne repe učinkovitost bioprocesa je iznosila 78.8 %, a u šaršnom procesu s pritokom supstrata 93.4 %. Istovremeno kod proizvodnje etanola na sirovim rezancima šećerne repe u HRCB s inokulumom od 16.7 % v/m (sirovih rezanača šećerene repe) i intervalnu rotaciju HRCB (3 min / 10 min⁻¹) ostvarena je najveća učinkovitost bioprocesa od 79.5 %. Rezultati ovih istraživanja jasno pokazuju da se oba međuproizvoda obrade šećerne repe mogu uspješno primijeniti u proizvodnji etanola. Međutim, procesni parametri HRCB imaju sigifikatni utjecaj na odvijanje bioprocesa te je stoga nužan proces njihove optimizacije da bi se dobile najveće učinkovitosti bioprocesa.
Želimir Kurtanjek was born in 1946 in Zagreb, Croatia. He graduated in 1971 with the engineering degree in physics from the Department of Sciences, University of Zagreb. After graduation he was employed as an assistant in the Laboratory for Process Control at the Faculty of Technology, Department in Sisak, University of Zagreb. He completed postgraduate studies in Technical Cybernetics at Faculty of Technology, University of Zagreb. In 1975 he enrolled at the postgraduate study at the Department of Chemical Engineering, The University of Houston, TX, USA. He completed his doctoral thesis with his mentor Prof. Dan Luss in the Laboratory for Reaction Engineering. In 1979 he received his Ph.D. degree in chemical engineering.

In 1980, he was employed at the Faculty of Food Technology and Biotechnology, University of Zagreb.

He completed his postdoctoral studies with Prof. G. Froment at the Department of Chemical Engineering, University of Gent, Belgium.

Through the EU project TEMPUS during 1991 he was a visiting professor at the Department of Biological Sciences, University of Ulster, Coleraine, Northern Ireland.

Presently he is employed at Faculty of Food Technology and Biotechnology, University of Zagreb as a professor of chemical engineering and is teaching reactor engineering, mathematical modelling and process control to students of biotechnology.

In his scientific work he is interested in modelling and control of reactors, modelling of bioprocesses and food engineering, and application of AI methods in process control. He has published over 50 papers in international and national journals.

Since 1976, he is a member of American Institute of Chemical Engineers, he is a delegate of Croatia in European Federation of Biotechnology, and also
Systems biology and biotechnology

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Modern biotechnology is considered “genome based biotechnology” and is based on knowledge of genome, proteome and metabolome of numerous industrial microorganisms. Available are huge sources of information in open access internet databases and also with original data resulting from experimental research with “high throughput” instrumentation of molecular biology. Systems biology is a mathematical theory (BST) of life systems which integrates a complete scope of available information on molecular level into a computer model. From biochemical engineering viewpoint systems biology provides engineers with ability to investigate biological potential of a microorganism, a potential spectrum of products, and optimization of process parameters in a production system. Especially is important application of systems biology as a rational basis for computer analysis of opportunities of genetic engineering and planning of genetic modification of microbial metabolism. Some of the basic BST methodologies are EPA (elementary path analysis), FBA (flux balance analysis), MFA (metabolic flux control analysis). In this work are presented our original computer simulation results of control flux analysis of \textit{E. coli} central metabolism upon glucose impulse. The results are obtained by application of Fourier amplitude sensitivity test (FAST) for global metabolic network sensitivity analysis. Explained are transients in control between PTS system and PFK and appearance of multiple regulation of glycolise products. Also are given results of synergy analysis of glycogenolysis flux and cofactors on a whole cellular level. As a third example are presented results obtained by \textit{Cell Designer} software of synergy interaction between the synthesis paths for ergosterol and sphingolipids. Local sensitivity analysis results include ergosterol transport to cellular membrane, along with hydrolosis of IPC-g i MIPC-g as the most sensitive reactions of the model. Upon establishing steady state values, the key enzymes are KDHS reductase, 4-hydroxylase and ceramid synthase.
Sustavska biologija i biotehnologija
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Moderna biotehnologija zasniva se na poznavanju genoma, proteoma i metaboloma industrijskih mikroorganizama. Raspolaže se s veliki brojem informacija pohranjenim s otvorenim pristupom u mrežnim bazama podataka i originalnih informacija dobivenih eksperimentalnim radom s “visoko-protočnim” metodama molekularne biologija. Sustavska biologija je matematička teorija o živim sustavima koja integrira veliki broj raspoloživih informacija na molekularnoj razini u računalni model. S gledišta biokemijskog inženjera sustavska biologija omogućava istraživanje matematičkih modela u cilju određivanja biološkog potencijala, spektra proizvoda i optimalne procesne parametre proizvodnje. Posebno se ističe primjena sustavnih računalnih modela mikroorganizama za racionalni pristup, primjenom matematičkih algoritama, u planiranju genetičke modifikacije metabolizma mikroorganizama. Osnovne matematičke metode sustavne analize mikrobiologijom su određivanje elementarnih tokova, analiza bilance tokova (FBA), analiza regulacije metaboličkih putova (MFA). U ovom radu dani su vlastiti rezultati analize regulacije centralnog metabolizma E. coli impulsnom pobudom glukoze. Rezultati su dobiveni matematičkom metodom Fourierove analize amplitude osjetljivosti metaboličkog modela. Objašnjenje su prijelazne promjene regulacije sustava fosfotransferaze (PTS), fosforuktinaza (PFK) i pojava višestruke regulacije produkata glikolize kao što je piruvat. Također su dani rezultati analize sinhergizma interakcije fluksa glukogenolize u stanici i kofaktora na razini cjelokupne stanice. Kao treći primjer sustavne analize prikazani su rezultati sinhergijskog međujelovanja putova sinteze ergosterola i sfingolipida. Dani su rezultati primjene analize računalnim sustavom za modeliranje stanica Cell Designer.

Primijenjene su metode determinističkog modela lokalne osjetljivosti i stohastičkog modela globalne osjetljivosti. Rezultati analize uključuju transport ergosterola u staničnu membranu, uz hidrolize IPC-g i MIPC-g od strane enzima IPCaze, kao najosjetljivije reakcije modela. Kao ključni enzimi prilikom uspostavljanja ustaljenih vrijednosti, ističu se KDHS reduktaza, 4-hidroksilazu i ceramid sintazu.
Anita Slavica was born in 1970 in Šibenik, Croatia. After degrees in Biotechnology (BSc and MSc) at Faculty of Food Technology and Biotechnology, University of Zagreb, she took PhD in Technical Chemistry at Graz University of Technology, Austria. She habilitated in 2006 in Physiology of Industrial Microorganisms at Department of Biochemical Engineering, the Faculty of Food Technology and Biotechnology, University of Zagreb. Since 2011 she is working as Associate Professor in Laboratory of Biochemical Engineering, Industrial Microbiology, Malting and Brewing Technology at the Department. She is lecturer at “Bioprocess Engineering” and “Molecular Biotechnology” MSc degree studies programme and “Biotechnology and Bioprocess Engineering” PhD programme; researcher in several national/international research projects; supervisor of undergraduate, graduate and PhD students; and member of international organizing and scientific committees. Scientific fields of her interest are: mass spectrometry; development and application of analytical methods related to monitoring of sustainable bio-processes for production of biochemicals; protein expression, purification and characterization; enzyme kinetics; biochemical engineering and industrial microbiology; TT and IPR.
Advances in biotechnological processes for lactic acid production

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Synthesis of lactic acid from petrochemicals has been cancelled and since 2011 the lactic acid has been exclusively produced by biotechnological processes – fermentation of glucose, sucrose or lactose by different microorganisms. Traditional use of the lactic acid and, nowadays, production of other chemicals and biodegradable polymers from the acid drive global lactic acid demand. Leading producers are investing in development of new bioprocesses for the lactic acid production from renewable raw materials by microorganisms which possess hydrolytic and fermentative activity.

Main objective of investigation was development of new bioprocess that meets the European Union climate and energy goals – “20/20/20” targets and the strategy “Innovating for Sustainable Growth: a Bioeconomy for Europe”.

Application of novel highly efficient and sustainable bioprocess and construction of industrial plant for the lactic acid production depends on type of raw material to be used in market established manner in the European Union area.

The new bioprocess of simultaneous saccharification of semi-solid substrate and fermentation to the lactic acid by amylolytic lactic acid bacterium was developed. The bacterium also possesses proteolytic activity and it can use proteins from the raw material as amino acid source. The developed bioprocess is suitable for scale up and it can be integrated in zero waste biorefineries in which other biochemical and biofuels can be produced from the renewable raw material.

Acknowledgement:

Financial support of the Ministry of Science, Education and Sports of the Republic of Croatia through a zProject “Use of integrated bioprocesses in lactic acid production” (058-0581990-1997) is acknowledged.
Razvoj biotehnoloških procesa za proizvodnju mliječne kiseline

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Sinteza mliječne kiseline iz petrokemikalija je napuštena i od 2011. godine ova se kiselina proizvodi isključivo biotehnološkim procesima i to fermentacijom glukoze, saharoze ili laktoze s pomoću različitih vrsta mikroorganizama. Potražnja za ovom organskom kiselinom u svijetu je u stalnom porastu jer se, osim tradicionalne primjene, u novije vrijeme mliječna kiselina koristi u proizvodnji drugih kemikalija i biorazgradljivih polimera. Glavni izvođači mliječne kiseline ulažu u razvoj novih bioprocesa u kojima bi se iz obnovljivih sировina proizvodila mliječna kiselina s pomoću mikroorganizama koji posjeduju hidrolitičku i fermentativnu aktivnost.

Ova istraživanja imala su za cilj razviti novi bioproces koji bi bio u skladu sa klimatsko-energetskim ciljevima Europske komisije “20/20/20” i strategijom “Innovating for Sustainable Growth: a Bioeconomy for Europe”.

Primjena inovativnog, visoko učinkovitog i održivog bioprocesa i izgradnja industrijskog postrojenja za proizvodnju mliječne kiseline zavisi o vrsti sировine koja bi se tržišno utemeljeno koristila na području Europske unije.

Razvijen je novi bioproces simultane saharifikacije polučvrstog supstrata i fermentacije jednostavnih ugljikohidrata do mliječne kiseline koji je proveden s pomoću amilolitičke bakterije mliječne kiseline. Odabrana bakterija posjeduje i proteolitičku aktivnost i može koristiti i proteine iz sировine kao izvore aminokiselina. Ovaj bioproces prikladan je za uvećanje do industrijskog mjerila i može se integrirati u biorafineriju u kojoj bi se iz odabrane sировine uz 100%-tno iskorištenje proizvodile i druge biokemikalije i biogoriva.

Zahvala:

Znanstveni projekt “Primjena integriranih bioprocesa u proizvodnji mliječne kiseline” (058-0581990-1997) podupire Ministarstvo znanosti, obrazovanja i sportsa Republike Hrvatske.
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Višnja Gaurina Srček is Associate Professor in Laboratory for Cell Culture, Applications and Biotransformation at the Faculty of Food Technology and Biotechnology, University of Zagreb, Croatia. She received her PhD in 2003 from the University of Zagreb in the field of Biotechnology. Her area of interest is production of biologically active compounds using animal cell technology; metabolism of animal cells in culture and application of in vitro cell cultures in toxicology. She published 20 scientific and professional papers, participated in more than 20 international and domestic congresses and 3 international courses on toxicology. Nowadays, she teaches 7 modules on undergraduate, graduate and doctoral studies at the Faculty of Food Technology and Biotechnology. Since 2005 she has been involved in intellectual property protection and worked as WIPO IP coordinator, educated and participated in CARDS and PHARE programs forcing IP protection in academia and R&D sector in the Republic of Croatia.

Aquaculture and fish cell technology

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Aquaculture is the fastest growing food production industry. An important part of increasing the aquaculture production is improving the biological productivity of farmed species of fish. Genetic improvement of
growth rate, survival and feed conversion efficiency have been shown to reduce space, water and feed requirements and could have a large impact on world aquaculture production if widely adopted.

Fish represent the most diverse group of vertebrates with approximately 20,000 different species occupying all aquatic niches. Most research projects using fish have been driven by commercial aquaculture and fisheries, mainly with the aim of identifying and growing viruses for fish vaccine production and as producers of other biopharmaceuticals. However, fish are the most frequently used animals in the environmental risk assessment in elucidating the effects of different stressors on fish health and disease and their use is continuously increasing in recent years. Within the context of EU’s framework REACH (EC 1907/2006; Registration, Evaluation, Authorization and Restriction of Chemicals) application of cell culture systems gain further importance in order to reduce number of animal testing. Therefore, the use of fish cell lines is of significant importance in the assessment of the environmental pollutants which are released into an aquatic environment by a variety of routes affecting different organisms, including humans. Fish cells can serve as a test-model for:

- studying ecotoxicants and water samples for regulatory purposes,
- development and safety evaluation of newly substances and products and
- environmental monitoring and assessment.

Akvakultura i tehnologija stanica riba

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Akvakultura je najbrÞe rastuæa grana proizvodnje hrane. Poveæanje proizvodnje u akvakulturi u znatnoj mjeri je vezano za poboljšanja biološke produktivnosti tijekom uzgoja riba. Tako bi genetièka poboljšanja u brzini rasta i preživljavanju te uèinkovitost hranjenja smanjili potrebe za prostorom, vodom i prehranom, te ukoliko se prihvate, mogu imati veliki utjecaj na svjetsku akvakulturu.
Ribe predstavljaju najrazličitiju skupinu kralješnjaka s oko 20.000 različitih vrsta i prisutne su u svim vodenim sustavima. Većina projekata vezana za istraživanja riba potaknuta su potrebama akvakulture i ribarstva, prvenstveno s ciljem identifikacije i uzgoja virusa za proizvodnju cjepiva za ribe te drugih biofarmaceutskih proizvoda za humanu primjenu. Međutim, ribe se često koriste i u procjeni rizika za okoliš u cilju utvrđivanja učinaka različitih tvari koje na njih djeluju, a njihovo korištenje kontinuirano raste posljednjih godina. U kontekstu EU REACH okvira (EC 1907/2006, Registration, Evaluation, Authorization and Restriction of Chemical substances) primjena kultura stanica dobiva na važnosti u cilju smanjenja testiranja na životinjama. Stoga je primjena staničnih linija riba od velikog značaja u procjeni utjecaja tvari koje se različitim putovima ispuštaju u vodeni okoliš i pri tom djeluju na brojne organizme, uključujući i ljude. Stanice riba tako mogu poslužiti kao test-modeli za:

- proučavanje ekotoksikanata i uzoraka vode u regulatorne svrhe,
- razvoj i procjenu sigurnosti novosintetiziranih tvari i proizvoda te
- praćenje i procjenu stanja u okolišu.
SATURDAY, May 11, 2013

Co-Chairpersons: Vladimir Mrša, Blaženka Kos
Vladimir Mrša
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Vladimir Mrša received his Ph.D. in Biochemistry from the Faculty of Science, University of Zagreb in 1984. He joined Laboratory of Biochemistry, Faculty of Food Technology and Biotechnology, University of Zagreb in 1980 and has been the full Professor at the same Faculty since 1999. He has conducted research in the field of protein glycosylation and secretion, as well as cell wall biosynthesis in fungi. Professor Mrša has published 41 scientific papers which have been cited over 800 times according to Web of Science. Besides, he has published 2 patents and has been an invited lecturer at a number of domestic and international meetings. He is an active member of the Croatian Society for Biochemistry and Molecular Biology, Croatian Microbiological Society and Croatian Society for Biotechnology. Professor Mrša has served as the dean of the Faculty of Food Technology and Biotechnology, University of Zagreb in two mandates (2003–2007). Presently, he is the vice-dean for international relations at the same faculty.

High education for the requirements of industrial production
– European experience and Croatian challenges

High education system in Croatia has developed in the last several decades without a long-term strategy and clearly defined aims and vision. This brought about an apparent diversification of high education institution and an increase in their number without clear quality criteria which they should meet for successful education of new experts. A consequence of this trend was a significant increase in the number of students accompanied by an equally significant drop of quality of the education process and, consequently, the
quality of experts migrating from the academic institutions to the labour market. This goes especially for the studies for careers in the field of production, or careers in the STEM areas.

Being aware of the weaknesses in the education process, not only at the high education level, the government of Republic of Croatia started a project of creation of Education and Science Strategy in order to stop the negative trends in our education and to insure better development conditions for the institutions in our high education system. The work on the strategy has been organised through four working groups one of which deals exclusively with the development strategy of the Croatian high education. This lecture will bring a short analysis of the state-of-art in Croatian high education with the identification of the observed weaknesses, and will propose some of the solutions for its up-grade and improvement.

Visokoškolsko obrazovanje za potrebe gospodarstva – europska iskustva i hrvatski izazovi

Sustav visokog obrazovanja u Republici Hrvatskoj u posljednjih se nekoliko desetljeća razvijao bez dugoročne strategije i jasno definirane vizije i ciljeva. To je dovelo do jasno uočljive diversifikacije visokih učilišta i porasta njihovog broja bez jasnih kriterija kvalitete koje bi ona trebala zadovoljiti za uspješno obrazovanje novih stručnjaka. Posljedica ovakvog trenda bila je značajno povećanje broja studenata, ali uz jednako tako značajan pad kvalitete obrazovnog procesa i u konačnici, kvalitete stručnjaka koji iz akademskih klupa kreću na tržište rada. To se posebno odnosi na studije za izobrazbu stručnjaka za proizvodna zanimanja ili zanimanja STEM grupacije znanstvenih područja.

Uočivši slabosti procesa obrazovanja, ne samo na visokoškolskoj razini, Vlada RH započela je projekt izrade Strategije razvoja obrazovanja i znanosti kojom bi se u narednom razdoblju zaustavili negativni trendovi našeg obrazovanja, a osobama i institucijama u sustavu zagarantirali bolji uvjeti za razvoj. Izradi strategije pristupilo se kroz rad u četiri radne grupe od čega se jedna bavi isključivo strategijom razvoja visokog obrazovanja. U ovom predavanju bit će dana kratka analiza stanja hrvatskog sustava visokog obrazovanja uz identifikaciju uočenih slabosti, kao i neka od mogućih rješenja za njegovo unaprijeđenje.
Martina Tijardović (PhD student) was born 1980 in Zagreb where she was graduated in 2005 from Faculty of Forestry at University in Zagreb. She worked three years at “Croatian Forests” Ltd. and from January 2008 works as PhD student-assistant on Croatian Forest Research Institute (project: “Conservation of stability and productive ability of forest cultures”, code: 024-0682041-2098). In 2009 starts PhD on Faculty of Forestry at University in Zagreb (dissertation: “Substitution of spruce monocultures in Croatia”).

She participated in several scientific conferences, workshops and shorter specializations in Croatia and abroad. In 2008 she participated in workshop at Mediterranean Agronomic Institute (Chania, Greece) and in 2009 completes short specialization at Finnish Forest Research Institute (Metla). She is co-leader of project “Condition of forest cultures with special overview on substitution activities” and associate member of several national projects (“Management of forest cultures in Croatia”, “Establishment of monitoring of forest ecosystems condition in Velebit nature park”, ...). She is also associate member on international COST projects: FP0703 ECHOES, FP1202 MaPFGR and FP1206 EuMIXFOR. During 2008 participates in FP7 project in the frame of “Cooperation” programme – Preposition no. 226410 EDES (Enhancement of Dendromass Energy from Short Rotation Carbon Forestry). She is a member of Croatian forestry society and participates actively in several courses in field education at Faculty of forestry.
Overview of the past and the look on the future of forest reproductive material production in Croatia

Sanja Perić¹ PhD, Martina Tijardović³ PhD student, Tomislav Dubravac¹ PhD, Mirjana Grahovac-Tremski forest engineer²

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Forest reproductive material represents basis for all activities related to forest establishment (afforestation of available forest areas and abandoned agricultural land, burned areas, landfills, queries, protective belts and help to natural regeneration). Increase of negative influences on natural ecosystems together with increase of public need for all functions which forests provide open the space for more intense nursery production in the future. This is the reason why afforestation in the future should have more pronounced significance in the Croatian economy regarding the fact that besides utilization of forest products, ecological and other functions forests also provide economical function in the frame of signed EU agreements. Croatian forest research institute (CFRI) was established during 1960-s with the aim of forest culture establishment with different purposes and was the leading institution in production of forest reproduction material. From the 1992 CFRI continually conducts expert supervision of nursery production which points to real needs not only in artificially established forest cultures but in natural stands as well. Five year monitoring data according to nurseries, tree species and cultivation methods are significant for analysis of past production and point to negative production trend. Estimation of production in the future and all available areas for forest production will be harmonized with Strategy of rural development 2014-2020. Besides autochthonous species special overview on past and present production of pioneer species which are suitable for establishment of cultures for energy purposes (willows, poplars, acacia) and rare and endangered tree species which are used for improvement of forests will be presented. Guidelines for nursery production development will contribute not only to forestry but to other sectors as well.

84
Osvrt na prošlost te pogled na budućnost rasadničke proizvodnje šumskog reprodukcijskog materijala u Hrvatskoj

Dr. sc. Sanja Perišić,¹ Martina Tijardović dipl. ing. šum.,¹
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Ivana Bošnjak was born on June 3rd 1980 in Zagreb. In 2004 she graduated at the Faculty of Science, University of Zagreb – Bachelor of Science in Biology (Ecology). On November 26th 2010 she defended her PhD thesis, entitled “Protective role of the ABC transporter proteins during early embryonic development of sea urchin”, at the Faculty of Science, University of Zagreb. From 2004 Ivana is employed as a research assistant in the Laboratory for Biology and Microbial Genetics at the Faculty of Food Technology and Biotechnology, University of Zagreb. She is actively participating in teaching laboratory classes for undergraduate study modules “Biology I” and “Biology II”. Her scientific specialization fellowship for a period of one year (2006/2007) was performed at Hopkins Marine Station, Stanford University, California, USA, where she made one part of her doctoral thesis. Ivana is a member of the Croatian Genetic Society, Croatian Biological Society, Croatian Toxicological Society, Association of European Marine Biological Laboratories (Assemble). She has co-authored two papers represented in Current Contents, where she is the first author and participated in the 3 international and 4 national scientific meetings.
Toxicity of bisphenol A (BPA) on urchin embryo gene expression and morphology

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Global usage of bisphenol A (BPA) in production of polycarbonate plastic resulted with high concentrations and negative effect of this synthesized chemical in the environment. BPA is severe endocrine disrupting compound and it has high toxic impact on aquatic organisms. Our research focused on determination of toxic effects of BPA on embryonic stages (2-cell, pluteus) of rocky sea urchin (*Paracentrotus lividus*). The main goals were: (i) real time quantitative PCR (qPCR) measurement of target genes expressions involved in chemical defensome: multixenobitoic resistance (MXR) mechanism (P-glycoprotein/P-gp, Multidrug Resistance-associated Protein/MRP), endocrine disruption (orphan Steroid Hormone Receptor/SHR2), and cell-cycle regulation (Cyclin B, CDK); (ii) insight of ultramorphological changes by transmission electron microscopy (TEM). qPCR results indicate the importance of Pgp as the main member of MXR mechanism in defense against BPA while upregulation of SHR2 gene expression clearly indicate endocrine disruption. TEM results strongly support the hypothesis that higher sublethal concentration of BPA induces disorder in karyokinesis and developmental retardation. Our data present an excellent embryotoxicity model that can be assayed for determination of toxic effects of environmentally relevant contaminants.
Učinak bisfenola A (BPA) na gensku ekspresiju i morfologiju embrija morskog ježinca

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Globalna upotreba bisfenola A (BPA) u proizvodnji polikarbonatne plastike je rezultirala visokim koncentracijama te negativnim učincima ove sintetizirane kemikalije u okolišu. BPA je snažan endokrini disruptor i ima značajan toksičan učinak na vodene organizme. Naše istraživanje se fokusiralo na određivanje toksičnih učinaka BPA na embrionalne stadije (2-stanični, pluteus) hridinskog morskog ježinca (Paracentrotus lividus). Glavni ciljevi bili su: (i) metodom kvantitativnog PCR (qPCR) mjeriti ekspresiju ciljanih gena uključenih u staničnu kemijsku obranu: mehanizam multiksenobiotičke otpornosti (MXR) (P-glikoprotein/P-gp, mutidrug resistance-associated protein/MRP), endokrinu disrupciju ( orphan steroidni hormonski receptor/SHR2), i regulaciju staničnog ciklusa (ciklin B, CDK); (ii) uočavanje ultramorfoloških promjena pomoću transmisijskog elektronskog mikroskopa (TEM). qPCR rezultati ukazuju na važnost Pgp kao glavnog člana MXR mehanizma u obrani od BPA dok porast ekspresije SHR2 gena jasno upućuje na endokrinu disrupciju. TEM rezultati čvrsto podupiru hipotezu da više subletalne koncentracije BPA induciraju poremećaje kariokineze i razvojne retardacije. Naši podaci prezentiraju izvrstan model embriotoksičnosti koji se može primijeniti za determinaciju toksičnih učinaka okolišno relevantnih zagađivala.
Lucija Nuskern, born on 5th December, 1988 in Zagreb. Graduated in Environmental Science (module biological environmental protection with mandatory and elective courses form geological and geographical environmental protection) from Faculty of Science, University of Zagreb, in September 2012. Graduation thesis titled “Removal of phosphates and heavy metals from wastewater by bacteria *Acinetobacter junii* and *Pseudomonas aeruginosa* immobilised on natural zeolitic tuff” was made in Department of microbiology and mentored by prof. Jasna Hrenović, PhD. Main professional interests – bioremediation, biotechnology, applied microbiology, waste and wastewater treatment.

Removal of heavy metals and phosphates from wastewater by bioparticles.

*Lucija Nuskern, Jasna Hrenović*

Department of Microbiology, Division of Biology, Faculty of Science, Zagreb, Croatia

The aim of the study was to design bioparticles which could tolerate exposure to high concentrations of heavy metal ions (copper, zinc and nickel) and simultaneously remove heavy metals and phosphates from wastewater. Since natural zeolitic tuff is non toxic, easily available and relatively cheap material with high ion exchange capacity, it has been chosen as a carrier material for bioparticles. The bacteria used for creation of bioparticles were *Acinetobacter junii*, a known phosphate accumulating bacterium, and *Pseudomonas aeruginosa* isolated from metalworking fluids. We created two types of bioparticles: with immobilised *A. junii* and with immobilised *P. aeruginosa*. 

89
Results showed that immobilised bacteria in form of bioparticles were more tolerant to heavy metal toxicity, and removed significantly more metals than planktonic bacteria. Bioparticles with *A. junii* and bioparticles with *P. aeruginosa* could survive, multiply and remove heavy metals and phosphates when in the same system. The highest removal rates for heavy metals and phosphates were obtained in experiment with real wastewater using combination of both types of bioparticles. Bioparticles composed of immobilised bacteria *A. junii* and *P. aeruginosa* on natural zeolitic tuff could be used to simultaneously remove heavy metals and phosphates from wastewater.

**Uklanjanje teških metala i fosfata iz otpadnih voda biočesticama**

*Lucija Nuskern, Jasna Hrenović*

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Cilj ovog istraživanja je bio dizajnirati biočestice koje mogu podnositi izloženost visokim koncentracijama iona teških metala (bakar, cink i nikal) te istovremeno uklanjati teške metale i fosfate iz otpadnih voda. Budući da je prirodni zeolitni tuf netoksičan, lako dostupan i relativno jeftin materijal s visokim kapacitetom kationske izmjene, izabran je kao nosač bakterija za pripremu biočestica. Za pripremu biočestica su korištene bakterije *Acinetobacter junii*, učinkovita fosfat-akumulirajuća bakterija i *Pseudomonas aeruginosa* portijerom iz otpadnih voda metaloprerađivačke industrije. Pripremljena su dva tipa biočestica: s imobiliziranim bakterijama *A. junii* i s imobiliziranim bakterijama *P. aeruginosa*. Rezultati su pokazali da su imobilizirane bakterije u obliku biočestica otpornije na toksično djelovanje teških metala i da su uklanjale značajno više teških metala nego planktonske bakterije. Biočestice s *A. junii* i biočestice s *P. aeruginosa* mogu preživjeti, umnožavati se, te uklanjati teške metale i fosfate kada se nalaze u istom sustavu. Najveći stupanj uklanjanja teških metala i fosfata je postignut u pokusu s realnom otpadnom vodom kombinacijom oba tipa biočestica. Biočestice sastavljene od imobiliziranih bakterija *A. junii* i *P. aeruginosa* na zeolitni tuf mogle bi se koristiti za simultano uklanjanje teških metala i fosfata iz otpadnih voda.
I was born in Kranj (Slovenia) in 1985. After finishing high school (Gimnazija Bežigrad) in Ljubljana I have decided to study microbiology on Biotechnical faculty, University of Ljubljana. In 2007 (during the study) I decided to participate on international student competition in synthetic biology (iGEM) held at MIT, USA. The team lead by prof. dr. Roman Jerala (National institute of Chemistry, Ljubljana) won first prize in Health and Medicine category. In 2009 I graduated under the mentorship of doc. dr. Hrvoje Petković in Department of Food science and Technology, Biotechnical Faculty lead by prof. dr. Peter Raspor. Title of my graduation thesis was: “Development of an Chalcone Synthase Based Actinomycete Reporter System”. After graduation I enrolled interdisciplinary PhD study of Biomedicine in University of Ljubljana under the mentorship of prof. dr. Roman Jerala. In 2010 I was one of the mentors of student team that overall won the iGEM competition held by MIT, USA. In 2011 I was one of the mentors of student team that participated on International Bio Molecular Design Competition (BioMod) held by Harvard University, USA and in 2012 I was one of the mentors of student team that again participated on iGEM competition MIT, USA. 2011 BioMod team won second place for online presentation and third place for YouTube movie. 2012 iGEM team won overall second prize and first place in Health and Medicine category. At the moment I am finishing my PhD research.
DNA guided assembly line

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Living organisms can efficiently synthesize complex compounds. Natural optimization of biosynthetic pathways evolved through organization of enzymes in multimeric units that allow substrate channeling. Synthetic biology uses molecular biology tools to design completely new biosynthetic pathways through combination of heterologous enzymes with the goal to improve the yield and the production of novel compounds. Similar to optimization of biosynthetic pathways through substrate channeling, artificial enzyme scaffolding could bring the enzymes of a biosynthetic pathway in close proximity, thereby improving metabolic flux, and decreasing the leak of potentially toxic intermediates. We hypothesized that a DNA based biosynthetic scaffold, in which DNA binding protein domains are fused to different biosynthetic enzymes, can be used to organize the enzymes of a biosynthetic pathway in a defined order. We designed chimeric enzymes for biosynthesis of a plant antioxidant trans-resveratrol in which each chimeric enzyme of the resveratrol biosynthetic pathway was genetically fused to a DNA binding zinc finger domain. When expressed together with a DNA scaffold containing the binding sites for the zinc finger domains, the yield of resveratrol in Escherichia coli increased up to 5 fold compared to the control without DNA scaffold. Taken together, our data suggest that organization of biosynthetic pathways by a DNA scaffold represents a new powerful tool of synthetic biology that can be used as an addition to classical metabolic engineering techniques for industrial strain improvement.
Teuta Murati was born on June 17, 1982 in Varaždin, Croatia, where she completed her elementary and secondary education. Her B. Sc. diploma she received from Faculty of Food Technology and Biotechnology, University of Zagreb, in 2006 after finishing the graduate thesis in Laboratory for toxicology. Since June 2007 to September 2008 she worked as a junior scientist – biochemist in GlaxoSmithKline Research Center Zagreb, in the Laboratory for in vitro and cell biology. In October 2008 she began to work as a scientific assistant in Laboratory for toxicology at Faculty of Food Technology and Biotechnology on the project of Ministry of Science, Education and Sports of the Republic of Croatia No. 058-0582184-2232: Endocrine active substances found in food and the damage to the reproductive system. Currently, Teuta Murati is the posgraduate student on doctoral study in Biotechnology and Bioprocess Engineering. Her scientific research interests include potential toxic effects of food contaminants with endocrine activities, especially pesticides and polychlorinated biphenyls. Mechanisms of their action at the cellular level she is attempting to explain by using alternative in vitro systems.

**Endocrine disruptors and animal-free toxicology**

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Endocrine disruptors, hormonally active compounds which can modulate the endocrine and immune systems, have captured public’s attention like no
other toxicity concern since the publication of Rachel Carlson's *Silent Spring* in 1962. Because the endocrine system is vital to reproductive health and the maintenance of species, the research of thousands of chemicals produced and used in commerce that may damage this system, remains very high on the scientific agenda. For decades, toxicological risk assessment has used animal experiments as the main approach to ensure human health and safety of the environment, respecting the so-called 3Rs (Reduce, Replace, Refine) principle. Beside ethical and economical considerations, there are also scientific reasons why the future studies are seen in the strength of development and incorporation of stepwise testing strategies, combining experimental data from a range of alternative methods (physicochemical techniques, computerized modeling based on quantitative structure-activity relationships – QSAR, the -omics technologies, metabolic and kinetic modelling and *in vitro* approach). The use of *in vitro* systems (subcellular systems, primary cell cultures, cell lines, stem cells, whole tissues and perfused organs) provide a detailed insight into the mechanisms of toxicity. At the same time, high-throughput and high-content measurements on various cell models provide a sensitive and robust approach for screening new chemical entities. To fill remaining gaps of knowledge, targeted testing in animals would then be performed as an additional step. This would change toxicology from being a predominantly observational craft and regulatory support discipline back to a natural science with all its dimensions. According to the fact that Croatia becomes from July 1 a member of EU, all appropriate testing regulations must be harmonized. Concerning the toxicity validation it means that animal-free testing methods will be obligated and applied in Croatia without any further delay as much as it is possible.

**Endokrini modulatori i toksikološko testiranje bez uporabe pokusnih životinj**

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Od objavljivanja knjige Rachel Carlson *Tiho proljeće* 1962. godine ništa nije više zaokupilo pozornost javnosti nego moguće toksično djelovanje
endokrinih modulatora, hormonski aktivnih spojeva, koji negativno utječu na endokrini i imunološki sustav. Budući da je endokrini sustav od vitalnog značaja za reproduktivno zdravlje i održavanje vrsta, istraživanja vezana uz tisuće novih spojeva, koje mogu oštetiti taj sustav, zauzimaju vrlo visoko mjesto u znanstvenim programima. Već desetljećima u svrhu procjene rizika, a u cilju očuvanja zdravlja ljudi i sigurnost okoliša, kao glavna strategija testiranja toksičnosti spojeva koriste se pokusi na životinjama, poštujući takozvani 3R (Reduce, Replace, Refine) princip. Osim etičkih i ekonomskih promišljanja, postoje i znanstveni razlozi zašto buduće studije razvijaju i uvode stupanjano testiranje toksičnosti, kombinirajući eksperimentalne podatke dobivene primjenom niza alternativnih metoda (fizikalno-kemijske tehnike, računalno modeliranje koje se temelji na odnosu strukture tvari prema njoj aktivnosti – QSAR, tzv. -omics tehnologija, metaboličko i kinetičko modeliranje i in vitro pristup). Uporaba in vitro sustava (substancijskih sustava, primarnih staničnih kultura, staničnih linija, matičnih stanica, dijelova tkiva i cijelih organa) može pružiti detaljan uvid u mehanizme toksičnosti. U isto vrijeme, mjerenja na različitim staničnim modelima pružaju osjetljiv i robustan pristup za provjeravanje novih kemijskih entiteta. Kao dodatan korak za popunu preostalih praznina u znanju, provodilo bi se ciljano testiranje na životinjama kao posljednja faza prije ispitivanja npr. nekog novog lijeka na ljudima. To mijenja pogled na toksikologiju sa pretežito deskriptivne i regulatorne discipline po novno na prirodnu znanost sa svim svojim dimenzijama i do substaničnih sadržaja. Obzirom na činjenicu da Hrvatska 1. srpnja postaje punopravna članica EU, svi odgovarajući propisi testiranja moraju biti usklađeni i u primjeni u Hrvatskoj. To znači obaveznu primjenu metoda provjerene i potvrđene valjanosti.
Ana Bielen was born on July 31st 1979. She works as a teaching assistant in the Laboratory for Biology and Microbial Genetics, Faculty of Food Technology and Biotechnology, University of Zagreb, and participates in realisation of several modules, Biology I and II being the most extensive. She has graduated in 2003 at Faculty of Science, Division of Biology, with average grade 4.98. She has obtained PhD degree in 2011 at Postdoctoral studies of natural sciences – Biology, University of Zagreb, with the thesis entitled “Molecular and functional characterisation of GDS(L) lipases from Streptomyces rimosus and Streptomyces coelicolor”. She has presented her research at 12 scientific congresses and published two original scientific papers. She has been professionally trained at the Technical University in Graz (Austria) and University of Pau (France). She has attended 11 courses and summer schools. She is a co-author of two chapters in the book “Methods in molecular biology”. She has won an award of The Society of University Teachers, Scholars and Other Scientists in Zagreb for the year 2009.
Scanning for genes encoding GDS(L) hydrolases in Actinobacteria from wide diversity of ecological niches

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Due to their multifunctionality, GDS(L) hydrolases have great potential for application (e.g. in food, pharmaceutical and detergent industry). The member of this protein family often exhibits various catalytic activities like lipase, phospholipase, esterase and thioesterase and has broad substrate specificities, which makes them very interesting for biotechnology. However, finding new GDS(L) enzymes using BLAST is difficult due to their low sequence similarity. A novel HMM-based method (Inverse Posterior Probability Assignment – IPPA) was applied for GDS(L) motifs scanning. In addition, searching for novel members of GDS(L) family was accelerated using automated GDS(L) detection pipeline. We have shown that Actinobacteria from wide variety of ecological niches possesses high number of genes encoding for GDS(L) enzymes. We have found 257 GDS(L) enzymes in 52 actinobacterial proteomes (up to 24 per proteome), majority coming from soil-inhabiting species. Clustering and phylogenetic analysis divided these enzymes into 8 well defined groups. Further, horizontal gene transfer had a significant impact on evolution of actinobacterial GDS(L) genes and it can be hypothesized that these enzymes facilitated adaptation to novel ecological niches, e.g. saprophytic lifestyle in soil. Moreover, we have found interesting variations in active site amino acids that possibly reflect novel enzyme properties of biotechnological interest.
Identifikacija GDS(L) hidrolaza u genomima aktinobakterija iz raznolikih ekoloških niša

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Zahvaljujući multifunkcionalnosti GDS(L) hidrolaze imaju veliku mogućnost primjene (npr. u prehrambenoj, farmaceutskoj i industriji detergenata). Članovi ove enzimske porodice često pokazuju različite katalitičke aktivnosti poput lipazne, fosfolipazne, esterazne i tioesterazne, kao i široku supstratnu specifičnost, što ih čini biotehnološki vrlo zanimljivima. Međutim, pronalazak novih GDS(L) enzima uz pomoć BLAST metode otežan je niskom sličnošću aminokislinskih sljedova. Nova metoda nazvana IPPA (eng. Inverse Posterior Probability Assignment) primijenjena je za skeniranje GDS(L) motiva, a pretraga za novim članovima GDS(L) porodice je ubrzana automatizacijom (eng. GDS(L)-detection pipeline). Pokazali smo da aktinobakterije iz širokog raspona ekoloških niša imaju velik broj gena koji kodiraju za GDS(L) enzime. Pronašli smo 257 GDS(L) enzima u 52 aktinobakterijska proteoma (do 24 po proteomu), od kojih većina potječe iz vrsta koje nastanjuju tlo. Klasteriranjem i filogenetskom analizom enzimi su podijeljeni u 8 dobro definiranih grupa. Nadalje, horizontalni prijenos gena je imao značajan utjecaj na evoluciju aktinobakterijskih GDS(L) gena i može se pretpostaviti da su ti enzimi potpomogli prilagodbu na nove ekološke niše, npr. saprofitski način života u tlu. Osim toga, pronašli smo zanimljive varijacije aminokiselina aktivnog mjesta koje vjerojatno odražavaju nova enzimska svojstva s biotehnološkim značajem.
Mario Franić was born on January 28, 1986 in Požega, Croatia. After graduating from gymnasium in Požega in 2004 he was enrolled at the Department of biology and chemistry (later just the Department of biology). During his studies he participated in two practical trainings abroad – in 2007 (Athens, Greece) and in 2008 (Gliwice, Poland). After graduating from the Department of biology in 2010 he worked as a school teacher until 2012 when he was employed at the Agricultural institute Osijek as a research assistant. During 2012 he was enrolled at postgraduate university study programme Molecular biosciences.

**Phytoextraction of cadmium using recombinant DNA technology in maize.**

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Heavy metals belong to a group of most significant pollutants in the environment. Cadmium, as a heavy metal, is an environmental problem due to toxicity to plants, animals and humans. Techniques used for remediation are generally expensive and have an adverse effect on soil fertility which is not the case with phytoremediation. In order to remove a heavy metal from soil it is necessary to have a plant that will hyperaccumulate the metal in its tissues.
Such process is called phytoextraction. Hyperaccumulation in tissues is possible with the limiting factor of biomass quantity. Gene for cadmium accumulation in maize leaf (*Zea mays* L.) has been found – *ask2* which codes for protein aspartate kinase2. Since it is only one gene there is great potential for manipulation of the gene to increase cadmium accumulation in maize plants. Gene was obtained from *Arizona Genomics Institute* from ZM_BFc003612C cDNA library. HA and FLAG tags were added to the 5’ end of the gene using polymerase chain reaction (PCR) for easier immunodetection and isolation of the protein from plants. *Ask2* gene, with HA and FLAG tags, was inserted into TOPO vector which was used to transform chemically competent *E.coli* cells. Selection of transformants was carried out on kanamycin plates and the conformation of gene insertion was done with electrophoresis and PCR. After confirming the insertion of *ask2* gene in TOPO vector gene was inserted into a Gateway vector suited for monocots transformation (pANIC) using LR reaction and was later used to transform DH5α chemically competent *E.coli* cells. Due to large maize biomass and distribution the idea of phytoremediating heavy metals with maize seems very appealing.

**Fitoekstrakcija kadmija pomoću rekombinantne DNA tehnologije kod kukuruza**

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o jednom genu velik je potencijal za manipulaciju tim genom u svrhu povećanja (ili smanjenja) akumulacije kadmiya u biljci. Gen dobiven iz *Arizona Genomics* instituta iz cDNA biblioteke ZM_BFc003612C. Zbog lakše kasnije imunodetekcije i izolacije proteina iz biljke lančanom reakcijom polimerazom (PCR) dodani su HA i FLAG biljezi na 5 Primer gena. *Ask2* gen s HA i FLAG biljezima je kloniran u TOPO vektor kojim su transformirane kemijski kompetentne stanice *E.coli*. Selekcija transformanata je obavljena na pločama s kanamicinom, a potvrda ugradnje gena je izvršena elektroforezom i PCR reakcijom. Nakon potvrde ugradnje *ask2* gena u TOPO vektor gen je LR reakcijom rekombinacije ugrađen u Gateway vektor prilagođen za transformaciju monokotiledona (pANIC) te su njima transformirane DH5a kemijski kompetentne stanice *E.coli*. Zbog velike količine biomase kukuruza i njegove široke rasprostranjenosti ideja fitoremedijacije teških metala pomoću kukuruza se čini vrlo privlačnom.
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PhD Jasna Beganović, assist. prof. graduated with Biochemical Engineering from Faculty of Food Technology and Biotechnology, University of Zagreb in 2003. Since 2003 she is employed in Laboratory for antibiotic, enzyme, probiotic and starter culture technology, Department of Biochemical Engineering, Faculty of Food Technology and Biotechnology, Univ. of Zagreb. In 2006 she won 18 months fellowship for PhD students in Research Institute INRA, Paris, France where she was involved in PhD program “Identification of surface proteins of lactic acid bacteria by Mass Spectrometry” in the framework of Marie Curie research project LABHEALTH “Novel applications of lactic acid bacteria to improve food safety and health”. She defended her PhD thesis entitled “Application of proteomics and other molecular methods in characterization of functionality of the probiotic bacteria”, under the supervision of PhD Jagoda Šušković, full prof., on Faculty of Food Technology and Biotechnology. Univ. of Zagreb. In 2011 she was postdoctoral fellow on project “Immunomodulation effect of Lactobacillus strains mediated by S-layer proteins”, in Department of Veterinary Bioscience, Faculty of Veterinary Medicine, Univ. of Helsinki. She was twice awarded Biotechnical Foundation Award and Young scientist Award “Vera Johanides” from Croatian Academy of Engineering. She is member of Croatian Society of Biotechnology and Croatian Microbiological Society.
Role of S-layer proteins in probiotic activity of \textit{Lactobacillus} strains

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The biological role of surface (S-) layer proteins, present at the cell wall, in several species of the genus \textit{Lactobacillus}, has not yet been fully elucidated. Among 200 autochthonous species of lactic acid bacteria (LAB) strains isolated from different traditional fermented products, identified, characterised as probiotic and/or starter cultures and deposited in the Collection of Lactic Acid Bacteria (ZBMK in Laboratory of Antibiotic, Enzyme, Probiotic and Starter Cultures Technology, Faculty of Food Technology and Biotechnology), \textit{Lactobacillus} strains expressing S-proteins on their cell surface were selected. After characterisation of their S-proteins, the probiotic concept experiments were performed. The role of S-proteins in auto- and coaggregation of bacterial cells, in adhesion to intestinal epithelial cells, in competitive exclusion of pathogens, as well as protective role during production and application of active dried probiotic products was examined. Special focus is directed towards a role of S-proteins in immunomodulation activity of selected LAB strains aimed to set up basis for their possible development as mucosal vaccines. Hence, cytokine production and maturation of human monocyte-derived dendritic cells (moDCs) in response to bacterial and S-protein stimulation were analysed with enzyme-linked immunosorbent assay (ELISA) and flow cytometric analysis (FACS), respectively. Results indicate that S-layer expressing \textit{Lactobacillus} strains are able to induce moDC maturation and cytokine production, but the induction varied from one bacterial strain to another.
Uloga S-proteina u probiotičkom djelovanju bakterija iz roda Lactobacillus

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Biološka uloga S-proteina (engl. surface layer (S-layer) proteins) prisutnih na površini stanične stijenke nekih vrsta bakterija iz roda Lactobacillus, još nije u potpunosti razjašnjena. Između dvjestotinjak autohtonih sojeva bakterija mljićne kiseline (BMK), koje su izolirane iz tradicionalno proizvedenih fermentiranih proizvoda, a zatim identificirane i okarakterizirane kao probiotičke i/ili starter kulture, te pohranjene u Zbirci bakterija mljićne kiseline (ZBMK), Laboratorija za tehnologiju antibiotika, enzima, probiotika i starter kultura Prehrambeno-biotehnološkog fakulteta, odabrani su sojevi BMK iz roda Lactobacillus koji sadrže S-proteine na površini stanice. Nakon karakterizacije S-proteina odabranih bakterijskih sojeva, provedena su istraživanja u okviru probiotičkog koncepta. Ispitana je uloga S-proteina u auto- i koagregaciji bakterijskih stanica, adheziji na crijevne epitelne stanice, kompetitivnoj ekskluziji patogena, kao i uloga S-proteina, tijekom proizvodnje i primjene suhih aktivnih probiotičkih pripravaka. Posebno je istražena uloga S-proteina u imunomodulacijskom djelovanju odabranih sojeva BMK s ciljem istraživanja mogućnosti njihove primjene kao mukoznih vakcina. Ispitan je imunomodulacijski učinak, s naglaskom na ulogu S-proteina odabranih sojeva BMK u poticanju sazrijevanja odnosno proizvodnje citokina u humanim dendritičnim stanicama. Proizvodnja citokina i sazrijevanje dendritičnih stanica, kao rezultat imunostimulacije, bilo pomoću bakterijskih stanica ili njihovih izoliranih i pročišćenih S-proteina, su određeni ELISA metodom, te metodom protočne citometrije. Odabrani sojevi bakterija iz roda Lactobacillus koji sadrže S-proteine su inducirali nastajanje citokina i sazrijevanje dendritičnih stanica, a dobiveni rezultati su bili različiti u odnosu na različite sojeve.
Anamarija Štafa finished undergraduate studies in biotechnology at Faculty of Food Technology and Biotechnology and obtained her Ph.D degree in molecular genetics at Faculty of Science, (University of Zagreb, Croatia). She finished two postdocs, one at Institut National de la Santé et de la Recherche Médicale (Paris, France) studying aging in yeast and the other at Columbia University (New York, USA) studying DNA repair. At the moment, Anamarija Stafa is senior scientific assistant researching DNA repair in yeast *S. cerevisiae* and working as a teaching assistant on different genetics courses at Faculty of Food Technology and Biotechnology, University of Zagreb.

**Genetic side-effects during gene replacement in yeast *Saccharomyces cerevisiae***

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Gene replacement is one of basic techniques used for the construction of producer strains in biotechnology. This method is based on the introduction of linear, non-replicative transforming DNA fragments to replace the specific genomic region by homologous recombination, using cell’s endogenous proteins evolutionary conserved across all eukaryotic organisms. We have investigated gene replacement in yeast *Saccharomyces cerevisiae*, a model organism used for research of almost all processes in eukaryotic cells and one of the most important industrial microorganisms. Despite the general belief
that gene replacement in yeast occurs in more than 99% of cases, we have observed that up to 10% of transformants are due to aberrant genetic events that include duplication of targeted chromosome, additions of the transforming DNA fragment next to the targeted sequence and random, illegitimate integrations of the transforming DNA fragment. Moreover, it was observed that both the percentage and the spectrum of aberrant genetic events depend on the type of modification being introduced to the genome. Results of this research give guidelines that facilitate the increase in successful gene replacement and at the same time warn that newly constructed strains should be properly checked using appropriate molecular genetic techniques.

Genetičke promjene tijekom zamjene gena u kvascu *Saccharomyces cerevisiae*

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Zamjena gena jedna je od osnovnih tehnika koja se koristi za konstrukciju proizvodnih sojeva u biotehnologiji. Ova metoda temelji se na unosu linearnih, nereplikativnih transformirajućih fragmenta DNA u stanicu, koji procesom homologne rekombinacije zamjenjuju odgovarajuću sekvencu u genomu, a stanični proteini uključeni u ovaj proces konzervirani su u svim eukariotskim organizmima. U ovome radu istraživana je zamjena gena u kvascu *Saccharomyces cerevisiae*, modelnom organizmu za istraživanje gotovo svih bioloških procesa u eukariotskoj stanici, ali i jednom od najvažnijih industrijskih mikroorganizama. Unatoč opće prihvaćenom mišljenju da uspješnost zamjene gena u kvascu iznosi više od 99%, primjećeno je da udio aberrantnih genetičkih događaja tijekom zamjene gena može doći i do 10%, a detektirani aberranti događaji uključuju duplicaciju ciljnog kromosoma, integraciju transformirajuće DNA pored ciljne regije te nasumičnu integraciju transformirajuće DNA. Osim toga, primjećeno je da udio i spektar nepoželjnih genetičkih događaja ovise o vrsti modificiranja koja se unosi u genom. Rezultati ovog istraživanja daju smjernice koje omogućuju povećanje vjerojatnosti uspješne zamjene gena te istovremeno sugerišu da je naročitu pažnju potrebno posvetiti upravo detaljnoj molekularno genetičkoj provjeri konstruiranog soja.
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I did recently become Ph.D. in Biotechnology at the Biotechnical Faculty at the University of Ljubljana, previously B.sc. in Food Science and Technology at the Biotechnical Faculty at the University of Ljubljana. The graduation thesis was awarded with the prize Premio Collio Innovazione 2009.

Before my University studies I finished the Agricultural High School (Solski Center Nova Gorica).

From the 1st of April 2013 employed at IFinD S.r.l. (Italian Food Industries (Food Processing Equipment), Pero di Breda di Piave (TV) Italy) as Export Manager. Before, from the 1st of September 2010 until the 31st of March 2013 I was employed at Velo S.p.A. (Food Processing Equipment) as Export Manager.

During My PhD studies I did Research from the 1st February 2009 until the 14th of February 2010 at The Australian Wine Research Institute, Adelaide, Australia.
Development and Application of *Saccharomyces* Interspecies hybrids in wine industry

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Industrial food-grade yeast strains are selected for traits that enhance their application in quality production processes. Wine yeasts are required to survive in the harsh environment of fermenting grape must, while at the same time contributing to wine quality by producing desirable aromas and flavors. For this reason, there are hundreds of wine yeasts available, exhibiting characteristics that make them suitable for different fermentation conditions and winemaking practices. As wine styles evolve and technical winemaking requirements change, however, it becomes necessary to improve existing strains. This becomes a laborious and costly process when the targets for improvement involve flavor compound production. Here, we demonstrate a new approach harnessing preexisting industrial yeast strains that carry desirable flavor phenotypes – low hydrogen sulfide (H$_2$S) production and high ester production. A low-H$_2$S *Saccharomyces cerevisiae* strain previously generated by chemical mutagenesis was hybridized independently with two ester-producing natural interspecies hybrids of *S. cerevisiae* and *Saccharomyces kudriavzevii*. Deficiencies in sporulation frequency and spore viability were overcome through use of complementary selectable traits, allowing successful isolation of several novel hybrids exhibiting both desired traits in a single round of selection.
Regulation of biosynthesis of immunosuppressant FK506 (tacrolimus) by *Streptomyces tsukubaensis*.

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FK506 (tacrolimus) is a medically important immunosuppressant biosynthesized by a combined polyketide synthase / non-ribosomal peptide synthetase complex. Biosynthetic gene clusters often contain regulatory genes which can have a profound influence on the yield of target product. The objective of our study was to obtain the complete sequence of the FK506 gene cluster and identify the regulatory mechanisms of FK506 biosynthesis. Whole genome sequencing of *S. tsukubaensis* NRRL 18488, a progenitor of industrial FK506-producing strains, allowed us to identify three regulatory genes in the FK506 gene cluster, namely, *fkbN*, which belongs to the LAL family (large ATP-binding regulators of the LuxR family), *fkbR*, which belongs to the
LTTR family (LysR-type transcriptional regulators) and allN, which belongs to the AsnC family of transcriptional regulators. Using gene disruption and overexpression approaches we determined fkbN and fkbR to be positive regulators of FK506 biosynthesis while no functional role has been observed for the allN gene. Further on, a combination of RT-PCR and the rppA reporter system approaches revealed potential target genes of the FkbN and FkbR regulatory proteins. Interestingly, while inactivation of fkbN completely abolished FK506 biosynthesis the tested biosynthetic genes were still actively transcribed. In conclusion, significant increase in FK506 yield by fkbN and fkbR overexpression confirms that regulatory genes possess a clear applied potential and can play an important role in development of industrial bioprocesses.
Mojca Brložnik is from 2012 employed at the Department of Molecular and Biomedical Sciences, Jožef Stefan Institute, Jamova 39, SI-1000 Ljubljana Slovenia. Undergraduate education: Microbiology 2012, graduated on Effects of growth medium on biofilm morphology and exopolymer composition in Bacillus subtilis. Employment and Research Experience: July–September 2010: Student praxis, National Institute of Biology, Marine Biology Station Piran, Slovenia, 2011–2012: Junior Research Associate, Food Science and Technology University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia. Research interests: Regulation of lipid metabolism in Saccharomyces cerevisiae, polygenetic trait analysis, yeast as cell factory for biofuel production.

Identification of Genetic Variants Responsible for Biotechnologically Important Traits and the Design of New Generation Industrial Yeast Strains.

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Most heritable traits are polygenic, including the majority of microbial biotechnologically important characteristics. One of these is the ability to produce biofuels, where yeast Saccharomyces cerevisiae is the major
industrial organism. Besides bioethanol, yeast strains are also promising as producers of biodiesel. Extreme quantitative trait loci mapping (X-QTL) is a method developed in yeast that enables detection of all genetic loci even with small effects on a given trait. X-QTL was applied to identify QTLs responsible for high triacylglycerol (TAG) content in a \textit{S. cerevisiae} strain. An industrial strain with high TAG content was crossed with a reference laboratory strain and their progeny in which the genetic material from the parental strains is combined was isolated. Two subpopulations of these segregants, a random one representing the average phenotype, and an extreme one, were selected on the basis of fluorescent staining of TAG and flow cytometry sorting. Both segregant pools were genotyped using tiling microarrays and whole-genome sequencing, and for the most part the latter method proved superior. Within the identified QTLs, candidate causative genes were identified using a novel custom-made bioinformatics algorithm. Allele swaps were performed from the strain with high TAG content to the one lacking this phenotypic trait, resulting in increased TAG content in the latter strain.
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Bachelor's degree in biology, 2002–2008 and from 2008 PhD student at University of Ljubljana. Supervisor: Prof. dr. Ines Mandic-Mulec. Foreign experience at University of Groningen, Centre for Ecological and Evolutionary Science, Department of Microbial Ecology, NL9751 NNHaren, Netherlands and University of Bielefeld, Centrum für Biotechnologie, Bioinformatics Resource Facility. His interests, skills and teaching experience is with methods of molecular biology ranging from DNA extraction and PCR to cloning and sequencing. Interested also in analyzing the sequence data to gain biologically meaningful insights.

Employed from 2009 as research scientist on FP7 European project “METAEXPLORE” and a national project “Metagenomics for bioexploration and biomining of bacterial laccases for a sustainable environment” at the University of Ljubljana, Biotechnical faculty, Department of Food science and Technology.

Mining bacterial genomes for laccases

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Laccases are blue copper oxidases that couple oxidation of a variety of phenolic compounds with reduction of molecular oxygen to water. Fungal enzymes have been used in processes in the paper, food and textile industries.
However, the attention is turning to bacterial laccases in the recent years which seem to be robust and versatile.

Recent research, including our own, suggested that bacterial genes for laccase-like enzymes are abundant and diverse in natural and man-made environments. To assess the diversity of bacterial laccases in silico, we searched more than 2,000 bacterial genomes for laccase genes and found over 1,200 genes for putative laccase-like genes using custom probabilistic models (pHMMs). By using cloning and heterologous expression in *Escherichia coli* we mined this pool of potentials to biochemically characterize three enzymes.

Firstly, we analyzed CotA, which is a well-characterized laccase found in the spore coat of *Bacillus subtilis*. Variability of CotA was studied in natural isolates of *B. subtilis* and was found to be highly conserved at the level of gene sequence and enzyme activity. Secondly, a laccase from *Geobacter metallireducens* was successfully expressed in *E. coli* and purified. This demonstrated for the first time that functional laccases are present in anaerobic organisms, despite dependence of laccases on molecular oxygen as electron acceptor. Finally, a gene for a laccase-like protein from an obligate alkaliphilic bacterium *Thioalkalivibrio sp.* was expressed in *E. coli* with the goal to find a novel pH-tolerant laccase. The purified enzyme was indeed robust and could oxidize phenolics at pH8. This characteristic could be vital in some industrial applications as all known fungal laccases are active only at low pH.

In conclusion, this work provides novel insights into diversity and biochemical traits of bacterial laccases, tools for their discovery and potential for their biotechnological application.
Bojan Žunar (born 1988) received his Bachelor degree in Biotechnology and Master degree in Molecular Biotechnology at the Faculty of Food Technology and Biotechnology, University of Zagreb, as well as Master degree in Bioindustrial Techniques at the University of Orleans. During his education he received Rector's Award and Dean's Award for excellence in curricular activities. He is currently employed as junior teaching assistant at the Faculty of Food Technology and Biotechnology and is enrolled in postgraduate PhD study in Biotechnology and Bioprocess Engineering. Focus of his doctoral work is transformation and genetic recombination in yeast *Dekkera bruxellensis*.

**Genetic transformation of yeast**

*Dekkera/Brettanomyces bruxellensis*

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Yeast *Dekkera/Brettanomyces bruxellensis* is a notorious wine spoilage yeast, but it is also a potential industrial organism for production of bioethanol and acetic acid. However, for detailed genetic characterization and construction of new strains with potential application in biotechnology, it is necessary to develop protocol for genetic transformation, i.e. for the introduction of for-
eign DNA into the cell. We have developed procedure for transformation of yeast *D. bruxellensis* by varying parameters of the three frequently used methods for transformation of *Saccharomyces* and non-*Saccharomyces* yeasts: transformation by alkali cations, electroporation, and spheroplast transformation. Obtained transformation efficiency was up to 20 transformants/µg linear DNA. As a selective marker sequence *kanMX4* was used, allowing for selection of transformants on geneticin-containing medium. Molecular analysis confirmed presence of the transforming DNA fragment in the genome. Stability of transformants varied between 93.6% and 100%. To enable selection of transformants without the use of antibiotics, we have isolated strain with inactive *URA3* gene therefore allowing for selection using medium lacking uracil. These results represent the first and necessary step in the development of methodology for precise genetic modification of *D. bruxellensis* which could enable its use in biotechnological production.

**Genetička transformacija kvasca**

*Dekkera/Brettanomyces bruxellensis*

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Kvasac *Dekkera/Brettanomyces bruxellensis* je najpoznatiji uzročnik kva-
renja vina, ali je ujedno i potencijalni industrijski organizam za proizvodnju
bioetanola i octene kiseline. Međutim, za detaljniju genetičku karakterizaciju i
konstrukciju potencijalnih proizvodnih sojeva potrebno je razviti protokol za
genetičku transformaciju, odnosno za unos strane DNA u stanicu. Variranjem
parametara triju često korištenih metoda za transformaciju *Saccharomyces* i
ne-*Saccharomyces* vrsta kvasaca (transformacija alkalnim kationima, elektro-
poracijom i transformacija protoplastiranjem) transformirali smo kvasac *D. bru-
xellensis*, pri čemu je uspješnost transformacije iznosila do 20 transforma-
nata/µg linearne DNA. Kao selektivni marker korištena je sekvencija *kanMX4*
čime je omogućena selekcija transformanata na podlozi s antibiotikom geneti-
cinom. Molekularnom analizom potvrđeno je postojanje transformirajuće
DNA u genomu. Stabilnost transformanata iznosila je 93,6% do 100%. Kako bismo mogli provesti selekciju transformanata bez upotrebe antibiotika, izolirali smo soj s inaktivnim genomom *URA3* čime je omogućena selekcija transformanata na podlozi koja ne sadrži uracil. Ovi rezultati predstavljaju prvi korak u postupku razvoja metodologije za preciznu modifikaciju genoma kvasca *D. bruxellensis*, a to može omogućiti njegovu primjenu u biotehnološkoj proizvodnji.
Antonio Starčević finished undergraduate studies in biotechnology at Faculty of Food Technology and Biotechnology and obtained his Ph.D degree in natural sciences (biology) at Kaiserslautern Technical University. At the moment, Antonio Starčević is Assistant professor and head of Cabinet for bioinformatics at the Faculty of Food Technology and Biotechnology, University of Zagreb where he teaches courses in bioinformatics for graduate and post-graduate students. Antonio has published 10 papers listed in ISI Web of knowledge which were cited 127 times (113 without self-citations) and has h-index of 5. Antonio was involved in several Croatian and international projects, and is currently engaged in an FP7 project named “Amylomics” as a project partner, and in Croatian science foundation funded project named “Clinical proteomics of microorganisms” which he actively leads.

Mass spectrometry based clinical proteomics

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Rapid sequencing of peptides using MALDI-TOF is still a challenge since it is considered as a critical capability in modern proteomics research. It is a tool of utmost importance for rapid identification of pathogenic cells, as
well as for high throughput characterization of cell-based products in pharmaceutical and food processing industry. Coupling this technology with newly developed computer software it is possible to deliver new services capable of “one step” identification of target proteins in a microorganism or a cell. In order to achieve this goal, we propose a faster, cheaper, more selective and reproducible analytical method for the determination of amino acid sequence of peptides (triptic digests) by derivatisation with disulphonic acid (Patent protected technology of Ruđer Bošković Institute) coupled with novel software developed at Faculty of Food Technology and Biotechnology. Above mentioned methods applied to proteomic analyses could eventually be applied in clinical practice as fast, reliable and efficient tools for microorganism detection and identification.

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Klinička proteomika mikroorganizama bazirana na spektrometriji masa

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Sekvenciranje peptida iako povijesno ostvareno prije sekvencioniranja DNA/RNA, tehnološki uvelike zaostaje za potonjim i stoga još uvijek predstavlja izazov iz više razloga. Prvenstveno zbog svoje važnosti pri brzoj identifikaciji patogenih stanica, ali i pri visoko učinkovitim metodama karakterizacije proizvoda farmaceutske i prehrambene industrije koji se baziraju na staničnim linijama. Sprezanje tehnologije sekvenciranja peptida pomoću MALDI-TOF masenog spektrometa s novo razvijenim softverom za analizu
Masenih spektara sigurno je jedan od smjerova koji može pružiti nova rješenja s mogućnošću gotovo trenutačne identifikacije ciljnih proteina u stanici ili mikroorganizmu. Kao kompetitivnu mogućnost, predlažemo brzu, jeftinju, selektivniju i reproducibilniju analitičku metodu određivanja slijeda amine kiseline u peptidu (triptičkom digestu) pomoću derivatizacije disulfonskom kiselinom (patent Instituta Ruđer Bošković) i uz pomoć računalnog softvera razvijenog na Prehrambeno-biotehnološkom fakultetu Sveučilišta u Zagrebu. Gore spomenuta kombinacija primijenjena na problematiku analize proteoma mogla bi u konačnici vidjeti svoju primjenu i u suvremenoj kliničkoj praksi kao brza, pouzdana i učinkovita metoda detekcije i identifikacije mikroorganizama.

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Ana Butorac was born in Zagreb. She graduated at Faculty of Food technology and Biotechnology, University of Zagreb. Her diploma work titled “Growth of mixed bacterial cultures Lactobacillus brevis L62 and enterobacteria during prolonged stationary phase” was finished in 2008 under supervision of Visnja Bačun-Družina. Since 2009 she is an assistant in the Laboratory for Biology and Microbial Genetics, Faculty of Food Technology and Biotechnology, University of Zagreb where she contributes on subjects of “Genetics of industrial organisms” and “Mechanisms of evolution”. She is engaged in the projects: “Stress-induced diversity and evolution of mixed bacterial cultures” team leader Višnja Bačun-Družina, PhD, “Automatization of Proteomics-driven Biotypization”, team leader Mario Cindrić, PhD, and Leonardo da Vinci project “Raising the awareness on healthy food and healthy eating among children, coordinator in Croatia Ksenija Durgo, PhD. Her research experience covers investigation of molecular evolution in bacteria and phylogenetics, and bacterial evolution in mixed cultures. She was on internship-specialization of three months at Faculteit Bio-Ingenieurswetenschappen, Gent Belgium under supervision of Andreja Rajković, PhD. The results of her research work was published in six SCI indexed journals and presented at 8 international scientific meetings.
Evolution of bacterial population:
The metabolic engineering of *Lactobacillus brevis*

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Lactic acid bacteria in particular Lactobacilli are widely used in food and pharmaceutical industries. *Lactobacillus brevis* occupies a diverse range of environmental niches and has various metabolic capacities. Its genomic islands are consisted of numerous functional gene cassettes, especially for carbohydrates utilization, which can be mobilized, shuffled, deleted or substituted in response to niche demands.

We used laboratory evolution experiments to explore bacterial adaptation under well-defined selective pressure of prolonged stationary phase growth. Genomics analyses of *L. brevis* after long term-starvation indicated that cells underwent chromosomal rearrangements and plasmid loss. Furthermore, proteomics analysis revealed the key differences in metabolic pathways responsible for the existence of cells. After prolonged starvation of cells the numerous proteins engaged in glucose and amino-acid catabolizing pathways, glycerolipid metabolizing pathways, and stress-response mechanisms were all differentially expressed. Metabolomics analysis indicated that the studied cells produce less lactic acid in comparison to parental cells.

The understanding of experimental evolution, such as the long-term adaptation, may facilitate new strategies for development of cell lines with advantageous traits.

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Evolucija bakterijske populacije i metabolički inženjering

*Lactobacillus brevis*

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Bakterije mliječne kiseline, osobito laktobacili, često se koriste u prehrambenoj i farmaceutskoj industriji. Bakterija *Lactobacillus brevis* zauzima različite ekološke niše i ima višestruke metaboličke sposobnosti. Njegini genomski otoci se sastoje od brojnih funkcionalnih genskih kaseta, posebice za korištenje ugljikohidrata, koje mogu biti prenijete, izmiješane, izgubljene ili zamijenjene kao odgovor na posebnost niše.

Za istraživanje adaptacije bakterijskih stanica koristili smo eksperimente laboratorijske evolucije tijekom dobro definiranog selektivnog pritiska u produljenoj stacionarnoj fazi rasta. Genomske analize *L. brevis* nakon dugoročno izgladnjivanje ukazuju da je u stanicama došlo do kromosomskih preinaka i gubitaka plazmida. Osim toga, proteomske analize pokazuju bitne razlike u metaboličkim procesima odgovornim za opstanak stanica. Nakon produljenog izgladnjivanja stanica drugačija je ekspresija gena za brojne proteine uključene u kataboličke putove glukoze i aminokiselina, metaboličke putove glicerolipida i mehanizme odgovora na stres. Analize metaboloma su pokazale da proučavane stanice proizvode manje mliječne kiseline u usporedbi s roditeljskim stanicama.

Razumijevanje eksperimentalne evolucije, kao što je dugotrajna adaptacija, može poboljšati nove strategije za razvoj staničnih linija s povoljnim osobinama.

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Quang D. Nguyen has published in numerous journals such as Biomass and Bioenergy, Process Biochemistry, Enzyme and Microbial Technology, Food Technology and Biotechnology, Acta Alimentaria, Biochimica et Biophysica Acta, Biotechnology Letters, Journal of Biotechnology etc.

Enhancement of efficiency of ethanol production from Jerusalem artichoke

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Jerusalem artichoke (JA) is a perennial plant and can be cultivated in such soil unsuitable for cultivation of food crops such as wheat, rice, potato. Moreover, this plant has high production yield (20–80 t/ha) mainly in inulin (70–80 % of d.m.), thus it can be used as a raw material for bioethanol fermentation. The main drawback should be low efficiency of bioconversion. Mean-
while, the efficiency of starch-based ethanol production can reach 93–97%, it is very poor (65–70 %) in the case of JA. In this study, improvement of the efficiency of fermentation using mixed cultures is focused. Different fermentation techniques (SSF, batch and semi-continuous fermentation with mixed cultures) were investigated. In the cases of batch fermentation with mono and mixed cultures, about 55–62 % and 74–86 % of conversion were calculated, respectively. Higher value (about 90 %) was determined when fermentation medium was inoculated using mixed cultures in two parts: 2/3 amount for initiation and 1/3 after 1 day of fermentation. A semi-continuous fermentation technology was modelled by harvesting a portion of the ferment broth and supplementing fresh substrate on two days period. The fermentation has operated for more than 40 days proved that the yeast cells were capable to hydrolyse inulin and convert to alcohol for a long time. The ethanol concentrations varied between 4,1 and 6,5 % in the mash. In conclusion, \textit{S. cerevisiae} with high ethanol tolerance was beneficially combined with \textit{Kl. marxianus} with high inulinase activity for production of bioethanol. The application of the mixed cultures combination with semi-continuous fermentation technology for inulin-based production of ethanol seems to be very promising due to increased efficiency significantly, thus it has potential in white biotechnology.
Contents

Program of the Symposium ................................................................. 3

FRIDAY, May 10, 2013

Zlatko Kniewald
   Welcome Remarks............................................................................... 11

Srđan Novak and Ivo Friganović
   How to initiate a new phase of biotechnology development in Croatia? ............................................................................. 16
   Kako pokrenuti novu fazu razvoja biotehnologije u Hrvatskoj? ............................................................................................. 17

Peter Raspor and Vito Turk
   Biotechnology at the door of Horizon 2020.................................................. 20

Green Biotechnology

Slaven Zjalić
   Aflatoxins fifty years after: still unsolved challenge............................ 23
   Aflatoksini pedeset godina poslije: još neriješen problem ............... 24

Domagoj Šimić
   Green biotechnology in agriculture – food, biofuel and recombinant gene technologies................................................................. 27
   Zelena biotehnologija u poljoprivredi – hrana, biogoriva i rekombinantna DNA tehnologija................................................................. 28

Neža Čadež
   Culture collections for future: case yeasts from genetic to metabolic diversity................................................................. 29

127
Boris Kovač
Amylolytic activity inhibition of low Falling Number flours ..........31

Matjaž Deželak
Processing of a Gluten-free Fermented Beverages
Based on Malted Pseudocereals ......................................................33

Borislav Šćulac
Modernisation of production plant and production processes
implemented in Zagrebačka pivovara in period from
1995 till 2012 ..................................................................................34
Modernizacija proizvodnih pogona i proizvodni postupci
primijenjeni u Zagrebačkoj pivovari u periodu
1995 do 2012. godine .......................................................................35

Red Biotechnology

Dragan Primorac
Molecular genetics: the present and the future .........................40
Molekularna genetika: sadašnjost i budućnost .........................41

Hrvoje Petković
New approaches in the development of antibiotics
– return of tetracyclines .................................................................44

Goran Gajski
Safety assessment and antioxidant activity of sodium
copper chlorophyllin ....................................................................45
Procjena sigurnosti i antioksidacijska aktivnost natrijeva
bakrenog klorofilina .......................................................................47

Aleš Podgornik
Production of bacteriophages for human applications ................48

Blue Biotechnology

Dušica Vujaklija
Molecular study of dominant soil bacteria: streptomycetes
in nature and application to biotechnology .................................52
Temeljna istraživanja dominantnih zemljišnih bakterija: streptomiceti u prirodi i primjena u biotehnologiji......................52

Bruno Zelić
Intensification of Agro and Food Industry Waste
Biodegradation Process .................................................................55
Intenzifikacija procesa biorazgradnje poljoprivrednog otpada
i otpada iz prehrambene industrije .................................................56

Maja Borić
Rheology of liquid media influences bacterial physiology ............57

Jure Šumi
Green roofs – future of urban life..................................................59

White Biotechnology

Gerhart Braunegg
Sustainable production of polyhydroxyalkanoates.......................64

Božidar Šantek
Development of integrated bioprocess for ethanol production
from sugar beet.............................................................................67
Razvoj integriranog bioprocесa proizvodnje etanola
iz šečerne repe...............................................................................68

Želimir Kurtanjek
Systems biology and biotechnology...............................................70
Sustavska biologija i biotehnologija..............................................71

Anita Slavica
Advances in biotechnological processes for lactic acid production...73
Razvoj biotehnoloških procesa za proizvodnju mliječne kiseline......74

Višnja Gaurina Srček
Aquaculture and fish cell technology............................................75
Akvakultura i tehnologija stanica riba.........................................76

129
SATURDAY, May 11, 2013

Vladimir Mrša
High education for the requirements of industrial production
– European experience and Croatian challenges.................................81
Visokoškolsko obrazovanje za potrebe gospodarstva
– europska iskustva i hrvatski izazovi .................................................82

Martina Tijardović
Overview of the past and the look on the future of forest reproductive material production in Croatia ......................................................84
Osvrt na prošlost te pogled na budućnost rasadničke proizvodnje šumskog reprodukcijskog materijala u Hrvatskoj .....................................85

Ivana Bošnjak
Toxicity of bisphenol A (BPA) on urchin embryo gene expression and morphology.....................................................................................87
Učinak bisfenola A (BPA) na gensku ekspresiju i morfologiju embrija morskog ježinca ...........................................................................88

Lucija Nuskern
Removal of heavy metals and phosphates from wastewater by bioparticles. ......................................................................................89
Uklanjanje teških metala i fosfata iz otpadnih voda biočesticama ....90

Rok Gaber
DNA guided assembly line...................................................................92

Teuta Murati
Endocrine disruptors and animal-free toxicology ...............................93
Endokrini modulatori i toksikološko testiranje bez uporabe pokusnih životinja .................................................................94

Ana Bielen
Scanning for genes encoding GDS(L) hydrolases in Actinobacteria from wide diversity of ecological niches ........................................97
Identifikacija GDS(L) hidrolaza u genomima aktinobakterija iz raznolikih ekoloških niša .................................................................98
Mario Franić  
Phytoextraction of cadmium using recombinant DNA technology in maize. ................................................................. 99  
Fitoekstrakcija kadmija pomoću rekombinantne DNA tehnologije kod kukuruza .................................................. 100

Jasna Beganović  
Role of S-layer proteins in probiotic activity of *Lactobacillus* strains ................................................................. 103  
Uloga S-proteina u probiotičkom djelovanju bakterija iz roda *Lactobacillus* .............................................................. 104

Anamarija Štafa  
Genetic side-effects during gene replacement in yeast *Saccharomyces cerevisiae* ............................................................ 105  
Genetičke promjene tijekom zamjene gena u kvascu *Saccharomyces cerevisiae* ............................................................ 106

Etjen Bizaj  
Development and Application of Saccharomyces Interspecies hybrids in wine industry .............................................. 108

Dušan Goranović  
Regulation of biosynthesis of immunosuppressant FK506 (tacrolimus) by *Streptomyces tsukubaensis*. ............................ 109

Mojca Brložnik  
Identification of Genetic Variants Responsible for Biotechnologically Important Traits and the Design of New Generation Industrial Yeast Strains. ...................................................... 111

Luka Ausec  
Mining bacterial genomes for laccases ............................................. 113

Bojan Žunar  
Genetic transformation of yeast *Dekkera/Brettanomyces bruxellensis* ................................................................. 115  
Genetička transformacija kvasca *Dekkera/Brettanomyces bruxellensis* ................................................................. 116

131
Antonio Starčević

Mass spectrometry based clinical proteomics .................................................. 118
Klinička proteomika mikroorganizama bazirana na spektrometriji masa ............................................................... 119

Ana Butorac

Evolution of bacterial population: The metabolic engineering of Lactobacillus brevis .................................................................................. 122
Evolucija bakterijske populacije i metabolički inženjering Lactobacillus brevis ............................................................................... 123

Quang D. Nguyen

Enhancement of efficiency of ethanol production from Jerusalem artichoke ........................................................................ 124

Donators .................................................................................................................................................. 133
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