CBM

Congress of Baltic Microbiologists

Riga, Latvia, 31. 10. - 4. 11. 2012

Scientific Program

Book of Abstracts

1st Congress of Baltic Microbiologists

Supporters

Welcome Message

Dear colleagues,

It is our pleasure to welcome you at the 1st Congress of Baltic Microbiologists (CBM 2012), organised by Latvian Society for Microbiology with the support of Federation of European Microbiological Societies (FEMS).

This Congress is organized as a first, comprehensive event dedicated to all microbiologists in the countries of Baltic Sea region.
As this is the first event of such type – organizers did not place any restriction for participants regarding particular fields of the microbiology. We suppose that such approach would give unique opportunity to Congress delegates to get an overview on the main current research directions in microbiology of Baltic Sea region countries.

The aims of the CBM2012 event are:
- to bring together microbiology professionals (scientists, engineers, students and partners from industry),
- to promote scientific knowledge sharing,
- to assist the development of active collaboration in the near future.

We hope a lot that this 1st Congress will serve as a efficient initial step towards closer research contacts in our region. You have to be a part of this activity and we encourage your support to help us to establish new networks and coordination groups. We hope that such congresses will become regular and would promote to further development of general and applied microbiology in the world.

Have a nice time at CBM2012!

Indrikis Muiznieks, Chairman
Alexander Rapoport, Chairman
Vaira Saulite, President of the Latvian Society for Microbiology

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This abstract book is compiled and printed with the support of State Research Programme
Nr. 2010.10-4/VPP-5 NatRes the 1st Project „Mineral Resources"

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PROGRAM

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Thursday, 1st of November

Venue Aspazijas bulvāris 22

Time	Code	Presenter	Title
09:30			**Opening**
			Plenary session I Chair Dr. hab. A. Rapoport
10:00	I 01	M. Kivisaar	Mutagenic processes in bacterial chromosome
10:30	I 02	R. Daugelavičius	Studies of microbial membranes in Lithuania
11:00			**Coffee break**
			Physiology and cultivation I Chair Dr. U. Kalnenieks
11:30	I 03	V. Siksnys	Molecular basis of CRISPR-encoded immunity in bacteria
12:00	O 01	R. Nahku	Stock culture heterogeneity rather than new mutational variation complicates short-term cell physiology studies of *Escherichia coli* K-12 MG1655 in continuous culture
12:20	O 02	K. Peebo	Trade-off between acetate overflow, citrate cycle and biomass yield in *Escherichia coli*
12:40	O 03	T. Visnapuu	Key catalytic residues for substrate binding and polymerization reaction of levansucrase from *Pseudomonas syringae* pv. tomato DC3000
13:00			**Lunch**
14:00			**Poster session P 01 - P 28; P 62**
			Microbial tools for toxicology Chair Dr. M. Kivisaar
15:00	I 04	H.J. Heipieper	Microbial adaptation to toxic organic solvents and other forms of stress - mechanisms and applications
15:30	I 05	A. Kahru	Comparison of the biological effects of different types of silver nanoparticles and nanoporous silica to bacteria and selected invertebrate species
16:00	O 04	K. Kasemets	*Saccharomyces cerevisiae* – a simple unicellular eukaryotic model organism for toxicological profiling of chemicals: copper oxide nanoparticles study
16:20	O 05	J. Liepins	FT-IR based *Saccharomyces cerevisiae* biomass quantification and it's application in ZrO_2 nanoparticle toxicity studies, proof of principle
16:40			**Coffee break**
			Applied aspects of molecular microbiology Chair Dr. D. Eze
17:00	I 06	A. Balode	Changing from CLSI to European (EUCAST) breakpoints: what happens with antimicrobial surveillance of resistance rates in national network
17:30	O 06	M. Bukowski	A novel highly specific method for species identification within the *Staphylococcus* genus

17:50	O 07	E. Viiard	Microbial composition of rye sourdoughs determined by Rep-PCR fingerprinting, denaturing gradient gel electrophoresis (DGGE) and pyrosequencing of barcoded 16S rRNA gene amplicons
18:10	O 08	L. Blank	Bacterial population dynamics in long-ripening cheese
18:30	O 09	M. Besmeltseva	Competence Center of Food and Fermentation Technology (CCFFT) – emergency for industrial bakeries
18:50	O 10	M. Malakauskas	Genetic comparison of *C. jejuni* strains isolated from wild birds and human campylobacteriosis cases
19:10	O 26	E. Miklasevics	Molecular history of Staphylococcus aureus in Latvia

Friday, 2nd of November

Venue Aspazijas bulvāris 22

Time	Code	Presenter	Title
		Plenary session II Chair Dr. hab. I. Muiznieks	
9:00	I 07	K. Haukka	Cyanobacteria – bioactive compounds, genomics and ecology
9:30	I 08	M. Itavaara	Deep biosphere microorganisms provide information of geobiological processes of earth crust
10:00	I 09	J. Truu	Environmental microbiology and environmental biotechnology in Estonia
10:30	I 10	L. Kalediene	Microbiological aspects of environmental bioremediation
11:00		**Coffee break**	
		Environmental microbiology and biodegradation I Chair Dr. J. Truu	
11:30	I 11	O. Muter	Recent trends in applied microbiology for agro- and remediation technologies in Latvia
12:00	O 11	L. Grantina-Ievina	Microbiological quality of vermicompost and its impact on soil microbial populations
12:20	O 12	J. Halimona	The impact of coniferous trees biomass extracts on *Phytophthora cactorum* and *Botrytis cinerea in vitro*
12:40	O 13	R. Kalnins	Use of natural microbial consortia for hydrogen production by carbohydrate degradation
13:00		**Lunch**	
14:00		**Poster session P 29 - P 61; P 63**	
		Environmental microbiology and biodegradation II Chair Dr. M. Itavära	
15:00	I 12	J. Truu	Microbial community biomass and structure in phosphorous removal filters based on oil shale ash from power plants
15:30	O 15	H. Nõlvak	Dynamics of antibiotic resistance genes during the stabilisation phase of microbial community in newly established municipal wastewater treating horizontal subsurface flow constructed wetland filter material
15:50	O 16	I. Krustok	Bacterial community activity, structure and succession in hybrid constructed wetland treating domestic grey water
16:10	0 17	L. Alksne	Cellular and molecular interactions between *Trichoderma* strains and conifer pathogens *Heterobasidion annosum* and *H. parviporum*
16:30	O 18	Z. Rutkovska	Microbial fuel cells in Latvia: Use of natural microbial consortia from bodies of water in Latvia in microbial fuel cells enriched with glucose and acetate
16:50		**Coffee break**	

Physiology and cultivation II Chair Dr. B. Schink			
17:10	I 13	V. Bankovsky	Revers spiner - real time cells growth logger - innovation in mini bioreactors
17:40	O 19	I. Stulova	Characterization of growth of lactic acid bacteria in milk: microcalorimetric approach
18:00	O 20	N. Kabanova	The use of microcalorimetry in studies of solid state fermentation processes
18:20	O 21	J. Jasko	Anaerobic fermentation of untraditional substrates for bioenergy application
18:40	O 22	L. Laurinovica	Influence assessment of several substrates and inhibitors for methane production in anaerobic fermentation process

Saturday, 3rd of November

Venue Raina bulvāris 19

Time	Code	Presenter	Title
		Plenary session III Chair Dr. R. Daugelavicius	
9:00	I 14	B. Schink	Energetic limitations in methane production from biomass
9:30	I 15	V. Passoth	Non-conventional yeasts in biofuel production
10:00	I 16	A. Rapoport	Anhydrobiosis in yeasts: mechanisms and applications
10:30	I 17	T. Tonjum	Genome dynamics in *Mycobacterium tuberculosis*
11:00		**Coffee break**	
		Yeasts and systems biology I Chair Dr. V. Passoth	
11:30	I 18	E. Lastauskiene	Yeast signalling research in Lithuania: from cell aging to food industry
12:00	I 19	T. Alamae	Sugars as signals affecting gene expression in yeasts: *Hansenula polymorpha* as alternative model
12:30	I 20	U. Kalnenieks	Modeling the central metabolism of industrial microorganisms: activities of the Latvian interuniversity systems biology team
13:00		**Coffee and snacks**	
		Yeasts and systems biology II Chair Dr. T. Alamae	
13:30	O 23	E. Stalidzans	ConvAn: convergence, consensus and stagnation analyzer for optimization of biochemical networks
13:50	O 24	D. Borovikova	New approach for yeast immobilization
14:10	O 25	A. Kokina	Trehalose metabolism in yeast *S. cerevisiae* - effects of common auxotrophies
14:30	I 21	K. Kogerman	MALDI Biotyper - Microbial Identification for the 21st century
15:00	I 22	I. Muiznieks	Molecular Microbiology in Latvia: going 3D
15:30		**Concluding remarks**	

INVITED SPEAKERS

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I 01

Mutagenic processes in bacterial chromosome

T. Juurik, H. Ilves, R. Teras, T. Ilmjärv, K. Tavita, K. Ukkivi, A. Teppo, K. Mikkel, M. Kivisaar

Institute of Molecular and Cell Biology, Tartu University and Estonian Biocentre, 23 Riia Street 51010 Tartu, Estonia
E-mail: maia.kivisaar@ut.ee

Keywords: mutation rate; mutation detection systems; bacterial chromosome; mutagenesis in growing and starving bacteria; evolution of bacteria; *Pseudomonas putida*

It has been known for many years that the mutation rate can vary dramatically between sites. Additionally, recent analysis of large genomic data sets of eukaryotic genomes suggests that the mutation rate can vary over many different scales, from the adjacent sites to whole chromosomes. Compared to the complex organization of eukaryotic chromosomes, bacterial chromosomes are smaller and structurally simpler. Therefore, it is still an open question whether mutation rate can vary across the bacterial chromosome. In this study, the occurrence of mutations within the same mutational target sequences at different chromosomal locations of *Pseudomonas putida* was monitored. Generation of mutations in the *P. putida* chromosome was investigated both in growing and in stationary-phase bacteria. Our results revealed that the mutation frequency and the spectrum of mutations vary at different chromosomal positions. It is also noteworthy that certain mutational hot spots were detected only at particular chromosomal positions and especially in growing bacteria. Thus, it seems plausible that regional differences in chromosome structure and organization influence mutagenic processes in growing bacteria more strongly than previously assumed. At the same time, since the mutants continued to accumulate in starving populations of *P. putida*, some cells could still grow slowly and replicate their chromosome under the starvation conditions. Nevertheless, it is also possible that mutations in the chromosome of *P. putida* stationary-phase cells have mainly occurred during the course of DNA repair synthesis. The fact that mutation frequency and spectrum of mutations vary across the bacterial chromosome could play an important role in divergence of bacterial populations in nature. Depending on the location of the potential target genes in the chromosome some mutational pathways may prevail over the others in the evolution of bacteria.

Acknowledgements
This work was supported by Estonian Science Foundation grants 7376 (MK), 7019 (RT) and 7458 (HI), by funding of Targeted Research Project SF0180031s08 to MK from Estonian Ministry of Research and Education, and

by grant #55005614 to MK from the Howard Hughes Medical Institute International Research Scholars Program.

I 02

Studies of microbial membranes in Lithuania

Rimantas Daugelavičius

Dept. of Biochemistry and Biotechnologies, Vytautas Magnus University, Kaunas, Lithuania
E-mail: r.daugelavicius@gmf.vdu.lt

Studies of microbial membranes in Lithuania started in early 1970ies, golden-age of membrane bioenergetics, when young researchers L. Grinius and Y. Kadziauskas came back from Moscow Lomonosov University to Vilnius University and brought their ideas on membrane transport and bacterial energetics. The most famous results of that period are papers from prof. L. Grinius scientific group on the energetics of DNA transport during processes of bacteriophage infection, bacterial conjugation and genetic transformation.

Mechanisms of bacteriophage infection are still a hot topic nowadays, the period of antibiotic resistance in bacteria. Initiation of bacteriophage infection requires the delivery of viral genome through envelope into the host cytoplasm. The viral nucleic acid penetration varies from dsDNR injection to dsRNA entry inside phage nucleocapsid after the endocytosis-like events. Two scientific groups at Vytautas Magnus University (Kaunas) and Vilnius University in collaboration with colleagues from University of Helsinki and CNRS (Gif-sur-Yvette, France) are working on this problem.

One of the main reasons of antibiotics resistance in many pathogenic bacteria is multidrug resistance (MDR) efflux pumps. It is of crucial importance to develop rapid methods to evaluate the activity of these pumps, to estimate their efficiency in clinical isolates and to develop means for overcoming of this problem. Polycationic antimicrobial peptides are an important component of the innate defense of all species of life. These compounds are active against a broad range of bacterial strains, including antibiotic resistant isolates, and are synergistic with conventional antibiotics. This and earlier mentioned topics will be discussed in the lecture on studies of microbial membranes in Lithuania.

I 03

Molecular basis of CRISPR-encoded immunity in bacteria

Virginijus Siksnys

Institute of Biotechnology, Vilnius University, Graiciuno 8, Vilnius, LT-02241, Lithuania
E-mail: siksnys@ibt.lt

Bacterial viruses (bacteriophages) provide a ubiquitous and deadly threat to bacterial populations. To survive in hostile environments, bacteria have developed a multitude of antiviral defense systems. Clustered regularly interspaced short palindromic repeats (CRISPR), together with CRISPR-associated genes (*cas*) constitute an adaptive microbial immune system which provides acquired resistance against viruses and plasmids. The CRISPR/Cas system hijacks short fragments of invasive DNA (spacers) and subsequently uses them as templates to generate specific small RNA molecules (crRNA) which combine with Cas proteins into effector complexes that trigger degradation of foreign nucleic acid, thereby preventing proliferation and propagation of invasive genetic elements. CRISPR/Cas systems have been categorized into three main types which differ by the structural organization and function(s) of nucleoprotein complexes involved in crRNA-mediated silencing of foreign nucleic acids. The *Streptococcus thermophilus* DGCC7710 model organism contains three different CRISPR systems. We have identified effector complexes and molecular mechanisms involved in the invading DNA silencing by the CRISPR2/Cas and CRISPR3/Cas systems of *S.thermophilus*. In the CRISPR2/Cas system crRNAs are incorporated into a multisubunit effector complex, which binds to the target DNA and triggers degradation by the signature Cas3 protein. We have established that in the CRISPR3/Cas system, a complex comprised of a single Cas9 protein and crRNA provides CRISPR-encoded immunity. Sequence specificity of the Cas9-crRNA complex is dictated by the crRNA while DNA cleavage is executed by two distinct active sites (RuvC and HNH) of Cas9 protein. The simple modular organization of the Cas9-crRNA complex, where specificity for DNA targets is encoded by a small crRNA and the cleavage machinery consists of a single Cas protein, provides a versatile platform for the engineering of universal RNA-guided DNA endonucleases. Indeed, by altering the RNA sequence within the Cas9-crRNA complex, programmable endonucleases can be designed both for *in vitro* and *in vivo* applications. These findings pave the way for the development of novel molecular tools for RNA-directed DNA surgery.

See also: Gasiunas, G., Barrangou, R., Horvath, P., Siksnys V. *PNAS,* September 25, 2012, vol. 109, 39, E2579-E2586

Microbial adaptation to toxic organic solvents and other forms of stress - mechanisms and applications

H.J. Heipieper

Department Environmental Biotechnology, Helmholtz Centre for Environmental Research – UFZ, Permoserstrasse 15, 04318 Leipzig, Germany
E-mail: hermann.heipieper@ufz.de

Keywords: adaptation, mechanisms, stress, solvents, membrane lipids

Many organic solvents also known as potential environmental pollutants such as monoaromatic compounds (e.g. BTEX and phenols), *n*-alkanols and terpeniols are known to be extremely toxic not only to humans, animals and plants but also to microorganisms that are capable to degrade them. Therefore, environments contaminated with high concentrations of such compounds cannot be effectively bioremediated due to the inhibitory effects of the pollutants on the microbiota.

In addition, the toxicity of organic solvents plays a role in the biotechnological production of fine chemicals in whole cell biotransformations One major problem of successful applications of biotransformations using living bacteria as biocatalysts is the high toxicity of potential substrates and products. Fine chemicals often show hydrophobicity very close to those of biological membranes. For that reason such substances have toxic effects on the cell membranes and disturb their functioning, lead to growth inhibition or even cell death. These facts limit the economic application for biotechnological syntheses of a broader range of fine chemicals. One possible solution of this problem could be the application of fermentation systems with two phases. Hereby, an organic solvent is added as a second phase which functions as a kind of reservoir for potential substrates and/or products within a biotransformation process. However, applicable solvents are often toxic to potential biocatalysts themselves.

A solution for this problem is the application of so-called solvent-tolerant bacteria, often belonging to the genera *Pseudomonas, Rhodococcus and Arthrobacter.* These bacteria are able to adapt to supersaturated concentrations of many toxic solvents and environmental pollutants, respectively. In the last two decades, many attempts have been made to elucidate and characterise the cellular mechanisms of these bacteria enabling them to adapt to such hazardous conditions.

The microbes respond to such stress with a cascade of mechanisms, whereby most of these adaptive responses to toxic organic compounds are connected with changes in the composition of the cytoplasmic membrane. The major adaptive response mechanisms of bacteria to adapt to toxic organic compounds will be presented.

References
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Comparison of the biological effects of different types of silver nanoparticles and nanoporous silica to bacteria and selected invertebrate species

A. Kahru, O. Bondarenko, I. Kurvet, A. Käkinen, M. Mortimer, I. Blinova, K. Kasemets, H. Vija, L. Kanarbik, K. Juganson, V. Aruoja, M. Sihtmäe, K. Künnis, A. Ivask

National Institute of Chemical Physics and Biophysics, Akadeemia tee 23, Tallinn 12618, Estonia
E-mail: anne.kahru@kbfi.ee

Keywords: FP7 NanoValid, ecotoxicology, microbial screening, nanosilver, collargol

Silver and silica nanoparticles (NPs) are used in a growing number of commercial products. Nanoporous silica particles (Ps) have great potential for a variety of diagnostic and therapeutic applications in medicine and nanosilver is already widely used in various bactericidal materials and coatings. For both particle types the ecotoxicological information is very scarce.

We studied the toxic effect of 2 SiO_2 Ps (different porosities, SSA ~1000 m^2/g) and 3 Ag NP preparations: non-coated Ag, PVP-Ag and protein-coated Ag (collargol) to bacteria, including Gram(-) *Vibrio fischeri, Escherichia coli, Pseudomonas fluorescens, P. putida, P. aeruginosa* and Gram(+) *Bacillus subtilis* and *Staphylococcus aureus* as well as to daphnids, algae and protozoa. Soluble silver and silica salts were used as ionic controls. The particle hydrodynamic size and z-potential were measured using Malvern ZS. Various oxidative stress (OS) sensitive *E. coli* mutants (donated by Prof. James Imlay, USA) were used for phenotypic profiling of the role of OS in observed toxic effects. The preliminary data show that to bacteria the SiO_2 Ps were not toxic: minimal inhibitory concentration, MIC, after 4 h exposure to Ps exceeded 1000 mg/l. SiO_2 Ps were also quite harmless to aquatic species: EC50 for crustaceans > 1000 mg/l and for algae EC50=73-109 mg/l. On the contrary, PVP-silver and collargol (but not uncoated Ag NPs) were remarkably more toxic than studied silica Ps. The MIC of PVP-Ag and collargol for different bacteria varied from 5-100 mg Ag/l and there was no clear difference between toxic effects to Gram positive and negative bacteria. The most remarkable was the high antimicrobial potency of collargol to *P. aeruginosa*: MIC=10 mg Ag/l (~5-fold lower than for *E. coli*) and comparable to toxic effect of Ag-ions showing high bioavailability of Ag in collargol. The coated silver NPs were especially toxic to crustaceans: 24-48 h LC50=0.02 mg Ag/l. The 2h EC50 of PVP-Ag and collargol to protozoa was 30-70 mg Ag/l. Combining analysis with recombinant sensor bacteria 'sensing' silver ions, analytical chemistry and silver ion selective electrode (ISE) we showed that most probably the toxic effect of nanosilver is connected with its solubility, *i.e.* manifested *via* Ag-ions.

Acknowledgements

This study was supported by projects SF0222601Bs03, ETF8561 & 8066 and FP7 project NanoValid (contract No 263147). PVP-Ag was synthesized in the laboratory of Prof. Heikki Tenhu, Helsinki University.

I 06

Changing from CLSI to European (EUCAST) breakpoints: what happens with antimicrobial surveillance of resistance rates in national network

A. Balode

Pauls Stradins Clinical University hospital Pilsonu street 13, Riga, Latvia, LV – 1002
E-mail: arta.balode@stradini.lv

Keywords: bloodstream infections, antimicrobial resistance, *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*

Objectives

Trends of antimicrobial resistance, reported by European antibiotic Resistance Surveillance network (ERAS-Net) depend on pathogen type, antimicrobial substance and geographic region. While an increase of antimicrobial-resistance among Gram negatives (GN) is observed in many European countries including Latvia, resistance rates of Gram positive (GP) bacteria became stabilizing. In EUCAST definitions of susceptible and resistant microorganisms the key phrase is ''by a level of antimicrobial activity'' and difference in breakpoint values can influence the interpretation of individual results and resistance rates of the country.

Methods.

All primary invasive isolates of *S.aureus, E.coli, Kl. pneumoniae*, *Ps.aeruginosa* from blood and liquor samples from 11 microbiological laboratories of Latvia were subjected to unified resistance detection protocol based on EARS-Net recomendations from 2006 – 2011. Antimicrobial susceptibility was evaluated acording actual CLSI standart.

Results.

From 2006 to 2012, 2497 isolates were detected in blood and liquor. Among them 1045 (41,9%) were *S.aureus,* (n=), 544 (21,8%) *E.coli*, 277 (11,1%) *Kl. pneumoniae*, 94 (3,7%) *Ps.aeruginosa*, 332 (13,3%) *Enterococcus spp.* and 205(8,2%) *S. pneumoniae*. (n= 940) Methicillin resistant *S. aureus* proportion in positive blood samples decreased from 18,6%(n=172) in 2006 to 9,9% (n=167) in 2011.Contrary resistance against III generation cefalosporins for *E.coli* incereast from 6% in 2006 to 17% in 2011, *Kl.pneumoniae* from 365 to 38%. Ffluorquinolone resistance increased for *E.coli* from 10% in 2006 to 17% in 2011 and accordingly for *Kl.pneuumoniae* from 26% to 38%. Aminoglicoside resistance of *E.coli* increased from 5% in 2006 to 11% in 2011 and for *Kl.pneumoniae* from 25% to 34%. In oposite resistance of *Ps. aeruginosa* in all antibiotic groups continuously decreased for Ceftazidim 33%in 2006 to 25% in 2011, for fluorquinolons and aminoglicosides from 33% to 25%. Carbapenem resistance of all tested Gram negatives remaind <1%.

Conclusion.

An increase of the antimicrobial-resistance among GN and stabilization of the resistance rates of GP bacteria is observed over the years 2006 -2011. Changes in the breakpoint values according EUCAS T standard will influence the resistance rates utmost and the comparison of the data utmost will be available by the value of the certain antibiotic not by evaluation result in SIR system.

Cyanobacteria – bioactive compounds, genomics and ecology

K. Haukka

Department of Food and Environmental Sciences, Division of Microbiology, P.O. Box 56, FI-00014 University of Helsinki, Finland
E-mail: kaisa.haukka@helsinki.fi

Keywords: Cyanobacteria, Baltic Sea, lakes, molecular techniques

In my talk I will present the research on cyanobacteria conducted in the group of Prof. Kaarina Sivonen at the University of Helsinki. The group has a long tradition in studying the taxonomy and physiology of cyanobacterial strains isolated from the Finnish lakes and the Baltic Sea. The group was the first to characterize the cyanobacterial toxins and their producers in the Baltic Sea. Also the ecology of the aquatic microbes, including cyanobacteria, has been studied in these environments. In recent years the emphasis of the research has been especially on studying bioactive compounds produced by cyanobacteria. Cyanobacteria are a rich source of natural products of e.g. pharmaceutical interest and we have discovered numerous new ribosomal and nonribosomal peptides. The discovery of new bioactive metabolites is done using a combination of mass spectrometry and genome mining. Through the use of whole-genome sequences we have discovered new families of protease inhibitors and antifungal peptides as well as new enzymatic machinery for making cyclic peptides.

In our recent ecological studies, we have applied new molecular techniques for detection of e.g. toxic cyanobacterial strains. We developed molecular detection methods for microcystin (*mcy*), nodularin (*nda*) and anatoxin-a (*ana*) synthetase genes to detect and identify hepatotoxin- and anatoxin-producing cyanobacteria in environmental samples. PCR, quantitative-PCR as well as microarray methods were developed. Also, we are interested in the akinete differentiation and germination in filamentous cyanobacteria. These resting cells are likely to play an important role in the life cycle of cyanobacterial blooms. We want to use proteomics and transcriptomics techniques in combination with whole-genome analysis to reveal the cellular changes during the cell differentiation.

We collaborate widely with both Finnish and international scientists and welcome contacts from people interested in new initiatives. More information on the research conducted in our group can be read from the following web pages:
http://www.biocenter.helsinki.fi/groups/sivonen/

Deep biosphere microorganisms provide information of geobiological processes of earth crust

M. Itävaara

VTT Technical research centre of Finland Institute
E-mail: merja.itavaara@vtt.fi

Keywords: deep terrestrial biosphere, geomicobiology, methanogens, sulphate reducers

Existence of life in deep earth crust aquifers has been under intensive studies. Scientific reasons for studying deep biospheres are connected to evolution of life and energetic mechanisms of deep microorganisms. Earth crust microorganisms are able to utilize energy form rock and geogases through redox processes. Which microorganisms dominate and what are their functions has been studied considerably in our projects.

There has been found that living microorginsms exist almost everywhere where there is water available and suitable temperature to support life. Our studies demonstrate the gradual decrease in the cell number of microorganisms downwards in the earth crust aquifers. High pressure, increase in salinity and increase in temperature select the surviving microbial communities.

Our studies demonstrate the changes in microbial communities and increasing number of methanogens and sulphate reducing bacteria downwards in the earth crust aquifers. Research questions are also connected to the safety of the final disposal of high radioactive wastes in the bedrock of Finland and biocorrosion of copper.

Several boreholes and fracture zones at several depths has been studied to study endemic bedrock aquifer microbial communities. Special emphasis has been put to study the microbial diversities of Outokumpu deep borehole (2.5km deep) located in Eastern Finland since 2007, the borehole has been drilled during 2004-2005. The Outokumpu deep borehole is an interesting sampling site for geomicrobiology research, the age of the water is expected to be several billions of years old and the gases such as methane and hydrogen are evolving from the earth crust fractures into air.

Acknowledgements

Deep borehole research team in Finland at VTT (Technical research centre of Finland) and GTK (Geological survey of Finland) and Aalto University (Computing science) is acknowledged as well as Deep carbon observatory DCO, for funding and collaborating acitivities of Deep Life and Deep Energy network.

Finnish Academy is acknowledged for funding the Deep Life and Deep metapathway projects. KYT research program is acknowledged Geomol and Geomicro projects. Posiva Ltd is acknowledged for providing equipments for packer sampling of Outokumpu deep borehole and providing samples from Olkiluoto boreholes.

Environmental microbiology and environmental biotechnology in Estonia

J. Truu

Institute of Ecology and Earth Sciences, University of Tartu, 46 Vanemuise St, 51014, Tartu, Estonia
E-mail: jaak.truu@ut.ee

Keywords: bioaugmentation, bioremediation, constructed wetlands, phytoremediation

One of the main directions of environmental microbiology research in Estonia is phylogenetic diversity of functional genes and microbial populations involved in degradation of organic pollutants in environment. Laboratory and field experiments have been carried out during last 20 years in order to test the effect of different bioremediation options (phytoremediation, biostimulation, bioaugmentation) for remediation of organic pollutants in oil shale chemical industry solid waste, in explosive polluted soils, and oil polluted subsurface and sea water. Based on these studies the integrated environmental biotechnology approach for remediation of oil shale chemical industry solid waste dump area was proposed. In addition to active bioremediation techniques a passive approach based on monitored natural attenuation have been a study subject. Another main direction of environmental biotechnology studies is microbial community structure and its functioning but also factors affecting microbial activity in different types of constructed wetlands. Research in this field targets different aspects of the microbial activity in constructed wetlands with respect to wastewater properties, specific wetland type and environmental parameters.

Acknowledgements
This study was supported by Target Project No SF0180127s08 of the Ministry of Education and Research of the Republic of Estonia, by the European Regional Development Fund through ENVIRON (Centre of Excellence in Environmental Adaptation) and state program „Aid for research and development in environmental technology“ grant 3.2.0801.11-0026.

Microbiological aspects of environmental bioremediation

L.Kalėdienė

Faculty of Natural Sciences of Vilnius University, M.K.Čiurlionio Street 21/27
Vilnius, Lithuania
LT-03101
E-mail: lilija.kalediene@gf.vu.lt

Keywords: bioremediation, biodegradation, hydrocarbons, microorganisms

The biodegradation of hydrocarbons by natural populations of microorganisms is one of the primary ways by which crude oil is eliminated from contaminated sites. Indeed, bacterial strains able to degrade many of the compounds present in oil are known to be ubiquitous in nature. For this reason, bioremediation has been considered a potentially useful tool in the cleaning of oil spills and the treatment of oil residues. The applicability of bioremediation depends upon the site characteristics, the nature of the pollutants, and the metabolic capabilities of microorganisms. Nevertheless, the presence of hydrocarbon-degrading strains in sites contaminated with oil, or oil residues does not guarantee that oil components will be metabolized. Biodegradation of petroleum hydrocarbons in soil can be limited by many factors, including nutrient availability, microbial biomass, and pollutant toxicity. The evaluation of pollutant biodegradation is especially complicated, since the distribution of pollutants in the environment typically is patchy; therefore, a high number of replicate samples must be obtained for results to be statistically valid.

Microbiological processes can reduce hydrocarbons concentrations in soil to level that no longer pose an unacceptable risk to the environment or to human health. From an environmental perspective, bacteria, which grow on hydrocarbons and mineralize them, may be especially useful for soil bioremediation. The selected indigenous bacterial consortium has been shown to assist in bioremediation and has an advantage of being resistant to variations in natural environment.

Recent trends in applied microbiology for agro- and remediation technologies in Latvia

O. Muter

Institute of Microbiology and Biotechnology, University of Latvia, 4 Kronvalda Blvd., Riga, Latvia, LV-1010
E-mail: olga.muter@inbox.lv

Keywords: bacteria consortium, hydrocarbons, nutrient amendments, peat sorbent.

Soil microorganisms play a vital role in fixing, solubilizing, mobilizing, recycling of nutrients as well as biodegradation of different types of contaminants. The current use of biopreparations in agro- and remediation technologies is limited by the poor capabilities of microbial communities in the field, lesser activity on spatial and temporal scales, and absence of bench-mark values for efficacy testing for their widespread application in the field [1]. Besides, many remediation technologies are insufficiently understood because of variable and complex environmental conditions and improper evaluation of the level and content of contamination, especially in situations involving multiple-element and mixed-mode pollution [2].

Our research group has a long-term fruitful cooperation with research institutions and commercial partners. Study on microbial competition and stress response under model and real environmental conditions, on one hand, and scaling up experiments in cooperation with Latvian SMEs, on the other hand, are brought together for development of new formulations, including composition of microbial consortia and nutrient amendments, carriers for immobilization/incapsulation, application mode, etc. [3-4]. Evaluation of the process of biofilm formation as well as the measurement of biofilm activity in different biotechnological systems remains to be among those areas, which need further development.

Evironmental responsibility, professional expertise, management, manpower, and related facilities are the basis for the development and realization of efficient remediation strategies. In the coming years, the management of contaminated sites will evolve rapidly from ecological risk assessment toward restoration of ecosystem services [5]. Further research on the application of biopreparations is needed, especially in the areas of mass balance and cost reduction.

Acknowledgements

This study was financed by the projects Z-6172-Z-270 supported by Latvian Council of Sciences and Y3- 26493 of the State Research program Nr. 2010.10-4/VPP-5 NatRes.

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Microbial community biomass and structure in phosphorous removal filters based on oil shale ash from power plants

J. Truu[1], M. Truu[1], J. Juhanson[2], S. Kutti[3]

[1] Institute of Ecology and Earth Sciences, University of Tartu, 46 Vanemuise St, 51014, Tartu, Estonia
[2]Department of microbiology, Swedish University of Agricultural Sciences, Box 7025, 75007 Uppsala, Sweden
[3]Tartu College, Tallinn University of Technology ,78 Puiestee St, 51008, Estonia
E-mail: jaak.truu@ut.ee

Keywords: constructed wetland, microbial biomass, microbial community, oil shale ash

The aim of this study was to assess the microbial biomass and community structure of the oil shale ash filters used for phosphorous removal in tertiary wastewater treatment systems. Ca-rich oil shale ash is derived from burning of low-caloric value solid fuel oil shale (kukersite) in the Estonian electric power plants. Oil shale combustion in electric power plants results in generation of large amounts of oil shale ash waste. The amount of ash deposited in waste plateaus ranges between 5 and 7 Mt each year and the total ash waste volume is close to 300 Mt and less than 5% of ash is recycled. Earlier studies have shown that calcareous oil shale ash have efficient phosphorus retention capacities. Two identical pilot-scale experimental wastewater treatment systems, one treating municipal wastewater and another landfill leachate consisting of vertical and horizontal flow filter units were operated in parallel at two locations. We observed large differences in microbial community structure and biomass of filter material between two studied systems. In case of municipal wastewater both microbial biomass and structure were altered, while landfill leachate had less pronounced effect on microbial biomass in ash filters. The within filter unit variation in microbial community structure was high in case of municipal wastewater treatment system while system treating landfill leachate was characterized by higher between filter variation. The large differences in microbial community composition between two treatment systems were most probably related to different composition of wastewater at two locations, but the treatment system operation period length may also be source of this dissimilarity.

Acknowledgements
This study was supported by Target Project No SF0180127s08 of the Ministry of Education and Research of the Republic of Estonia, by the European Regional Development Fund through ENVIRON (Centre of

Excellence in Environmental Adaptation) and state program „Aid for research and development in environmental technology“ grant 3.2.0801.11-0026.

Reverse – Spin technology - innovative principle of microbial cultivation

V. Bankovskis

SIA BioSan , Ratsupites 7, build.2, Riga , Latvia LV-1067
E-mail: science@biosan.lv

Keywords: single used tubes bioreactors, reverse spin technology, RT cells growth

In present paper theoretical and experimental data of cellular growth at the use of a Reverse Spin Technology (**RST**) are described. Reverse-Spinner is a thermostating device which gives the possibility to mix cells' growth media by a rotation of tube reactor around it axis. It leads to highly efficient Vortex Type Mixing (**VTM**) with consumption of very low amounts of energy.

Absence of stirrers inside a reactor gives the opportunity to use a Reverse-Spinner as a rotating spectra-cells which measure optical densities in reactor in Real-Time.

Special software makes possible to set up optimal parameters of fermentation and to register all necessary parameters (mixing intensities, temperature of the process, optical densities and cells concentrations, rate of the growth etc).

The appearance of the **VTM** and depth of Vortex depend on 1) angular speed of RS-Reactor, 2) time from the start of rotation of RS-Reactors, 3) growth media viscosities, 4) temperature. These parameters determine also the angular speed of rotating Vortex Layer (**VL**) and their transition states from the Irrotational Vortex, when angular speed of the **VL** is proportional to the radius to the **RV** states when the angular speed of the **VL** is the same and **VL** looks like a monolithic Vortex crater. Common rules regulating Vortex type mixing processes may be formulated as follows: the longer is the time from Vortex has been appeared, the more obvious is a transition from **IRV** to the **RV**. The concept of Reverse-Spin mixing is based on these assumptions.

RST is efficient for anaerobic and aerobic microorganisms. Special adapter (including antibacterial filters) has been developed for aerobic cultivation. To increase an OD measurement range we investigated near IR spectra and showed that area of 860 nm as a sufficient one to measure increased cells concentration. Such wavelength shift (from traditional 600 nm to 860 nm) strongly expanded a range of correct OD measurement and as a result excludes sampling and dilution procedure which are especially dangerous for anaerobes, harmful pathogenic organisms as well as for microorganisms which live in extreme conditions of the environment (like a Thermophillus).

In present study we showed for the first time experimental results of cells growth kinetics obtained by application of single used tubes reactors agitated on a principle of **RST**. Growth conditions for several microorganisms models

(facultative anaerobe *E.coli BL21*, extreme aerobic microorganism *Thermophillus sp*. and anaerobic bacteria *Lactobacillus acidophilus*) have been optimized. The basic and applied valuable aspects of single used tube type RS-reactors and their potential niche in a different biotechnological fields are discussed.

Energetic limitations in methane production from biomass

B. Schink

University of Konstanz, D-78457 Konstanz, Germany
E-mail: Bernhard.Schink@uni-konstanz.de

Keywords: Energy metabolism, methanogenesis, syntrophic associations, reversed electron transport

The conversion of biomass to methane and CO_2 is a well established process for producing a reliable energy carrier for technical use. Different from aerobic degradation, anaerobic microbial degradation processes proceed in most cases in cooperation of different trophic groups (guilds) of microorganisms including Bacteria and Archaea which cooperate closely via metabolite transfer. The last members in these cooperations, i. e., obligately syntrophically fermenting bacteria and methanogenic archaea, have to share very small amounts of energy, and only fractions of an ATP unit are available to the respective reactions catalyzed. The biochemical basis of energy sharing under these conditions includes substrate-level phosphorylation steps combined with reversed electron transport reactions. Examples will be discussed with butyrate and acetate oxidation. Also glucose degradation can depend on syntrophic cooperation of fermenting and methanogenic partner organisms to allow efficient sugar degradation. These aspects of cooperation within methanogenic microbial communities will be discussed with respect to limitations of anaerobic degradation in natural methanogenic ecosystems, as well as to the limitations in applicability of this process for biogas production.

Non-conventional yeasts in biofuel production

V. Passoth

Swedish University of Agricultural Sciences (SLU), Department of Microbiology
E-mail: volkmar.passoth@slu.se

Sustainable biofuel production requires the utilisation of non-food, lignocellulosic biomass as raw materials. Lignocellulose conversion is currently limited by identifying appropriate pretreatment methods and utilisation of sugars not fermented by S. cerevisiae. Currently, lignocellulose is treated in two steps; thermochemical pretreatment represents a substantial input of energy and releases fermentation inhibitors, the subsequent enzymatic pretreatment substantially contributes to the process costs. Pentose fermentation to ethanol has yet not been established in commercial scale. We tested a new method of pretreatment, integrated storage and pretreatment (ISP), where during the storage process biopreservation and pretreatment were combined. A model lignocellulose (moist wheat straw) was inoculated with the biocontrol yeast Wickerhamomyces anomalus or Scheffersomyces stipitis and stored at low temperatures, reflecting Scandinavian storage conditions. Mould growth was identified as a major problem in non-inoculated controls, but was strongly diminished in parallels inoculated with W. anomalus and S. stipitis. Substantially higher ethanol yields were obtained from moist stored straw compared to dry controls, with the highest values from S. stipitis conserved samples. Non fermentable sugars can also be used for other processes than ethanol fermentation, like biogas generation or animal feed production using non-conventional yeasts. Some examples of these integrated approaches will be discussed.

Anhydrobiosis in yeasts: mechanisms and applications

A. Rapoport, G. Khroustalyova, L. Rozenfelde, D. Borovikova

Laboratory of Cell Biology, Institute of Microbiology and Biotechnology, University of Latvia, Kronvald Blvd., 4, Riga LV-1586, Latvia
E-mail: rapoport@mail.eunet.lv

Keywords: yeast, anhydrobiosis, dehydration/rehydration

Yeasts belong to those microorganisms which are rather resistant to different natural stress treatments including also strong desiccation which lead to their transfer into the state of anhydrobiosis. This state of live organisms is linked with temporary reversible delay or suspension of their metabolism which can be restored at return of normal conditions of the environment. Studies of this unique phenomenon of live nature give us a lot of earlier un-known information on the cryptic potential possibilities of eukaryotic systems. They reveal also important intracellular protective reactions which can be initiated by cells in various extreme conditions and which may be activated artificially if it is necessary, for example in medicine. Investigations performed during last decades showed us main structural-and-functional changes of cell wall, plasma membrane, mitochondria, vacuoles and nucleus. Models on the changes of molecular organization of lipid components of intracellular membranes during dehydration/rehydration treatments were proposed. Studies on the importance of protein components of membranes and cell wall have been started, Recently it was finally reached the state of anhydrobiosis also for yeast grown in anaerobic conditions. On the basis of these findings some non-conventional applications (besides “traditional” preparations of dry active yeasts) were proposed. They include new possibilities for the extraction of valuable intracellular compounds, purification of the environment, obtaining of immobilized yeast preparations and some others.

Genome dynamics in *Mycobacterium tuberculosis*

T. Tonjum

Centre for Molecular Biology and Neuroscience and Institute of Microbiology, University of Oslo, Norway, Oslo University Hospital, 0027 Oslo, Norway
E-mail: tone.tonjum@medisin.uio.no

Keywords: Tuberculosis, Mycobacterium tuberculosis, genome instability, DNA repair, helicases

Mycobacterium tuberculosis (Mtb), the etiologic agent of the re-emerging disease tuberculosis, continues to cause mortality and morbidity worldwide claiming 1.5-2 million people's lives annually. Mtb is an intracellular pathogen that survives and replicates inside macrophages. Intracellularly, Mtb is exposed to abundant oxidative and nitrosative stress, inducing macro-molecular damage. The ability to repair DNA damage caused by exposure to reactive oxygen and nitrogen intermediates produced by the phagocytic host cell is likely to play an important role in this species. Genome maintenance functions such as DNA repair, replication and recombination (3R) play important roles in the pathogenicity and survival of this pathogen. Hence, characterization of 3R components will enlighten Mtb genome dynamics and pathogenesis.

Helicases, motor proteins that catalyze separation of double stranded helices into single strands, are involved in almost all aspects of nucleic acid transactions. In *E. coli*, Ercc3, DinG and RecG homologs have been found to unwind a variety of branched DNA substrates *in vitro*, including holliday junction, D-loops and R-loops.

We have cloned, overexpressed and purified the Mtb Ercc3, DinG and RecG helicases. The purified proteins were assessed with regard to their ability to bind and unwind a range of DNA substrates mimicking replication and recombination intermediates and for ATPase activity. Mtb Ercc3, DinG and RecG were found to exhibit ATP dependent helicase activities with various substrate specificities, were able to unwind full and partial replication fork substrates and converted holliday junctions to flayed duplexes.

This is the first characterization of Mtb helicase homologs. The results indicate that the Mtb Ercc3, DinG and RecG are active helicases and ATPases that most probably serves as general guardians of the Mtb genome. These findings and on-going structure-function analysis at the atomic level will shed new light on the roles of helicases in mycobacterial pathogenicity and survival in their host.

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Yeast signalling research in Lithuania: from cell aging to food industry

E. Lastauskienė, A. Zinkevičienė, A. Timonina, D. Čitavičius

Department of Microbiology and Biotechnology, Faculty of Natural sciences, Vilnius university, M.K. Čiurlionio 21/27,Vilnius, Lithuania, LT – 03101.
E-mail: egle.lastauskiene@gf.vu.lt

Keywords: Ras/PKA pathway, cell aging, apoptosis, medium acidification, alcohol production.

Constantly changing environment is the major factor controlling the growth and development of the microorganisms. For quick generation of the cell response, information about changes in the cell environment is rapidly transmitted to the inner molecules of the cell. One of the universal signaling systems is Ras/PKA signal transduction pathway. This system helps cells recognize the nutrient sources present in the growth medium. Ras/PKA signal transduction pathway controls variety of the cell functions: cell proliferation, sporulation, aging, cell death, response to stress and others.

Environmental pH is one of the main factors influencing the growth, physiology and differentiation of yeast. Extracellular acidification leads to intracellular acidification which causes stress to the yeast cells and activates Ras/PKA signal transduction pathway. Ras/PKA signal transduction pathway regulates cell aging as response to environmental pH and leads to the cell apoptosis.

Many aging and apoptosis features are conserved between yeast and multicellular microorganisms, and this makes them perfect model organisms in cell death researches. Yeasts are also suitable also for acidosis related disease studies.

Saccharomyces cerevisiae are valuable not only as a model organism for studying molecular processes of the higher mammals. They are widely used in food and alcohol industry. Growth of the yeast cells are followed by an acidification of the medium. In some branches of industry such acidification is highly welcome in other – totally undesirable. It is known, that quality of the sparkling wines is basically related to acidification of the surrounding medium and autolysis of the cells. Ability to control these processes will allow extending the use of yeast in the food and alcohol production.

Sugars as signals affecting gene expression in yeasts: *Hansenula polymorpha* as alternative model

T. Alamäe, S. Suppi, T. Michelson

Institute of Molecular and Cell Biology, University of Tartu, Riia 23, 51010 Tartu, Estonia
E-mail: talamae@ebc.ee

Keywords: glucose repression, methylotrophic yeasts, maltose, sucrose, *Hansenula polymorpha*

Yeasts live in sugar-rich environment and prefer sugars over other substrates. When a high amount of sugar in the medium is sensed by the yeast, the signal is processed and utilization of alternative carbon sources such as alcohols and organic acids is prevented by transcriptional down-regulation of respective genes. This phenomenon is usually called glucose repression and its mechanisms have mostly been studied in *Saccharomyces cerevisiae* in which the HXK2 (one of hexokinase isoforms) and MIG repressor proteins have specific roles. Sugar repression also exists in methylotrophic yeasts: in the presence of abundant sugar, expression from methanol-specific promoters is strongly inhibited whereas some of them considerably activate at sugar limitation. Study of gene regulation in methylotrophic yeasts is very important from the aspect of biotechnology as these yeasts are widely used in heterologous expression of proteins from powerful methanol-specific promoters. Among methylotrophic yeasts, *Hansenula polymorpha* has a special position as it grows on maltose and sucrose, utilization of which is repressed by monosaccharides, glucose and fructose [1-2]. So, *H. polymorpha* is a suitable alternative yeast model to study sugar-related effects on promoters. We have shown that hexokinase protein has no specific role in glucose repression in *H. polymorpha* [3], suggesting a metabolite-mediated control over target promoters. Here, we proceed with sugar repression studies of *H. polymorpha* by screening reporter gene expression from four sugar-repressed *MOX*, *FMD*, *MPP1* and *MAL1* promoters in a set of sugar catabolism-specific disruption strains. In this study, yeasts are grown on solid medium that easily creates sugar limitation conditions, and reporter enzyme activity is measured in a high-throughput way – in permeabilized yeast suspensions on microtitre plates. Novel features of sugar sensing and signaling revealed by the study will be presented.

Acknowledgements
This study was supported by grants 7528 and 9072 from Estonian Science Foundation.

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I 20

Modeling the central metabolism of industrial microorganisms: activities of the Latvian interuniversity systems biology team

U. Kalnenieks[1], E. Stalidzans[2]

[1] Institute of Microbiology and Biotechnology, University of Latvia, Kronvalda Blvd. 4, Riga, Latvia LV-1586
[2]Biosystems group, Latvian University of Agriculture, Liela street 2, Jelgava LV-3001
E-mail: kalnen@lanet.lv

Keywords: systems biology, central metabolism, *Zymomonas mobilis*, *Saccharomyces cerevisiae*

During the last three years an interdisciplinary collaboration between microbiologists, bioengineers and computer scientists has taken place in the framework of the Latvian systems biology team. Following the research interests of the involved microbiologists from the institute of Microbiology and Biotechnology, University of Latvia, central metabolism of ethanologenic microorganisms has been chosen for model building and simulations. Kinetic and stoichiometric modeling approaches have been applied to analyze the uncoupled growth phenomenon in *Zymomonas mobilis*, to investigate the stoichiometric limitations for metabolic design of novel product pathways and to optimize the ethanol yields on sucrose for this bacterium, as well as for modeling of the physiological reactions determining stress-resistance in yeast. The results of the systems biology endeavour, the lessons learned from the collaboration, and the prospects for future research are analyzed from the microbiological point of view.

Acknowledgements
Research was funded by the ESF project 2009/0207/1DP/1.1.1.2.0/09/APIA/VIAA/128

Molecular Microbiology in Latvia: going 3D.

I. Muiznieks

Faculty of Biology, University of Latvia (FB UL), 4 Kronvalda Blvd. LV-1586, Riga, Latvia
E-mail: indrikis.muiznieks@lu.lv

Employment of molecular methods, in particular molecular cloning, in Microbiology has resulted in well published results on construction of virus-like particles at Latvian Biomedical Research and Study Centre and on regulation of bacterial glycolisis and amino acid synthesis at the Institute of Microbiology and Biotechnology, UL. Regarding respectfully to those established research areas I would like to focus my talk on the work addressing the role of molecular architecture of recombinant plasmids in regulation of their replication and gene expression, which is pursued at FB UL.

Under physiological conditions plasmid molecules in bacterial cells are plectonemically supercoiled and the binding sequences for proteins that are separated on 2D circular map may come close together and cooperate at driving replication or transcription. 3D structure of plasmids is dynamic, supercoiling density (SD) changes during replication and transcription. Distribution of loops and flexibility of the molecule is influenced by the presence of intrinsically bent DNA sequences, which reduce the possibilities of intramolecular sliding of DNA segments. We have shown that plasmid maintenance stability and copy number is changed if 3D structures of the supercoiled molecules carry *in vivo* or *in vitro* insertions of intrinsically bent sequences that change the ratio of regulatory RNAI and RNAII concentrations through long distance molecular interactions. mRNA synthesis and protein expression of the cloned genes coincide with the decrease of SD; the experimental reduction of SD stimulates the expression of cloned genes and intramolecular recombination. Also the regulation of plasmid gene expression by environmental stress factors may take place through changes of SD.

The 3D molecular architecture should be considered at construction of recombinant replicons to achieve the goals of efficient recombinant DNA or protein production.

ORAL PRESENTATIONS

Supporters

O 01

Stock culture heterogeneity rather than new mutational variation complicates short-term cell physiology studies of *Escherichia coli* K-12 MG1655 in continuous culture

R. Nahku[1,2], K. Peebo[1,2], K. Valgepea[1,2], J. E. Barrick[3], K. Adamberg[2,4] and R. Vilu[1,2]

[1]Tallinn University of Technology, Department of Chemistry, Akadeemia tee 15, 12618 Tallinn, Estonia
[2]Competence Centre of Food and Fermentation Technologies, Akadeemia tee 15a, 12618 Tallinn, Estonia
[3]The University of Texas at Austin, Department of Chemistry and Biochemistry, Institute for Cellular and Molecular Biology, Austin, TX 78712, USA
[4]Tallinn University of Technology, Department of Food Processing, Ehitajate tee 5, 19086 Tallinn, Estonia
E-mail: ranno@tftak.eu

Keywords: High-throughput sequencing, *E. coli*, A-stat, genetic heterogeneity

Nutrient-limited continuous cultures in chemostats have been used to study microbial cell physiology for over 60 years. Genome instability and genetic heterogeneity are possible uncontrolled factors in continuous cultivation experiments. We investigated these issues by using high-throughput (HT) DNA sequencing to characterize samples from different phases of a glucose-limited accelerostat (A-stat) experiment with *Escherichia coli* K-12 MG1655 and a duration regularly used in cell physiology studies (20 generations of continuous cultivation). Seven consensus mutations from the reference sequence and five subpopulations characterized by different mutations were detected in the HT-sequenced samples. This genetic heterogeneity was confirmed to result from the stock culture by Sanger sequencing. All the subpopulations in which allele frequencies increased (*betA*, *cspG/cspH*, *glyA*) during the experiment were also present at the end of replicate A-stats, indicating that no new subpopulations emerged during our experiments. The fact that ~31% of the cells in our initial cultures obtained directly from a culture stock centre were mutants raises concerns that even if cultivations are started from single colonies, there is a significant chance of picking a mutant clone with an altered phenotype. Our results show that current HT DNA sequencing technology allows accurate subpopulation analysis and demonstrates that a glucose-limited *E. coli* K-12 MG1655 A-stat experiment with a duration of tens of generations is suitable for studying cell physiology and collecting quantitative data for metabolic modelling without interference from new mutations.

Acknowledgements

This research was supported by EU project EU29994, Estonian targeted and science foundation projects SF0140090s08 and G8165, the US National Institutes of Health (R00 GM087550 to J. E. B.), and the US National Science Foundation BEACON Center for the Study of Evolution in Action (DBI-0939454 to J. E. B.).

O 02

Trade-off between acetate overflow, citrate cycle and biomass yield in *Escherichia coli*

K.Peebo[1,2], R.Nahku[1,2], G.Riis[1,2], M.Õun[1,2], A.Maser[1,2], K.Valgepea[1,2], K.Adamberg[1,3], R.Vilu[1,2]

[1] *Competence Centre of Food and Fermentation Technologies, Akadeemia tee 15a, 12618, Tallinn, Estonia*
[2] *Tallinn University of Technology, Department of Chemistry, Akadeemia tee 15, 12618, Tallinn, Estonia*
[3] *Tallinn University of Technology, Department of Food Processing, Ehitajate tee 5, 19086, Tallinn, Estonia*
E-mail: Karl@tftak.eu

Keywords: *E. coli*, acetate overflow metabolism, citrate cycle, continuous cultivation, A-stat, acetyl-CoA synthethase

The biotechnology industry has extensively exploited *Escherichia coli* for producing recombinant proteins, biofuels etc. However, high growth rate aerobic *E. coli* cultivations are accompanied by acetate excretion *i.e.* overflow metabolism which is harmful as it inhibits growth, diverts valuable carbon from biomass formation and is detrimental for target product synthesis.

Growth rate dependent acetate overflow metabolism of *E. coli* was continuously monitored using advanced continuous cultivation method A-stat. The first step in acetate overflow switch (at $\mu = 0.33\ h^{-1}$) is the repression of acetyl-CoA synthethase (Acs) activity triggered by carbon catabolite repression resulting in decreased assimilation of acetate produced by phosphotransacetylase (Pta), and disruption of the PTA-ACS node[1]. In order to avoid Acs down-regulation, its native promoter was swapped with an artificial promoter that provided constitutive specific growth rate independent over-expression. The latter mutation resulted in no reduction of acetate overflow and was likely caused by inactivation of Acs by protein lysine acetyltransferase (Pka). To remove the Acs inactivation *pka* gene was deleted. The mutation caused postponed acetate overflow ($\mu = 0.39\ h^{-1}$), but no significant difference in final acetate concentration at maximal growth rate. This indicated that in addition to increased Acs activity it is necessary to enhance citric acid cycle (TCA) and/or glyoxylate shunt activity. The latter was accomplished by deletion of *arcA* and/or *iclR* + *fadR* in *Δpka* mutant *E. coli* strain, respectively. Indeed, it was shown that increased TCA flux (CO_2 production and gene expression) resulted in lower acetate excretion but also in lower biomass yield. This highlights the problem of that approach – increased TCA activity results in loss of carbon to CO_2, therefore, the carbon is not converted to biomass. The constructed strains with lower acetate excretion could be used in acetate sensitive bioprocesses.

Acknowledgements

The financial support for this research was provided by the Enterprise Estonia project EU29994, and Ministry of Education, Estonia, through the grants SF0140090s08 and ETF8165.

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O 03

Key catalytic residues for substrate binding and polymerization reaction of levansucrase from *Pseudomonas syringae* pv. tomato DC3000

T. Visnapuu, K. Mardo, T. Alamäe

Institute of Molecular and Cell Biology, University of Tartu, Riia 23, 51010 Tartu, Estonia
E-mail: visnapuu@ut.ee

Keywords: mutational analysis, catalytic residues, fructooligosaccharides, levansucrase, *Pseudomonas syringae*

Levansucrases (EC 2.4.1.10) are bacterial extracellular enzymes that are classified to family 68 of glycoside hydrolases (http://www.cazy.org). They split sucrose and synthesize levan, a ß-(2,6)-linked polyfructan. Structure-function relationships of levansucrases from *Pseudomonas* bacteria are poorly studied though they are promising catalysts for biotechnology and their specific role in plant pathogenesis is waiting to be solved.

We have expressed in *Escherichia coli* three genomic levansucrase genes, *lsc1*, *lsc2* and *lsc3* of *P. syringae* pv. tomato DC3000 which infects tomato and *Arabidopsis thaliana*. Study of purified Lsc3 revealed that besides levan it also synthesized potentially prebiotic fructooligosaccharides (FOS) and heterooligofructans when transfructosylating a wide range of nonconventional acceptors [1, 2].

According to mutational and structure analysis of levansucrases, three acidic residues, two aspartates and a glutamate, constitute catalytic triad of the active site. According to sequence alignment and 3D modelling catalytic residues were predicted for Lsc1, Lsc2 and Lsc3 [3]. Asp62, Asp219 and Glu303 were proposed as nucleophile, transition-state stabilizer and acid-base catalyst of Lsc3, respectively. Site-directed mutagenesis and biochemical assay of mutant proteins confirmed these positions as catalytic triad residues of *P. syringae* pv. tomato levansucrase.

Additional analysis of mutants in conserved regions bordering catalytic triad residues showed that several positions located in the close proximity of nucleophile, transition-state stabilizer and acid-base catalyst were also crucial for Lsc3 functions. Mutations adjacent to Glu303 of Lsc3 affected polymerization reaction and adjacent to Asp62 – substrate binding.

As Lsc3 enzyme is perfectly stable and has very high catalytic activity (k_{cat} = 3 x 10^4 min^{-1}) [2], it makes a perfect candidate for the design of novel variants of the enzyme with desired properties.

Acknowledgements
This study was supported by grants 7528 and 9072 from Estonian Science Foundation.

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O 04

Saccharomyces cerevisiae – a simple unicellular eukaryotic model organism for toxicological profiling of chemicals: copper oxide nanoparticles study

K. Kasemets, S. Suppi, A. Kahru

Laboratory of Molecular Genetics, National Institute of Chemical Physics and Biophysics, Akadeemia tee 23, Tallinn 12618, Estonia
E-mail: kaja.kasemets@kbfi.ee

Keywords: nanoparticles, toxicological profiling, single-gene deletion mutants, dissolution, metal-specific biosensors

There is a growing need to develop high-throughput assays for rapid screening of large number of toxicants (i.e. synthetic nanoparticles, NPs). The yeast *Saccharomyces cerevisiae* is a promising unicellular eukaryotic organism for the toxicological evaluation of chemicals as its cellular structure and functional organisation has many similarities with higher-level organisms. In addition, available mutated strains allow mechanistic studies.

The current study focuses on phenotypic profiling of CuO NPs using *S. cerevisiae* BY4741 wild- type (wt) and mutants from EUROSCARF collection defective on genes related to oxidative stress (OS) response (*sod1Δ*, *sod2Δ*, *cta1Δ*, *ctt1Δ*, *gsh1Δ*, *glr1Δ*, and *ccs1Δ*) and copper resistance (*cup2Δ*). $CuSO_4$ (solubility control), bulk CuO (size control), H_2O_2 and menadione (positive controls for OS) were studied in parallel. Yeasts were studied for the growth inhibition in rich YPD medium and for the viability in deionized water (DI) at 30°C for 24 hours. Recombinant metal-sensing bacteria were used to quantify the dissolved copper ions.

Phenotypically the most sensitive single-gene mutants of *S. cerevisiae* in both test conditions for H_2O_2 and menadione, i.e., the OS-reference chemicals were *sod1Δ*, *sod2Δ* and *yap1Δ* (difference with wt > 3-times). However, these mutants were not more sensitive than wild-type to nCuO, bCuO and $CuSO_4$ indicating that Cu-compounds exerted toxicity *via* different mechanisms than menadione and H_2O_2. The most vulnerable strain to Cu-ions, but also to nCuO and bCuO was *cup2λ* (up to 15-fold compared to wt), indicating that the toxicity of nCuO ($EC_{50,wt}$ 643 mg/l in YPD and 4.8 mg/l in deionised water, DI) and bCuO ($EC_{50,wt}$ >20 000 mg/l in YPD and 155 mg/l in DI) was assumingly caused by the solubilized Cu-ions.

Acknowledgements
This study was supported by projects SF0690063s08, ETF9001, FP7 project NanoValid (contract No 263147).

O 04

O 05

FT-IR based *Saccharomyces cerevisiae* biomass quantification and it's application in ZrO_2 nanoparticle toxicity studies, proof of principle

J. Liepins[1], M.Gavare[1], A. Kokina[1], M. Grube[1], K. Smits[2]

[1]*Institute of Microbiology and Biotechnology, University of Latvia, Kronvald Blvd. 4, Riga, Latvia LV-1586*
[2]*Institute of Solid State Physics, University of Latvia, 8 Kengaraga Str., Riga, Latvia LV-1048*
E-mail: Janis.liepins@lu.lv

Keywords: *S. cerevisiae*, FT-IR spectroscopy, toxicology

Yeast cell population growth and viability are parameters useful for integral toxicology studies. This is very popular measurement used by microcultivation studies in drug and chemical toxicity screens. Usually increase in cell number is measured spectrophotometrically as change in "optical density" at 600 nm (OD600). Other assays for yeast growth dynamics assessments are related to specific enzymatic activity visualised by specific dyes (teterasolim salts, fluorodiacetate, etc.). In both cases visible light spectroscopy are used as a main tool.

There are situations when biomass determination via OD600 or dye absorbance at similar wavelength might be interfered by additional substances present in broth (non soluble particles/ precipitated broth components, specific nanoparticles). In these cases different yeast cell features should be exploited for biomass determination. In the same time – biomass assessment should be simple and robust to various contents of the broth.

Yeast contains macromolecules distinct from broth. The amount of those elements is proportional to total cell mass. Thus, if there were simple method to assess amount of these macromolecules – it could be used for estimation of biomass growth dynamics as well.

Fourier transform infrared (FT-IR) spectroscopy has gained wide interest as simple and rapid method to estimate organic macromolecules and fermentation products [1].

Absorptions of several typical (amides, nucleotides, proteins, lipids, carbohydrates) markers were estimated and linear range between biomass dry weight and specific macromolecule's absorbancies determined over fermentation and respiratory growth as well as for different *S. cerevisiae* strain backgrounds. To test the method – biomass estimation for yeasts cultivated together with ZrO_2 nanoparticles where done [2].

Lipid spectral region turned to form the best calibration curve over vast range of biomass concentrations. Besides, it was indifferent to growth mode (fermentative or respiratory) or strain background.

We conclude that developed FT-IR method for biomass quantification gives results similar to traditional optical methods and gravimetrical methods and thus can substitute them.

Acknowledgements
The present investigation was supported by European Social Fund.
K Smits contract Nr.2009/0202/1DP/1.1.1.2.0/09/APIA/VIAA/141
J Liepins, M Gavare, M Grube and A Kokina contract
Nr.2009/0202/1DP/1.1.1.2.0/09/APIA/VIAA/128

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O 06

A novel highly specific method for species identification within the *Staphylococcus* genus

M. Bukowski[1], K. Polakowska[2], K. Nytko[1], A. Sitarska[1], B. Wladyka[1], J. Miedzobrodzki[2], A. Dubin[1]

[1]Department of Analytical Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, 7 Gronostajowa St. 30-387 Krakow, Poland
[2]Department Microbiology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, 7 Gronostajowa St. 30-387 Krakow, Poland
E-mail: m.bukowski@uj.edu.pl

Keywords: *Staphylococcus*, staphylococci, typing, epidemiology

The determination of the microbe species colonising samples of environmental and clinical origin is the basic step in any epidemiological research. *Staphylococcus* strains are widely-spread animal and human opportunistic pathogens. Although many well-grounded methods of staphylococci species typing are in use, high genetic diversity frequently poses a problem in quick and simple species determination, thus necessitating the development of new approaches.

The study was focused on searching for a gene suitable for species determination within the *Staphylococcus* genus, by means of the RFLP (restriction fragments length polymorphism) analysis. The initial stage was focused on the analysis of the occurrence of four-nucleotide restriction sequences within selected genes, which are conserved across species of the Staphylococcus genus based on genomes available in the GenBank database. Further *in silico* analysis of corresponding digestion patterns was carried out. As a result, a gene of a desired pattern of conservation and a group of four restriction enzymes were selected and tested *in vitro* for their usefulness in staphylococci typing. At present, the optimised primers allowed for obtaining products of PCR reaction in case of 85 strains of 12 staphylococcal species. Those products were subsequently subjected to the RFLP analysis.

The digestion patterns obtained during the course of the research were characterised by high repeatability and uniqueness across all of the analysed species, as well as by the ease of their interpretation. Taking into account the current state of research in the field, the proposed method presents a new opportunity for the relatively quick and cheap identification of species in case of microbes of the *Staphylococcus* genus. However, further development, including analyses of other staphylococci species and adaptation of the method for use in daily routines, is needed.

Acknowledgements

This project was financed by funds granted by the National Science Centre (NCN, Poland) on the basis of the decision No DEC-2011/01/N/NZ1/01167 (to MB).

O 07

Microbial composition of rye sourdoughs determined by Rep-PCR fingerprinting, denaturing gradient gel electrophoresis (DGGE) and pyrosequencing of barcoded 16S rRNA gene amplicons

E. Viiard[1,2], M. Bessmeltseva[1,2], T. Paalme[1,2], I. Sarand[1,2,3]

[1]Department of Food Processing, Tallinn University of Technology, Ehitajate tee 5, 19086, Tallinn, Estonia
[2]Competence Center of Food and Fermentation Technologies, Akadeemia tee 15A, 12618, Tallinn, Estonia
[3]Department of Gene Technology, Tallinn University of Technology, Ehitajate tee 5, 19086, Tallinn, Estonia
E-mail: ene@tftak.eu

Keywords: lactic acid bacteria, rye sourdough, DGGE, pyrosequencing

Culture dependent techniques and traditional molecular methods, such as DGGE and Sanger sequencing, have so far been used to characterize sourdough populations. However, such methods may provide an inaccurate description of the whole microbial structure. Lactic acid bacteria (LAB) in rye sourdoughs can have complex nutrient requirements due to their high adaptation to the cereal environment. They are often sensitive to oxygen and are therefore difficult to cultivate in laboratory conditions. DGGE, which is based on the analysis of whole DNA extracted from the sourdough, may also give indefinite results, for it only allows detecting species that form roughly more than 10% of the microbial population. These limitations make studying the composition of LAB populations challenging.

Our aim was to determine the composition of LAB in rye sourdoughs from four bakeries and a spontaneous laboratory rye sourdough and to compare the results obtained by different approaches. We implemented in parallel three methods: plating followed by Rep-PCR of isolated colonies and 16S rRNA gene Sanger sequencing, DGGE analysis of the 16S rRNA genes and pyrosequencing of barcoded 16S rRNA gene amplicons.

Our results showed that technological parameters of the sourdough propagation (temperature, cycle length, type of flour etc.) have a major impact on the microbial composition of the sourdough. The species *L. helveticus, L. amylovorus, L. pontis, L. sanfransiscensis, L. vaginalis* and *L. panis* were found dominating in various combinations in different bakeries. The spontaneous laboratory rye sourdough contained species *L. plantarum, L. brevis, L. paralimentarius* and *L. curvatus.* Many of the species were only identified by DGGE and pyrosequencing analysis, for they did not grow on a selection of media. Pyrosequencing enabled us to identify dominating LAB species in the sourdoughs, but also to detect bacteria that were present at low concentrations, which cannot be seen with other methods.

Acknowledgements

The financial support for this research was provided by the European Regional Development Fund project EU29994, Estonian Ministry of Education and Research grant SF140090s08, Estonian Science Foundation grant ETF9417 and by European Social Fund's Doctoral Studies and Internationalisation programme DoRa.

O 08

Bacterial population dynamics in long-ripening cheese

L. Blank[1,2], M. Panke[1,2], J. Saareleht[1], T-.M. Laht [1,2]

[1] Tallinn University of Technology, Department of Chemistry, Akadeemia tee 15, 12618, Tallinn, Estonia
[2] Competence Center of Food and Fermentation Technologies, Akadeemia tee 15B, 12618, Tallinn, Estonia
E-mail: liisi.blank@tftak.eu

Keywords: cheese, lactic acid bacteria, pyrosequencing, population dynamics

Lactic acid bacterial community dynamics during manufacture and ripening of newly developed commercial cheese Old Saare was studied. Controlled industrial trials of high-temperature cooked long-ripening cheese were made in Saaremaa Dairy Plant (Estonia) using pasteurized cow`s milk and two types of commercial DVS starters: DCC-260 and EMFOUR containing multiple strains of different mesophilic and thermophilic lactic acid bacteria. Three industrial trials of cheese were made from ten tons of milk. Samples for microbiological and molecular analyses were taken from vat process and from cheeses after each ripening month. Samples were diluted according to IDF standard and plated on MRS and M-17 agar to determine the number of main LAB. Free amino acids were analyzed using UPLC. DNA and RNA were extracted using commercially avalailable kits from Sigma-Aldrich and Qiagen. The 16S V1-V2 region of DNA and cDNA were sent to pyrosequencing to determine species present in vat process and cheese. Data analysis of sequences were done using NCBI database.

High cooking temperature in vat process influenced plate counts. More detailed data was got from sequencing analysis which revealed almost complete lysis of lactococci in grain heating process. Formation speed and amount of free amino acids was high already after two months of ripening probably because of enzymes released from lactococci. The number of streptococci and lactobacilli was growing in vat process and several strains of *Streptococcus thermophilus* and *Lactobacillus helveticus* were dominating in samples but no large changes in population were observed after vat process. Comparison of DNA and RNA profiles revealed differences between metabolic activities of dominating strains. Species present in low numbers can have very high metabolic activity and therefore influence proteolysis and metabolic profiles in higher extent than strains which are present in high numbers but have low metabolic activity.

O 08

O 09

Competence Center of Food and Fermentation Technology (CCFFT) – emergency for industrial bakeries

M. Bessmeltseva[1,2], E. Viiard[1,2], I. Sarand[1,2]

[1] *Competence Center of Food and Fermentation Technology (CCFFT), Akadeemia tee 15A, 12618 Tallinn, Estonia*
[2]*Department of Food Processing, Tallinn University of Technology, Ehitajate tee 5, 19086 Tallinn, Estonia*
E-mail: marianna@tftak.eu

Keywords: sourdough, rye bread, microbial population

In February 2012 small-scale Estonian bakery contacted CCFFT due to the problems with rye bread quality. In present study we show the ability of CCFFT team to detect the problem in the industrial sourdough fermentation and to solve it using laboratory-scale simulations of sourdough propagation.

Preliminary studies revealed insufficient microbiological activity of the industrial sourdough in complex with season-dependent fluctuation of fermentation temperature. Eight model sourdoughs were set up and propagated at 20 or 30°C with 12h fermentation cycle during 10 days. Four model sourdoughs were started by mother sponge from the industrial sourdough (I-batches) and other four model sourdoughs were initiated with *Lactobacillus plantarum M30I-1* and *Lactobacillus brevis M30I-2* strains originated from CCFFT microbial collection (S-batches). Microbial activity of experimental sourdoughs was evaluated measuring pH and TTA values. Bacteria numbers were measured by plate counting. Changes in bacterial composition and stability of starters were studied using DGGE analysis.

The obtained results showed that TTA values of all S-batches were above the required level, while in I-batches fermented at 20°C TTA values were under 16. S-batches also demonstrated significantly higher bacteria numbers independently of fermentation conditions. DGGE analysis with further sequencing of 16S rDNA showed that inoculated starter bacteria remained dominant in all S-batches. In S-batches fermented at higher temperature DNA fragment specific for *Lactobacillus pontis* was additionally found after 20 cycles of propagation. In I-batches only constant maintaining of fermentation temperature at 30°C resulted in dominating of *Lb. pontis* and *Lactobacillus helveticus* species, while in I-batches fermented at 20°C *Lb. pontis* was replaced by technologically unwanted *Lactobacillus zymae* species.

Acknowledgements
This investigation was financially supported through the grant ETF9417 of Estonian Science Foundation and by the European Regional Development Fund project EU29994.

O 09

O 10

Genetic comparison of *C. jejuni* strains isolated from wild birds and human campylobacteriosis cases

S. Ramonaitė[1], E. Kudirkienė[1], M. Ružauskas[3], E. Tamulevičienė[4], A. Malakauskas[2], M. Malakauskas[1]

[1]Department of Food Safety and Quality, Veterinary Academy, Lithuanian University of Health Sciences, Tilžės str.18, Kaunas, LT-47181, Lithuania
[2]Department of Infectious Diseases, Veterinary Academy,Lithuanian University of Health Sciences, Tilžės str.18, Kaunas, LT-47181, Lithuania
[3]Veterinary Institute, Veterinary Academy, Lithuanian University of Health Sciences, Tilžės str.18, Kaunas, LT-47181, Lithuania
[4]Clinic of Children Medicine Academy, Diseases, Lithuanian University of Health Sciences, Eivenių str. 2 Kaunas Kaunas, LT-50009, Lithuania
E-mail: ramonaite@lva.lt

Keywords: wild birds, genotyping, *C. jejuni*, campylobacteriosis, diversity, MLST

Campylobacter jejuni is the leading cause of human gastroenteritis, which according to EFSA has been increasing over the past years. Poultry products are well recognized source of human campylobacteriosis, however other sources may significantly contribute to the incidence of illness as well.
To obtain more knowledge on possible sources of human campylobacteriosis, this study was done to compare genetic diversity of humans *C. jejuni* isolates with isolates from wild pigeons and crows.
Species identification revealed that *C. jejuni* was the dominant species among both, the wild birds samples (97.8%) and human clinical isolates (90.5%). The genotyping of 198 wild birds, and 56 human isolates by *flaA*-RFLP revealed that the most *C. jejuni* genotypes were source specific. Two genotypes (12 isolates and 17 isolates) were dominant among human isolates, however not detected among wild birds isolates. Among wild birds campylobacter isolates one genotype (53 isolates) was dominant and although rarely (only 2 cases) but was detected in humans. Others even genotypes were shared by humans and wild birds, however in humans they were detected sporadically. Further characterization of *C. jejuni* isolates by MLST assigned 46 isolates to already establish clonal complexes (CCs) whereas other 13 isolates were grouped into new CCs. Two wild birds STs could not be assigned to a specific CCs. One CC was shared by humans and wild birds isolates. Further MLST typing of more *C. jejuni* strains from wild birds and human campylobacteriosis cases will enable us to quantify the number of human cases attributable to wild birds. However based on the preliminary results we speculate that wild birds as a source of human campylobacteriosis pose a minor risk in Lithuania.

Acknowledgements
This research was funded by a grant (No. SVE 05/2011) from the Research Council of Lithuania

O 11

Microbiological quality of vermicompost and its impact on soil microbial populations

L. Grantina-Ievina, D. Berkolde-Pire, V. Nikolajeva

Department of Microbiology and Biotechnology, Faculty of Biology, University of Latvia, Kronvalda Blvd. 4, Riga, Latvia LV-1586
E-mail: lelde.grantina@lu.lv

Keywords: vermicompost, *Escherichia coli*, coliforms, *Enterococcus*, *Salmonella*, *Aspergillus fumigatus*, actinobacteria

More than 100 small enterprises in Latvia are preparing vermicompost (VC) that is biologically active soil fertiliser produced by earthworms from different substrates. There are several investigations proving that as a result of earthworm activities the level of pathogenic microorganisms (MO) is significantly reduced but still VC can contain the pathogenic MO to plants and animals, and that VC contains potentially plant growth promoting and disease suppressing MO.

In first part of the investigation 15 VC samples were analyzed from four producers prepared from following substrates: composted cow manure, organic waste and cow manure with tree leaves, starchless organic potato pulp with composted grass and sewage sludge. MO were investigated with plate count method using seven media: tryptic soy agar for the enumaration of the bacteria, Rose Bengal agar with chloramphenicol for the fungi, Endo for the detection of *Escherichia coli* and coliforms, Enterococcosel for the enterococci, SS for *Salmonella* spp., Mycosel for potentially pathogenic fungi, and yeast extract – peptone – glucose – salt agar for actinobacteria. In Latvia as stated in the Rules of the Cabinet of Ministers the CFU number of *E. coli* and enterococci may not exceed 1000 CFU g^{-1}, and *Salmonella* must be absent in 25 g of sample. Fourteen VC samples met this standard. The exception was VC produced from sewage sludge that contained increased numbers of *E. coli.* Several samples contained potentially pathogenic filamentous fungi to humans and animals, for example, *Aspergillus fumigatus*, *Pseudallescheria boidii*, *Geotrichum* spp. and keratinolytic species *Aphanoascus terreus*.

In second part of the investigation 18 soil samples were analyzed from the field experiment established by State Priekuli Plant Breeding Institute where different amounts of the VC (0 – 12 t ha^{-1}) were amended in the field of organic potatoes. In these samples total number of bacteria, fungi and actinobacteria, as well fungal diversity was estimated.

O 11

O 12

The impact of coniferous trees biomass extracts on *Phytophthora cactorum* and *Botrytis cinerea in vitro*

J. Halimona[1], Z. Metla[1], R. Sešķēna[1], S. Minova[1], M. Daugavietis[2], L. Jankevica[1]

[1] *Institute of Biology, University of Latvia, Miera iela 3, Salaspils, LV-1586*
[2]*Latvian Forest Research Institute, Rīgas iela 111, Salaspils, Latvia, LV-2169*
E-mail: julija_halimona@inbox.lv

Keywords: *Botrytis cinerea*, conifer extracts, mycelial growth inhibition

Nowadays there still are different chemical pesticides applied in plant protection. To protect plants from harmful activity of pests and diseases, more attention should be paid to development and establishment of environmentally friendly regulation actions. Conifer trees produce a great diversity of compounds, such as an enormous array of terpenoids and phenolics that may impart resistance to a variety of herbivores and microorganisms [1]. The antifungal effect of pine (*Pinus densiflora*) needle extract against *Botrytis cinerea* is proved [2]. General aim in the framework of the study is development of new, innovative products – plant protection aids for agriculture – thus facilitating the integration of science and sustainable usage of forest resources and development of integrated and biological agriculture. Specific aim is to find plant extracts with fungicidal properties. Products of processing pine (*Pinus sylvestris*) and spruce (*Picea abies*) biomass were used. Different solvents (ethanol, Na_2CO_3 NaOH, butanol, water e. t. c) were used for extraction.

Botrytis cinerea and *Phytophthora cactorum* strains obtained from CBS (Centraalbureau voor Schimmelcultures, the Nedherlands) and National Collection of Microorganism Cultures of Latvia and isolates mainly obtained from diseased plants (strawberies, rapsberies, cabages, e.t.c) colected in Pure Horticultural Research Centre were used for *in vitro* experiments. The fungicidal influence of extracts on the mycelial growth of *B. cinerea* and *P. cactorum* strains, applying radial growth test, was tested. The influence of concentrations on sporulation and spore germination depending on regime of incubation were determined

Pine and spruce bark ethanol extracts had inhibitory effect on mycelium growth (inhibition rate >50%), using the concentration 20 g L^{-1} in medium.

Acknowledgements
This study has been supported by ERAF Nr. 2010/0249/2DP/2.1.1.1.0/10/APIA/VIAA/168.

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O 13

Use of natural microbial consortia for hydrogen production by carbohydrate degradation

R. Kalnins

Department of Microbiology and Biotechnology, Faculty of Biology, University of Latvia, Kronvalda Blvd. 4, Riga, Latvia LV-1586
E-mail: rudolfs.ka@gmail.com

Keywords: bio-hydrogen, dark fermentation, microbial consortia, lake sludge, silage

Hydrogen is considered to be a promising energy carrier for the future and it could be used for energy production. It can be produced in an environmentally friendly way by various biological processes, including dark fermentation.

The objective of this work was to investigate the use of natural microbial consortia for hydrogen production by carbohydrate fermentation.

All experiments were carried out in 50 and 100 milliliter serum bottles. Mainly two sources of microorganisms were used – lake sludge and silage, which was also used as the substrate for fermentation. The ability of microorganisms to ferment glucose, galactose, sucrose and glycerol as well as degrade and ferment the carbohydrates in silage biomass were studied.

Amount of produced gas was measured and its composition was determined by mass spectrometry. The dominant species of microorganisms from fermentation liquids were isolated and identified, and the amount of consumed chemical oxygen demand (COD) was determined.

The microorganisms from lake sludge were able to ferment all 3 sugars but not glycerol. Hydrogen yield from glucose, sucrose and galactose were respectively 12,95 ± 4,06 mmol, 16,38 ± 2,13 mmol and 22,13 ± 0,85 mmol per 1 gram of COD used.

The H_2 acquired from silage fermentation was 0.255 ± 0,011 mmol per 5 grams and 0,221 ± 0,008 mmol per 2.5 grams of silage. After the first days of fermentation, consumption of hydrogen was observed and the final pH of the media was increased, which is unusual for fermentation process.

Lake sludge was shown to be a suitable microbial source for hydrogen production since no pretreatment was necessary and facultative anaerobes dominated the fermentation liquids.

Silage was shown to contain hydrogen-producing microorganisms though some pretreatment or a different fermentation mode should be applied to increase the hydrogen yield. The fermentation liquids were dominated by a variety of *Bacillus, Paenibacillus, Yersinia* species and also *Clostridium* species.

Acknowledgements
This study was supported by the National Research Program LATENERGI, Project "Research of hydrogen extraction, storage and energy production methods and elaboration of prototypes".

O 14

Distribution of a novel plasmid mediated beta-lactam resistance genes (ampC) among environmental aquatic bacteria

V. Voolaid, T. Tenson, V. Kisand

Institute of Technology, University of Tartu, Nooruse 1, 50411 Tartu, Estonia
E-mail: veiko.voolaid@ut.ee

Keywords: new subclass of ampC FOX genes; freshwater lake and river bacteria; *Aeromonas* population structure; antibiotic resistance

Resistance to beta-lactam antibiotics is evolutionarily ancient and resistance to new beta-lactam drugs develops and spreads quickly. Most studies on beta-lactam resistance concentrate on pathogens in medical or veterinary settings.

We tested the presence of beta-lactamases among environmental aquatic bacteria isolated on various selective media with antibiotics (including ampicillin) from Lake Võrtsjärv and its run off river. The isolate collection was obtained from samples collected in years 2005 to 2008. The lake is potentially exposed to several antibiotics from the use in farming and diffuse countryside settlements, while there is a larger town (~100 000 inhabitants) at the runoff.

Isolates were phylogenetically characterized by 16S rRNA gene sequencing and the presence of various ampC subclasses (LAT, DHA, ACC, ACT, FOX) in bacteria genome was tested using multiplex PCR. Only the FOX ampC subclass was found, mostly among Gammaproteobacteria, total number of FOX containing isolates was 48. Most prominent group of FOX gene carriers was genus *Aeromonas* (n = 30). On the other hand, not all isolated *Aeromonas* bacteria (n ~ 70) were containing a FOX gene. In addition, the FOX gene was also abundant among *Pseudomonas* (n=12). Found FOX gene was novel according to its sequence and most closely related to plasmid mediated FOX (FOX-3, -4, -8 and -9) sequences found in Enterobacteria, *Escherichia coli* and *Klebsiella* spp. On the same time the 48 FOX genes grouped into four different clusters, meaning that not all the FOX genes were identical and one of the gene copies prevailed over others. Our study demonstrates how medically relevant antibiotic resistance genes can spread among various free living aquatic bacteria but preferentially among environmental non-human pathogens.

O 15

Dynamics of antibiotic resistance genes during the stabilisation phase of microbial community in newly established municipal wastewater treating horizontal subsurface flow constructed wetland filter material

H. Nõlvak, M. Truu, K. Tiirik, T. Sildvee, K. Oopkaup, I. Zaytsev, L. Sobak, Ü. Mander, J. Truu

Institute of Ecology and Earth Sciences, University of Tartu, 46 Vanemuise St, 51014, Tartu, Estonia
E-mail: hiie.nolvak@ut.ee

Keywords: antibiotic resistance genes, constructed wetland, municipal wastewater

The occurrence and spread of antibiotic resistant bacteria is a pressing concern worldwide. Wastewater treatment is a major route by which antibiotic resistance genes are introduced into natural ecosystems. Traditional wastewater treatment facilities are also recognized as concentration point and reservoir for antibiotic resistant bacteria where antibiotic resistance genes are selected, enriched and transferred to other bacterial species. Constructed wetlands – an ecological alternative to conventional wastewater treatment - have not yet been studied thoroughly in this respect

The aim of this study was to describe the presence, abundance and proportions of antibiotic resistance coding genes (*tetA*, *tetB*, *tetM*, *ermB*, *sul1*, *ampC*, *qnrS*) during the 150-day stabilisation phase of microbial community in a newly established municipal wastewater treating horizontal subsurface flow constructed wetland filter material. The results revealed that the abundance of targeted resistance genes in the filter material followed different dynamic patterns: *tetA*, *sul1*, *ermB* and *ampC* abundances showed slight increases throughout the experiment; *tetB*, *tetM* and *qnrS* abundances on the other hand revealed decreasing trend. At the same time the proportions of the individual resistance genes in the microbial community revealed no definite trends over the experiment duration. The proportion of targeted resistant bacteria of the total bacterial community ranged from 0,86% to 4,96% being higher at the start and generally decreasing towards the end of the experiment. During the study period a temporal system clogging occurred. It was revealed that during such malfunctions the abundance of the whole microbial community as well as antibiotic resistance genes increases significantly. This indicates the higher risk of resistance development and progress to the environment during such system maintenance malfunctions. After clogging was removed, resistance indicators dropped to the pre-clogging level.

Acknowledgements

This study was supported by Target Project No SF0180127s08 of the Ministry of Education and Research of the Republic of Estonia, Estonian Science Foundation grant 9387, by the European Regional Development Fund through ENVIRON (Centre of Excellence in Environmental Adaptation) and state program „Aid for research and development in environmental technology“ grant 3.2.0801.11-0026.

O 16

Bacterial community activity, structure and succession in hybrid constructed wetland treating domestic grey water

I. Krustok[1], J. Truu[2], M. Truu[2], , J-K. Preem[2], Ü. Mander[2], E. Nehrenheim[1], M. Odlare[1]

[1]School of Sustainable Development of Society and Technology, Mälardalen University, P.O. Box 883, SE-721 23 Västerås, Sweden
[2] Institute of Ecology and Earth Sciences, University of Tartu, Vanemuise 46, 51014 Tartu, Estonia
E-mail: ivo.krustok@mdh.se

Keywords: bacterial community, grey water, microbial activity, next-generation sequencing, hybrid treatment wetland

The dynamics of filter microbial communities was studied in ten month long survey carried out in a newly established experimental hybrid filter system treating domestic gray water (wastewater generated from laundry, dishwashing, and bathing) from November 2009 to August 2010. The study system comprised of four parallel filter systems. Each individual system consisted of three parallel vertical flow filters (VFF) followed by horizontal flow filter (HFF). Light expanded clay aggregates (LECA) varied by particle size in each VFF and the same uniform material for each HFF were used as filter materials in three systems whereas crushed oil-shale ash (aggregate size 5-20 mm) was used in all filters of the fourth system. Samples for microbiological analyses were taken from 0-5 cm active filter layers in two weeks interval.

Potential nitrification and dehydrogenase activity was measured from the materials. There were statistically significant differences in dehydrogenase activity between studied VFF filter materials being lowest in case of oil shale ash.

The dynamics of microbial community composition from the system units having the most effective purification efficiency was analyzed sequencing of the V6 region of 16S rRNA gene using Illumina HiSeq 2000. The most abundant bacterial groups in VFF material were Proteobacteria (71.7%), Bacteroidetes (18.9%), Actinobacteria and NKB19 (both 3.8%), in HFF material Proteobacteria (69.2), Bacteroidetes (11%), Firmicutes (7.6%), Actinobacteria (5.2%) and also in HFF large number of sequences remained unclassified to phylum level (4.7%).

Multivariate analysis showed similar bacterial community succession pattern in parallel vertical CW units and indicated stabilization of bacterial community structure after four months since beginning of operation. Similar successional pattern was observed in HFF material.

Acknowledgements

This study was supported by Target Project No SF0180127s08 of the Ministry of Education and Research of the Republic of Estonia, 7th EU Framework project No 232274 and by the European Regional Development Fund through ENVIRON (Centre of Excellence in Environmental Adaptation).

O 17

Cellular and molecular interactions between *Trichoderma* strains and conifer pathogens *Heterobasidion annosum* and *H. parviporum*

L. Alksne[1], V. Nikolajeva[1], Z. Petrina[1], D. Eze[1], T. Gaitnieks[2], L. Vulfa[1], A. Lielpetere[1]

[1] Microbial Strain Collection of Latvia, Faculty of Biology, University of Latvia, Kronvalda Blvd. 4, Riga, Latvia LV-1586
[2] Latvian State Forest Research Institute „Silava", Rigas str. 111, Salaspils, Latvia LV-2169
E-mail: lauruxla@inbox.lv

Keywords: *Trichoderma, Heterobasidion,* antagonism, interaction

Root rot of conifers caused by *Heterobasidion annosum s.l.* is one of the most destructive tree diseases of the European forestry. Biological method for the *Heterobasidion* control seems to be a good alternative to chemical treatment.

The aim of the research was to evaluate the influence of medium filtrates from six selected *Trichoderma* strains on the growth of *H. annosum* and *H. parviporum* and *Trichoderma* volatiles impact on pathogens and hyphal interactions with and without neutral red in different nutrient substrates.

Malt extract agar was supplemented with 10% of *Trichoderma* medium filtrate. *Heterobasidion* strains were incubated at temperature of 15 °C and 20 °C. Radial growth of *Heterobasidion* was measured every tree days.

Trichoderma and *H. annosum* s.l. strains were inoculated in glass Petri dishes with malt extract agar faced against each other, pathogen was always on top.

Two extract agar feeds were prepared – 2% and 0.2%, each was applied in a thin layer on sterile slides. On the one side was placed *Heterobasidion* mycelium, on the other – *Trichoderma.* Neutral red solution on the hyphae contact zone and microscope.

The results show that all *Trichoderma* strains were releasing in the medium water-soluble compounds with antagonistic effect on the radial growth rate of *Heterobasidion*. Media filtrates from *T. viride* strains MSCL 945 and MSCL 1026 were the most active inhibitors. The inhibition was more pronounced at 15 °C than at 20 °C. Perhaps *Trichoderma* spp. volatile substances didn't affect *Heterobasidion* growth rate, but there had been changes in the appearance of colonies. *Trichoderma* hyphal interactions with *H. annosum* s.l. were influenced by media available nutrients. Direct *Trichoderma* hyphae contact caused *Heterobasidion* cytoplasmic coagulation. Neutral red was uptaked in damaged *H. annosum* s.l. hyphae cells where they were crossed by *Trichoderma* hyphae.

Acknowledgements
This study was supported by the European Regional Development Fund (ERDF) (2010/0277/2DP/2.1.1.1.0/APIA/VIAA/129).

O 18

Microbial fuel cells in Latvia: Use of natural microbial consortia from bodies of water in Latvia in microbial fuel cells enriched with glucose and acetate

Z. Rutkovska [1], A. Gruduls[1], I. Dimanta[1], J. Kleperis[2] and V. Nikolajeva[1]

[1]*Department of Microbiology and Biotechnology, Faculty of Biology, University of Latvia, Kronvalda Blvd. 4, Riga, Latvia, LV-1586*
[2]*Institute of Solid State Physics, University of Latvia, 8 Kengaraga street, Riga, Latvia, LV-1063*
E-mail: rutkovska.zane@gmail.com

Keywords: microbial fuel cells, operational MFC construction, natural microbial consortia, additional substrates

Microbial fuel cells (MFCs) are devices that use bacteria as the catalyst to oxidize organic and inorganic matter and generate current [1]. MFC research has been rapidly evolved in the past decade, whereas studies in Latvia have emerged only in recent years.

An operational MFC prototype was designed. In experiments natural microbial consortia from bodies of water in Latvia were used. In three experiments with additionally added substrates energy readings were fixed and analysed using electrochemical voltametry system for MFC potential assessment. The highest voltage (0,753 V) was achieved by MFC containing Ķemeru swamp sludge as a substrate enriched with glucose (50% 5 ml/l), whereas the highest amperage (0,24 mA) was produced by MFC containing water from river Daugava enriched with acetate (50% 1,7 ml/l). Also glucose concentration change assessment was made by using high-performance liquid chromatography (HPLC). Natural microbial consortia from both substrates were analysed using various identification methods (BBL™ Crystal™ ID, ViaGram™). Microbial biofilm prevalence on MFC anodes were examined using scanning electron microscope.

Results in the report will be discussed in detail.

Acknowledgements

We acknowledge the support of Latvian National Research Programme Research Program in Energetics and Microbial Strain Collection of Latvia.

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CBM
Riga, Latvia 2012

O 19

Characterization of growth of lactic acid bacteria in milk: microcalorimetric approach

I. Stulova[1,2], N. Kabanova[1,2], T. Kriščiunaite[1,2], T.-M. Laht[1,2], R.Vilu[1,2]

[1] *Tallinn University of Technology, Ehitajate tee 5, 19086, Tallinn, Estonia*
[2] *Competence Center of Food and Fermentation Technologies (CCFFT), Akadeemia tee 15A, 12618, Tallinn, Estonia*
E-mail: irina.stulova@tftak.eu

Keywords: milk, lactic acid bacteria, microcalorimetry, heat treatment, irradiation, solid state fermentation

The ability to grow in milk is an important feature of lactic acid bacteria (LAB) used as starters in production of fermented milk products.

The methods used for quantifying the acidification activity of starter bacteria are usually based on measuring pH changes as function of time, or on determination of the accumulation of lactic acid (Zanatta & Basso, 1992). A more detailed analysis of the acidification processes is not carried out in absolute majority of the cases. Calorimetry, in particular isothermal calorimetry, is ideally suited for the detailed study of acidification processes as it offers many unique advantages in comparison with the other methods.

The specific objectives of the present work were to apply microcalorimetry to study: 1) peculiarities of growth of thermophilic starter bacteria as well as non-starter lactic acid bacteria (NSLAB) in differently pretreated milk samples (pasteurized, ultra-high temperature (UHT) treated milk with different fat content, reconstituted skim milk (RSM); 2) influence of low to high concentrations of H_2O_2 (10-250 mg L^{-1}) on the growth of thermophilic starter bacteria in UHT milk; 3) peculiarities of the growth of *Streptococcus thermophilus* at different inoculation rates in liquid and renneted RSM prepared from non-irradiated and irradiated by γ-irradiation at 10 kGy milk powder.

Heat produced during different growth stages (Q_{tot}, Q_{exp}), maximal specific growth rate (μ_{max}) and lag–phase (λ) duration were determined by processing calorimetric curves, and detailed analysis of growth of the bacteria in differently prepared milks were carried out on the basis of these data.

It was shown that isothermal microcalorimetry in combination with other methods (HPLC, UPLC, reological measurement, SFS) is one of the most promising high throughput method for the characterization of growth of starter bacteria in opaque media.

Acknowledgements

The financial support for this research was financially supported through the grant ETF7636 of Estonian Science Foundation, through the grant SF0140090s08 of Estonian Ministry of Education and Research, and by the EU Regional Development Fund project EU28912 and EU29994.

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O 20

The use of microcalorimetry in studies of solid state fermentation processes

N. Kabanova[1,2], I. Stulova[1,2] and R. Vilu[1,2]

[1] *Tallinn University of Technology, Ehitajate tee 5, 19086, Tallinn, Estonia*
[2] *Competence Center of Food and Fermentation Technologies (CCFFT), Akadeemia tee 15A, 12618, Tallinn, Estonia*
E-mail: natalja@tftak.eu

Keywords: *Lactococcus lactis*, solid state, microcalorimetry, colonial growth

Thermal activity monitor TAMIII was used to study the growth of *Lactococcus lactis* IL1403 in solid agar gels. Bacterial growth in structured samples was compared with the growth in broth. The bacterial growth was studied at different glucose concentrations of 2, 10 and 20 g L^{-1} and at different inoculation rates from 10^0 to 10^6 cfu mL^{-1} with the 10-fold increment. In parallel to calorimetric measurements the HPLC and pH measurements of culture media were carried out in order to obtain additional information for the interpretation of calorimetric power-time curves. The sizes of colonies were measured at the end of solid state growth using microscope. Maximal specific growth rates μ_{max} (W h^{-1}), heat produced during different growth stages Q_{TOT} (J mL^{-1}), Q_{EXP} (J mL^{-1}) and lag–phases λ (h) duration were determined by processing calorimetric curves. Changes of pH and lactic acid concentrations together with density and geometry of colonies were identified as factors determining the peculiarities of growth of colonies of different size. The data obtained together with calculated heat yield coefficient Y_Q (J cfu^{-1}) and microscopic measurements allowed to analyze and describe quantitatively the growth of individual colonies, to develop the model distribution of colonies in solid matrix and to construct the growth curve of a typical colony of *Lactococcus lactis* in 1% agar gel. The results obtained showed that microcalorimetric method used in combination with other relevant methods is a very powerful tool in studying solid state fermentations and growth in other opaque media – milk, biological fluid etc., and could be applied successfully for the study and optimization of industrial solid state fermentation processes. Calorimetric, essentially *on-line* measurements are very precise and easier to carry out in comparison with other methods currently used in studies of solid state fermentation processes.

Acknowledgements
This investigation was financially supported through the grant ETF9192 of Estonian Science Foundation, through the grant SF0140090s08 of Estonian Ministry of Education and Research, through the project 3.2.0701.11-0018 and by the European Regional Development Fund project EU29994.


CBM
Riga, Latvia 2012

O 21

Anaerobic fermentation of untraditional substrates for bioenergy application

J. Jasko[1,2], B. Tutere[1], E. Skripsts[1,2], V. Dubrovskis[2]

[1] Institute of Biomechanics and Physical Research, Maskavas iela 22 - 1, Rēzekne, Latvia, LV – 4604
[2] Latvia University of Agriculture, Liela iela 2, Jelgava, Latvia, LV-3001
E-mail: janis.jasko@gmail.com

Keywords: Biogas, anaerobic fermentation, straw, sawdust, hogweed

Usage of anaerobic fermentation process to produce renewable energy becomes more widespread in European Union and also in Latvia. According to Latvian Biogas Association in the mid of year 2012 there are 36 biogas stations in operation in Latvia. Large part of biomass used as a substrate for biogas production is energy crops, mainly maize and grass silage. In the same time, according to latest renewable energy policy trends in EU, cultivation of energy crops should not compete with food production for arable land. To replace energy crops with other biomass that would generate equivalent biogas amount, research on available biomass biochemical methane potential (BMP) is needed.

In this study biochemical methane potential of three local biomass types has been studied. Rape straw and deciduous tree saw dust were selected as they are common waste in agriculture and wood-processing industry respectively. Hogweed is an invasive plant species in Latvia that has took over large areas of land. Hogweed has high biomass yield thus has potential to be used for biogas production.

During the study average biochemical methane potential for rape straw, deciduous tree saw dust and hogweed biomass were determined. Batch fermentation in small volume glass reactors was carried out. BMP for rape straw was 259±3 $l*kg^{-1}$ TS and 271±3 $l*kg^{-1}$ VS. BMP for saw dust was 561±16 $l*kg^{-1}$ TS and 619±18 $l*kg^{-1}$ VS. BMP for hogweed was 459±29 $l*kg^{-1}$ TS and 513±33 $l*kg^{-1}$ VS.

Acknowledgements
This study was supported by ERDF project “New technology for bio-hydrogen production and separation in anaerobic digestion process”, contract number: 2010/0326/2DP/2.1.1.1./10/APIA/VIAA/132.


CBM
Riga, Latvia 2012

O 22

Influence assessment of several substrates and inhibitors for methane production in anaerobic fermentation process

L. Laurinovica[1], I. Dirnena[1], V. Nikolajeva[1], I. Dimanta[2]

[1]*Faculty of Biology, University of Latvia, Kronvalda Blvd. 4, Riga, Latvia, LV-1586*
[2]*Institute of Solid State Physics, University of Latvia, Kengaraga street 8, Riga, Latvia, LV-1063*
E-mail: lauma.laurinovica@gmail.com

Keywords: methane, anaerobic digestion, substrates, inhibitors

This study examines biogas substrate samples taken from two biogas fermenters in Latvia. Both biogas plants perform identical gas production technologies. Several substrate and inhibitor assessments were done to see their influence on methane production and to improve methane quantitative and qualitative analysing methods.

Two different methods were used to incubate samples. They were incubated in serum bottles and in bioreactor test system where gas samples were taken. Gas samples were analysed with gas mass spectrometer to determine methane concentrations after extra substrate (glucose, $CO_2 + H_2$) addition and physical condition changes of fermentation process. Additional substrates did not have identical influence on methane production in different incubation conditions. It was observed that in examples incubated over optimum temperature (47^0C) of methanogenic bacteria, after glucose addition methane gas concentration increased but carbon dioxide and hydrogen gas mixture decreased it.

As potential inhibitor to methane producing bacteria ammonia was observed. In this research determined ammonia concentration was about 25 times lower than theoretical, so it did not have inhibitory influence on methane gas production.

Microorganisms were cultivated in aerobic and anaerobic media and incubated in various environments – laminar flow box, anaerobic jar and controlled atmosphere chamber. Bacteria identification was performed with Crystal™ ID kits. Several microorganisms were identified - two *Bacillus* spp. and two *Lactobacillus* spp. but these genera do not provide unequivocal view about bacteria living in biogas fermenter. None of methanogenic bacteria were cultivated in anaerobic media perhaps of their high sensitivity to the oxygen. Their absence was proved performing the gas mass spectrometry results. For more precise methane gas analysis both incubation methods need to be optimized as well as microorganism cultivation in anaerobic environment.

Acknowledgements

This work was supported by the National Research Program in Energetic. One from us (L. Laurinovica) thanks to Microbial Strain Collection of Latvia, Laboratory of Hydrogen Energy Materials of Institute of Solid State Physics and Laimonis Jekabsons - engineer of Institute of Solid State Physics.

O 23

ConvAn: convergence, consensus and stagnation analyzer for optimization of biochemical networks

A. Kostromins[1], J. Sulins[1], I. Mozga[1], E. Stalidzans[1]

[1]*Faculty of Information Technologies, Latvia University of Agriculture, Liela iela 2, Jelgava, Latvia LV-3001*
E-mail: egils.stalidzans@gmail.com

Growing number of mathematical models of biochemical networks demands improved analysis and optimization approaches. Kinetic models usually are expressed as systems of nonlinear differential equations. Their optimization often is performed using global stochastic optimization methods that need significant computational power and time.

A software tool *ConvAn* (www.biosystems.lv/convan) is developed to analyze statistical properties of convergence dynamics for optimization runs with particular optimization method, model, software tool, set of optimization method parameters and number of adjustable parameters of the model. The convergence curves can be normalized automatically to enable comparison of different methods and models in the same scale.

ConvAn can be used with software tool *CoRunner* (www.biosystems.lv/corunner) for analysis of parallel optimization runs of identical optimization task using *COPASI*. The functionality of *ConvAn* enables processing and visualization of convergence dynamics of parallel optimization runs to detect fulfilment of consensus and stagnation criteria.

Consensus criterion is applied to parallel optimization runs for their early termination assuming that the best value of the objective function is reached.

Stagnation criterion is developed to terminate parallel optimization runs in case of long stagnation of runs at different values of objective function. Stagnation is an indicator that the optimization method does not perform well in particular optimization task and the optimization method or it's parameters have to be changed.


CBM
Riga, Latvia 2012

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New approach for yeast immobilization

D. Borovikova, A. Patmalnieks, A. Rapoport

Institute of Microbiology and Biotechnology, University of Latvia, Kronvald Blvd. 4, Riga, Latvia LV-1586
E-mail: borovikovadiana@inbox.lv

Keywords: yeasts, immobilization

Nowadays immobilization of cells achieves more and more application. Immobilized yeasts are used widely in various areas, mainly in the food industry, in biotechnological fuel production, pharmaceutical and chemical industries as well as in agriculture, electronics and medicine. Cells can be immobilized by different methods based on physical and chemical interactions between cells and carriers. Current attempts for the development of immobilization methods are linked with the improvement and/or combination of existing approaches. The application of natural adhesion to different surfaces gave the possibility for development and implementation of new approach of yeast immobilization. Our new method of yeast immobilization consists of two stages: 1) incubation of cells with carrier and their sedimentation on it which is linked with physical interactions and 2) stabilization of cells on the carrier surface by dehydration stimulating by this way the formation of chemical interactions between carrier and cells. Yeasts immobilized by this method can be used in continuing processes because it was shown that in this case cells' adhesion to carrier is stable and tight. Different carriers were studied and it was concluded that the structure of carrier surface has important role for cell adhesion. Immobilization of cells by our new method allows them easy utilize nutrients from the media and maintains the reproduction of cells.

Acknowledgements
This research was supported by the European Commission Project NMP3-CT-2003-504937 "Multifunctional Percolated Nanostructured Ceramics Fabricated from Hydroxyapatite" and European Social Foundation.

O 25

Trehalose metabolism in yeast *S. cerevisiae* - effects of common auxotrophies

A. Kokina, J. Liepins

Institute of Microbiology and Biotechnology, University of Latvia, Kronvalda Blvd. 4, Riga, Latvia LV-1586
E-mail: agnese.kokina@lu.lv

Keywords: trehalose, auxotrophy, storage carbohydrates, stress resistance

Trehalose is well known storage carbohydrate in yeast. There has been a wide debate if it acts a stress protectant as well [1]. It has been shown recently, that trehalose accumulation accompanies growth speed reduction in chemostat during growth under nutrient limitation [2].

Most laboratory yeast strains possess various auxotrophies, that are used as genetic markers and exploited in genetic engineering. Common auxotrophies are – histidine, uracil, leucine, tryptophan, less common – adenine and methionine. As synthesis of those metabolites in auxotrophs is obstructed, external supply of those nutrients is necessary to support growth. There are concerns, that in complex and even in synthetic media those required nutrients could limit growth well before carbon source depletion [3;4], thus changing their physiological responses drastically [5]. Based on those concerns and our previous observations – we decided to explore how commonly used auxotrophies influence yeast metabolism and physiology with emphasis on trehalose metabolism.

We assessed response of trehalose accumulation of three common laboratory yeast strains (CEN.PK, BY4741, W303) in case of adenine auxotrophy in complex media (YPD) and in synthetic broth lacking one of all the respective auxotrophic agents.

Trehalose accumulation was a strain specific phenomenon, that varied in different auxotrophic conditions and strain genetic backgrounds. This effect was most pronounced in adenine auxotrophs in CEN.PK strain background – the trehalose content reached up to 200 mg/gDW during adenine auxotrophy there. Correlation between increase in trehalose and stress resistance (oxidative, heat, desiccation) exists.

Based on results obtained, we would like to advise to take those effects in to consideration when investigating yeast physiology in complex media in auxotroph yeast strains.

Acknowledgements

Research was supported by the European Social Fund (ESF), project "Establishment of Latvian interdisciplinary interuniversity scientific group of systems biology", project Nr. 2009/0205/1DP/1.1.1.2.0/09/APIA/VIAA/128

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O 26

Molecular history of *Staphylococcus aureus* in Latvia `

E. Miklaševičs [1,2], A. Balode[1,2] , L. Čupāne[1,3] , D. Gardovska[1, 3]

[1] *Riga Stradins University, Dzirciema iela 16, Riga, Latvia*
[2] Pauls Stradiņš Clinical University Hospital, Pilsoņu iela 13, Riga, Latvia
[3] Children Clinical University Hospital, Riga, Latvia
E-mail: edvins.miklasevics@rsu.lv

Keywords: *S. aureus*, typing, PVL

Staphylococcus aureus is a major cause of hospitalt infections. The spectrum of staphylococcal infections varies from mild superficial to invasive life-threatening diseases due to *S. aureus* ability to produce a wide range of virulence factors, including toxins. Panton – Valentine leukocidin (PVL) is an extracellular pore forming *S. aureus* gamma toxin.

Molecular types and pathogenicity factors of *S. aureus* clones circulating in Latvia will be discussed. Special emphasys will be on PVL positive strains. Recent investigations suggest that PVL-positive *S. aureus* exhibits enhance virulence and are responsible for severe infections such as bone and joint infections and necrotising pneumonia. Due to PVL positive *S. aureus*, community acquired necrotizing pneumonia is an emerging infection. Pneumonia often arises from the blood born spread of organisms from infected tissues and can follow viral respiratory infections, especially influenza. Our study revealed that Panton-Valentine leukocidine (PVL) genes are carried by a high number (75%) of *S. aureus* isolates recovered from children hospitalised in the Children Clinical University hospital. Most of these isolates were associated with abscesess and other skin and soft tissue infections. Patients with PVL positive invasive infections stayed significantly longer in hospital than patients with PVL negative invasive infections. Clonal distribution of PVL positive *S. aureus* isolates were closely related, which provides evidence for the wide spread of PVL producing spa type t435 and ST121 staphylococci in community.


CBM
Riga, Latvia 2012

POSTER PRESENTATIONS

Supporters

Solid tumour diagnostics and immunotherapy by immunomodulators from natural sources

G. Vitols[1], L. Peshkova[1], S. Donina[1], S. Rasa[1], J.Jermolajevs[1]

[1] Riga Stradin's University A. Kirchenstein Institute of microbiology and virology Ratsupites 5, Riga, Latvia, LV-1067
E-mail: guntis@animalab.lv

P 01

Keywords: Immunotherapy, glycopeptides, immunomodulators, tumour, Thimidine kinase, cytotoxicity, lung cancer cells A 549

Efficiency of tumour immunotherapy depends on diagnostics of tumour early stages. Tumour patents were examined by Thyimidine kinase assay. Presence of active Thymidine kinase (TK) in serum and its correlation to tumour diseases was shown in 1982. Results obtained with TK ELISA tests (DiviTum) have demonstrated that levels of serum TK in tumour patients correspond to the amount of dividing tumour cells. Serum TK activity assays have found their major clinical application for the prognosis and the monitoring of therapy in blood malignancies. Elevated levels of TK activity have also been linked to solid tumours with metastases. Biologically active substances as β-glucans isolated from *Saccharomyces cerevisiae* and mushrooms: local chanterelle, medical mushrooms-shiitake and glycopeptides isolated from local cultivated *Lactobacillus helveticus* on local skim milk powder media were used for tumour immunotherapy and tested on cytotoxicity. Glycopeptides and beta-glucans together with other biologically active compounds have immunomodulator activity which helps to strenghten immune system and rehabilitate organism after X-Ray, chemical and drug therapy. Imunomodulators were done by our scientific laboratory and their quality for cytotoxicity were tested by tissue culture within 24 to 72 hours. Cells A549. Both immunomodulators; glycopeptides and beta- glucans were tested for cytotoxicity before clinical observation. Tumour patents were divided into two groups and tested by Thymidine kinase assay before treatment and after every 3 months. 60 patients were divided into two groups with equal stage of cancer. 30 patients have lung cancer and 30 patients have different kinds of women cancers. Another 60 patients were in placebo group and they haven't cancer. All patients received X-Ray or chemical therapy. Thymidine kinase level for all patients was between 500- 1000 U/L. All patients in first group received β- glucan 3 capsules per day during three months, other group received glycopeptides 3 capsules per day. Effectivity of immunomodulators therapy was checked by Thymidine kinase assay, immunologycal and biochemical analysis. The first results were obtained after 3 months of immunotherapy. Clinical observation will continue for 6 months.

Acknowledgements
Glycopeptides, beta - glucans isolation from local natural lactobacteria and mushrooms, clinical observation and Thymidine kinase testing was supported by ERAF project Nr.2DP/2.1.1.1.0/10/APIA/VIAA/125.

P 01

P 02

Production of hydrogen peroxide and lactic acid by vaginal lactobacilli

E. Lapp[1,2], A. Ahelik[1], I. Smidt[1], N. Borovkova[1,2], H. Oopkaup[1], J. Štšepetova[1], S. Oolep[1,2] and R. Mändar[1,2]

[1] Department of Microbiology, University of Tartu, Estonia
[2]Competence Centre on Reproductive Medicine and Biology, Tartu, Estonia
E-mail: elerilapp@gmail.com

Health of the female genital tract depends significantly upon the composition of vaginal microbiota. Vaginal lactic acid bacteria maintain the ecological equilibrium of the tract by protecting against pathogens and microbiota imbalance. Hydrogen peroxide and lactic acid are the most important antimicrobial compounds produced by vaginal lactobacilli, therefore these properties must be determined while screening the lactobacilli as potential vaginal probiotics.

Our **aim** was to assess the hydrogen peroxide and lactic acid production of vaginal lactobacilli of healthy women and women of infertile couples in case the latter being the partners of men with or without inflammatory prostatitis (IP).

Methods. Altogether 135 lactobacilli strains were investigated, of them 70 originated from women of infertile couples (48 from partners of healthy men, 22 from partners of men with IP) and 65 from healthy women. Lactobacilli were identified by sequencing of the 16S rDNA fragment. For hydrogen peroxide detection, the pre-grown strains were plated on TMB agar consisting of tetramethyl-benzidine, horseradish peroxidase, brucella agar base, hemin, starch and vitamin K, change of colour was assessed semi-quantitatively. The production of lactic acid was estimated using gas chromatography.

Results. All lactobacillus strains belonged to 3 species – *Lactobacillus crispatus, Lactobacillus jensenii* and *Lactobacillus gasseri.* Most of *L. crispatus* (88%) and *L. jensenii* (86%) strains while only 46% of *L. gasseri* strains produced hydrogen peroxide ($p<0.001$ in comparison with *L. crispatus* and $p=0.001$ in comparison with *L. jensenii*). The lactobacilli originating from healthy women ($p=0.037$) and partners of healthy men ($p=0.029$) expressed stronger production of hydrogen peroxide than the partners of IP patients. In case of *L. jensenii*, higher number of strong hydrogen peroxide producers originated from healthy women ($p=0.008$) or partners of healthy men ($p=0.047$) than partners of IP-patients. Lactic acid production did not correlate with hydrogen peroxide production and no differences were found between the donors' groups. The best lactic acid producers were *L. gasseri* (18.2±2.2 mg/ml) and *L. crispatus* (15.6±2.8 mg/ml) while *L. jennsenii* produced less lactic acid (11.6±2.6 mg/ml).

Conclusions. Our study suggests that hydrogen peroxide and lactic acid production ability are species-specific – *L. crispatus, L. jensenii* are stronger

hydrogen peroxide producers while *L. crispatus* and *L. gasseri* are stronger lactic acid producers. That fits with the former knowledge that *L. crispatus* is associated with more stable vaginal environment. At the same time large strain-specific and also donor-specific differences can be seen and therefore large and heterogeneous strain collection is needed when probiotic development is performed.

P 02

P 03

Antagonistic activity of probiotics and its glycopeptides against *Staphylococcus spp.*

G. Gulbe[1], A. Valdovska[2], A. Jemeljanovs[1], G. Vitols[3]

[1] Research Institute of Biotechnology and Veterinary Medicine „Sigra", Latvia University of Agriculture, Instituta str.1, Sigulda, Latvia, LV-2150
[2] Faculty of Veterinary Medicine, Latvia University of Agriculture, K.Helmana str.8, Jelgava, Latvia, LV-3004
[3] Institute of Microbiology and Virology, Riga Stradins University, Ratsupites str.5, LV-1067
E-mail: Anda.Valdovska@llu.lv

Keywords: Probiotics, in vitro, *Staphylococcus spp., Lactobacillus*; *Pediococcus*, glycopeptides

According to current definitions, probiotics are live microorganisms that exert a health benefit to the host. The action of probiotics in the host is exerted by prevention of bacterial translocation and modulation of the local immune response. However, each strain must be well identified and characterized *in vitro* before using. *Lactobacillus reuteri*, *Pediococcus pentosaceus*, glycopeptides of *L. helveticus* in titre 10^9 cfu/g (GP1), in titre 10^7 cfu/g (GP3) and glycopeptides of *L. helveticus* with ß-glucans (GP2) were tested *in vitro* to determine its antimicrobial effects againsts *Staphylococcus aureus, Staphylococcus saprophyticus, Micrococcus kristinae, Streptococcus uberi, E.coli* and mixed culture of above mentioned microorganisms isolated from raw milk of cows with subclinical mastitis. As control suspension of "Pen Strep" and amoksicillin (AMC30) were used. The antimicrobial activity of bacterial isolates was tested by well and disk diffusion assays. Results obtained in the experiment showed, that GP1 has a greatest inhibitory effect (13.73±0.23 mm), GP3 also works effectively (6.14±0.4 mm), while GP2 showed low antagonistic effect (0.80±0.00 mm) against bacterial cultures as a whole. Zone of antibacterial resistance of "Pen Strep" and AMC30 was 48.74±0.48 mm and 25.76±0.24 mm, resp. All bacterial strains were resistant to probiotics *L. reuteri* and *P. pentosaceus* containing test solutions. *S. aureus* and *S. saprophyticus* showed resistance against GP2 and GP3, *E.coli* was resistant only to GP3. GP2 has not inhibitory effect to mixed culture. Conclusion - the applied test solution GP1 which contains *L. helveticus* glycopeptides in titre 10^9 cfu/g has antagonistic activity against a variety of pathogens and it can be used as an alternative to antibiotic therapy.

Acknowledgements
Glycopeptides isolation was supported by ERAF project Nr.2DP/2.1.1.1.0/10/APIA/VIAA/125, but experiment – by Nr.2010/0226/2DP/2.1.1.1.0/10/APIA/ VIAA/099 foundations.

The link between *Gardnerella vaginalis* genotype and sialidase activity

M. Pleckaityte[1], M.Janulaitiene[2], R.Lasickiene[1], A.Zvirbliene[1]

[1] Institute of Biotechnology, Vilnius University, Vilnius, Lithuania
[2]National Public Health Surveillance Laboratory, Vilnius, Lithuania
E-mail: mildap@ibt.lt

P 04

Background: *Gardnerella vaginalis* is considered a substantial player in the progression of bacterial vaginosis (BV). Very little is currently known about the genetic composition of *G.vaginalis*, the diversity of strains and its physiology. We analysed 17 *G.vaginalis* strains isolated from the genital tract of women diagnosed with BV to establish a potential link between genotypes and expression of sialidase, a virulence factor, assumed to play a substantial role in the pathogenesis of BV.

Observations: DNA was extracted from 17 *G.vaginalis* clinical isolates and with the ARDRA technique (amplified 16s rRNA gene restriction analysis) using the restriction endonuclease TaqI two *G.vaginalis* genotypes were identified. The genotypes were not equally presented in the clinical isolates, with six strains of genotype 2 and 11 strains of genotype 1. The presence of sialidase gene in clinical isolates was identified by PCR using specific primers. The expected DNA fragment was observed for all 17 G.vaginalis isolates. Sialidase activity was assayed by a filter spot test using 4-methylumbelliferyl-alfa-D-N-acetylneuraminic acid as substrate. Sialidase activity observed as a fluorescent blue spot on filter paper after incubation with the substrate for 5 min was scored as a positive reaction. Fluorescence detected after 15 min incubation was recorded a weak positive. Six of 17 isolates showed sialidase activity, 11of 17 isolates expressed weak or no sialidase activity.

Conclusions: 35 percent of *G.vaginalis* isolates exhibited sialidase activity. The isolates of genotype 2 were characterized solely as sialidase positive. Thus, the link between genotype and sialidase production may serve as a possible marker for the identification of pathogenic potential of *G. vaginalis* strains.

P 05

Evaluation of bacterial colonisation of biomaterials and its effect on TNF-a,-b defensin-2 and IL-10 expression in tissues *in vivo* study, after a 2 and 4 week, and 3 month exposure in rabbit tissues

A. Reinis[1], M. Pilmane[2], J. Kroiča[1], J. Vētra[2], I. Skadiņš[1], A. Stunda[3], L. Bērziņa-Cimdiņa[3], N. Bērza[1], D. Rostoka[1]

[1] *Rīga Stradiņš University, Department of Biology and Microbiology*
[2] *Rīga Stradiņš University, Institute of Anatomy and Anthropology*
[3] *Riga Technical University, Biomaterial Development and Innovation Centre*
E-mail: aigars.reinis@rsu.lv

Objectives. The aim of the study is to explore 3 originally synthesised biomaterials and surface bacterial colonisation risk modification and their impact on the surrounding tissue: material A - raw materials and products are crystalline, B - the substance is an amorphous, crystalline product; B + - B etched in order to reduce the amorphous phase on the surface.

Methods. *Ps.aeruginosa* ATCC 27853, ATCC *S.epidermidis* 12228 for the preparation of pure cultures we used bacterial suspensions in 1 ml volume of TSB concentration of 10^2 and 10^3 CFU/ml. Samples were cultivated at 37°C for 2h to determine adhesion rates and ensure adhesion of the bacteria. Contaminated biomaterial samples were implanted in interscapular area of chinchilla rabbits (*in vivo* 2 and 4 weeks). The biomaterial was removed and, using plate count and sonification method the bacterial colonisation on the surface of the biomaterial was determined, however, we prepared preparations from the surrounding tissues, staining in haematoxylin-eosin and using immunohistochemistry methods thus determining TNF-A,-B defensin-2 and Il-10.

Results. Biomaterial samples contaminated with *S.epidermidis* showed a low degree of colonisation. Two samples (A and B) after 2 weeks of exposure were sterile. *Ps.aeruginosa* showed a higher degree of colonisation with intensity from 0.21 CFU/ml (B biomaterial) up to 8.7 CFU/ml (B + biomaterial). After 3 months of exposure we observed rare bacterial colonisation on samples A and B with *S.epidermidis*. And after 3 months of exposure with *Ps.aeruginosa* we observed rare bacterial colonisation on sample A.

The most intense inflammatory reaction was observed around the *Ps.aeruginosa* contaminated biomaterials. Many TNF-A and Il-10-containing inflammatory cells infiltrated tissues surrounding the aforementioned materials. Indicators of inflammation practically did not differ for the 2 and 4 week implants of biomaterials.

We observed some defensin-containing cells in tissue surrounding biomaterials after *Ps.aeruginosa* infection, while in other biomaterial contamination cases such cell amount was much more moderate. After 3

months of exposure we observed rare inflammatory mediators producing macrophages.

Conclusions. The study showed that *Ps.aeruginosa* compared with *S.epidermidis* more intensively colonised biomaterials in the *in vivo* study. *Ps.aeruginosa* infection tends to cause depletion of B-defensin 2 production in tissues, and therefore may lower the body's non-specific resistance. Cytokine expression is characterised by pronounced tissue around biomaterials, which are contaminated with *Ps.aeruginosa.* After 3 months of exposure in the rabbit tissue, were observed decreased expression of inflammation indicators.

Acknowledgements

This work has been partly supported by the European Social Fund within the project "Multidisciplinary Research in Biomaterials Technology of New Scientist Group", No.2009/0199/1DP/1.1.1.2.0/09/APIA/VIAA/090, (PVS ID 1380).

Dissemination of Lyme disease agents in Europe by migratory birds

R. Ranka[1], V. Capligina[1], O. Keiss[2], K. Brangulis[1], K. Vilks[1], V. Baumanis[1,3]

[1] Latvian Biomedical Research and Study Centre, Ratsupites Street 1, Riga, Latvia, LV-1067
[2] Institute of Biology, University of Latvia, Miera Street 3, Salaspils, Latvia, LV-2169
[3] University of Latvia, Kronvalda Blvd. 4, Riga, Latvia LV-1586
E-mail: renate_r@biomed.lu.lv

P 06

Keywords: Lyme disease, *Borrelia burgdorferi*, birds.

Lyme disease (Lyme borreliosis, LB) is the most prevalent vector-borne disease in North America and in countries with moderate climates in Eurasia. The disease is caused by spirochetes of the *Borrelia burgdorferi* sensu lato complex that are transmitted by hard ticks of the *Ixodes* family. Human Lyme disease generally occurs in stages with different clinical manifestations - most probably because of different bacteria species. LB spirochetes transit between their arthropod and vertebrate hosts during the enzootic cycle, with rodents and migratory birds as the principal reservoirs. In Europe, the range of LB spirochetes continues to expand as lands become reforested and due to the present climate modification with subsequent changes in *Ixodes* tick biology. The goal of this study was to evaluate the possible role of migratory birds for the transmitting of different species of *Borrelia burgdorferi* spirochetes by studying of infection rate of detached host-seeking ticks.

Ticks were collected from birds in Pape Nature Park in Latvia that is an important resting place for migratory birds. In total, 93 *Ixodes ricinus* ticks from 41 birds (order Passeriformes, 9 species) were collected, and total DNA was isolated from all tick samples. The infectivity rate was determined by real-time polymerase chain reaction targeted the 23S ribosomal RNA gene. In addition, polymerase chain reaction targeted 16S ribosomal RNA gene with subsequent sequencing was used for borrelia species identification.

Our results shows that *B. burgdorferi* DNA was detected in 18,3% of tick samples, the majority of infected ticks were from *Turdus* sp. birds. Among them, *B. valaisiana* was detected in 41,2% cases, *B. garinii* in 35,3% and mixed infection was detected in 23,5% cases.

In conclusion, migratory birds host epidemiologically important vector ticks and contribute to the geographic distribution of LB agents.

Acknowledgements
The research was supported by grants from the LCS No.09.1286 and by ERDF project No. 2DP/2.1.1.2.0/10/APIA/VIAA/004.

Evaluation of apoptosis in skin-derived mesenchymal cell cultures with RT-qPCR method

E. Berga

Laboratory of Bioanalytical and Biodosimetry Methods, Faculty of Biology, University of Latvia, Ratsupites Street 7/3, Riga, Latvia, LV-1067
E-mail: egijaberga@gmail.com

P 07

Keywords: mesenchymal cell cultures, apoptosis, gene expression, RT-qPCR

Development of cell cultivation in recent decades has provided an opportunity to establish significant research of mechanisms of apoptosis to gain a deeper comprehension of this complex process in body level, which have not yet been explored distinctly. The aim of the work was to study the effects of different apoptosis-inducing factors on human skin-derived mesenchymal cell cultures, using morphology and gene expression analysis.

There were several attempts to induce apoptosis in four cell cultures using copper (II) ions, dymethylsuphoxide, serum starvation, confluency and UV irradiation. After morphologic evaluation, RNA was isolated (extracted) for use in single-strand complementary DNA (cDNA) synthesis. Using the obtained cDNA and primers for apoptotic genes *bcl-2, bax, bar,* and *nf-κB,* RT-qPCR was done and expression data was interpreted by Livak's relative quantification method.

Morphologically in most cases were observed the formation of apoptotic bodies. However RT-qPCR data don't provide with unambiguous conclusions but they allow estimate the tendencies of apoptosis in cell cultures and they can be used as preliminary data for modulation of further studies.

Acknowledgements

This work was supported by The European Social Fund (ESF) project "Capacity building for interdisciplinary biosafety research" (Contacts Nr.2009/0224/1DP/1.1.1.2.0/09/APIA/VIAA/055). Author would like to thank Mag. biol. Janis Kungs for his help and support and Dr. biol. Janis Ancans for the opportunity to work out this study in his managed laboratory.

Ex vivo cytokine production in peripheral blood mononuclear cells after their stimulation with dsRNA

R.Veinalde[1], R.Petrovska[1], R.Brūvere[1], G.Feldmane[2], and D.Pjanova[1]

[1] Latvian Biomedical Research and Study Centre, Ratsupites street 1, Riga, Latvia, LV-1067
[2] Larifan Ltd., Kurbada street 2b, Riga, Latvia, LV-1009
E-mail: ruuta@biomed.lu.lv

P 08

Keywords: dsRNA, Larifan, immunomodulation, cytokines, Luminex xMAP technology

Double-stranded RNA (dsRNA) is a pathogen associated molecular pattern that induces activation of the host innate immune system and enhances communication between innate and adaptive immune system cells, which is essential to establish antitumoural response. This communication is ensured by cytokines and chemokines that are small signalling molecules – regulators of the immune response.

The aim of this study was to detect the spectrum of cytokines and chemokines in peripheral blood mononuclear cells (PBMCs) of healthy donors secreted after *ex vivo* stimulation with Larifan (dsRNA).

Material and methods. Blood samples from ten healthy donors were collected and isolated PBMCs were kept in culture. Supernatant from cultured cells was collected 6, 24, 48, 72, and 120h after cell stimulation with Larifan (200, 300, 400μg/ml). Collected supernatants were mixed together creating a pool from 10 persons. Concentration of 29 different cytokines was detected by Luminex® 200™ System using three Milliplex MAP Multiplex Assay Kits.

Results. Larifan concentration 200μg/ml appeared as having the maximal effect. Ten of the analyzed cytokines and chemokines showed relevant increase in the concentration. Five of them (IL-6, TNF-α, IL-10, IL-23, IL-1β) showed rapid increase followed by decrease in concentration. Five other (MIP-1β, I-309, IFN-γ, TARC, GM-CSF) showed late and gradual response to dsRNA. One cytokine IL-16 was considerably suppressed.

Conclusions. Larifan *ex vivo* in cultured PBMCs mainly induces production of pro-inflammatory cytokines and chemokines and suppresses the production of anti-inflammatory cytokine IL-16. The data can be used for modelling the Larifan effect on the immune cell communication network.

Acknowledgements
We thank Scientific Laboratory of the Pauls Stradins Clinical University Hospital Endocrinology Centre for the opportunity to use Luminex® 200™ System. This work was supported by the ERDF project No. 2010/0233/2DP/2.1.1.1.0/10/APIA/VIAA/076.

Spreading of *Streptococcus agalactiae* in Latvia among women of childbearing age

A. Berzina[1], V. Nikolajeva[2], A. Balode[1]

[1] National Medicine Service – Laboratory, Bikernieku str. 25a, Riga, Latvia LV-1039
[2] Microbial Strain Collection of Latvia, Faculty of Biology, University of Latvia, Kronvalda Blvd. 4, Riga, Latvia LV-1586
E-mail: agnese.berzina@hotmail.lv

P 09

Keywords: *Streptococcus agalactiae,* serotypes, resistance to antibiotics

Objectives. The demographical situation in Latvia is unsatisfactory and mortality rate between newborns is higher in Latvia than average level in the EU. Approximately 7.75 children per 1000 live births died in Latvia during 2009. In 2010 this rate slightly reduced and reached 5.72. According WHO European Health data average infant mortality rate in EU was 4.23 in 2009 and accordingly 4.18 per 1000 live births in 2010. There were little studies about spread of *S.agalactiae* among the women in Latvia till now.

Methods. The prevalence of *S.agalactiae* among the women of childbearing age, serotypes and antimicrobial susceptibility in 2011 was investigated and analyzed in National Medicine service laboratory. Vaginal swab and urine samples were collected from women of indeterminate gravidity between ages 16-40 apart from their health information. The obtained material was investigated for the presence of *S.agalactiae* using laboratory approved microbiological detection and identification methods. Antimicrobial susceptibility testing was carried out by the disc diffusion method according to the actual CLSI standard. Serotyping of GBS (group B streptococcus) isolates was performed with B group streptococcus ESSUM AB analysis GBS kit (Umea, Sweden).

Results. *S.agalactiae* was detected in 5.7% of urine (n = 1197) and 9.4% of vaginal swab samples (n = 1368) in 2011. The occurrence of *S.agalactiae* serotypes in 104 tested isolates was as following: III serotype - 34, Ia - 19, V - 16, IV – 15, II - 13 and Ib - 3. From 86 tested *S.agalactiae* strains all were susceptible against penicillin, ceftriaxone, vancomycin and linezolid but 17% were resistant against erythromycin and 16% against clindamycin.

Conclusion. *S. agalactiae* is common agent found in women's urine and vaginal swab samples. The most widespread serotypes of *S.agalactiae* are III and Ia serotypes.

Acknowledgements
The study was supported by the European Social Fund und Latvian University Students Council.

Development of microbial populations in packaged rye bread

E. Kozlinskis, T. Rakcejeva, L. Skudra, L. Dukalska, S. Muizniece-Brasava, A. Silvjane

Faculty of Food Technology, Latvia University of Agriculture, Liela iela 2, Jelgava, Latvia LV-3001
E-mail: emils.kozlinskis@gmail.com

P 10

Keywords: rye bread, microbiological safety, packaging

Microbiological parameters are essential due to the development of rye bread manufacturing and variety of preservation. The shelf life of most baked products is limited unless products are refrigerated or packed. Most bakery products are marketed fresh and are stored at ambient temperature. Unpreserved bread has a shelf of 3-4 days, at which point a visible mould becomes evident.

The aim of research was to analyze development of microbial populations and changes in pH, organic acids and ethanol of rye bread packed in different materials – traditional packaging using PP pouches in air ambience (control), Multibarrier 60 (60% CO_2, 40% N_2) pouches and thermo shrink film pouches with or without oxygen absorber.

Packaged rye bread samples were stored at +4±1 °C and +20±1 °C. Samples for microbiological analysis were prepared by standard dilution method, TPC (total plate count), yeast and mould plate count, and lactic acid bacteria count as well as organic acid content and ethanol content analysed using HPLC and pH was determined in conformity with standard methods. Dominating microflora of rye bread was identified using API biochemical test systems – API 50 for lactic acid bacteria, ID 32 C – for yeasts.

Results of present research reveals identified lactic acid bacteria species in rye bread: *Lactobacillus paracasei subsp paracasei 1*, *Lactobacillus paracasei subsp. paracasei 2*, and yeast *Saccharomyces cerevisiae* as well wild yeasts: *Candida pelliculosa, Candida famata.* Development of microbial populations was inhibited by thermo shrink flm pouch with oxygen absorber. This packaging provides the quality of rye bread during storage at + 20 ± 0.5 °C and + 4 ± 0.5 °C for eight weeks.

Acknowledgements

The research and publication has been prepared within the framework of the ESF Project “Formation of the Research Group in Food Science”, Contract No. 2009/0232/1DP/ 1.1.1.2.0/09/APIA/VIAA/122.

Fermented milk product “Labdaris”

V. Ramniece[1], G. Pupelis[2], G. Pirvits[3]

[1] Riga Stradins University A. Kirhensteins Microbiology and Virology Institute, Ratsupite str. 5, Riga, Latvia LV-1067
[2]Riga Eastern Clinical University Hospital “Gailezers”, Hipokrata str. 2, Riga, Latvia, LV-1038
[3]PLPKS “Dundaga”, E. Dinsberga str. 1, Dundaga, district Dundaga, Latvia, LV-3270
E-mail: vija.ramniece@inbox.lv

P 11

Keywords: *Lactobacillus helveticus* R-7, skimmed milk, health

“Labdaris” idea is based on many years scientific and practical work experience. The novelty of the product associated with lactic acid bacteria *Lactobacillus helveticus* R-7 and its fermentation techniques. It normalizes intestinal microflora, prevents constipation and diarrhoea, promote resurgence of intestinal activity after surgeries, help to recover from bacterial and viral infections, enhance you feeling and quality of living.

“Labdaris” is prepared from skimmed milk. Its texture is homogeneous, alike supple cream, without gas bubbles, and a small top layer of whey is allowable. Sweeteners and other food supplements are not added to the product. “Labdaris” may be called as a live because *L. helveticus* bacteria form lactic acid fast. Clinical experiments indicate that “Labdaris” can stabilize ecology of intestines.

Acknowledgements
Investigations were granted by ERAF project Nr. 2010/0238/2DP/2.1.1.1.0/10/APIA/VIAA/125.

Quality of probiotic dietary supplements present on the Polish market

A. Zawistowska[1], T. Zaręba[1], S. Tyski[1,2]

[1] Department of Antibiotics and Microbiology, National Medicines Institute, Warsaw, Poland
[2]Department of Pharmaceutical Microbiology, Medical University of Warsaw, Warsaw, Poland
E-mail: azawistowska@il.waw.pl

P 12

Keywords: probiotics, dietary supplement, *Lactobacillus, Bifidobacterium*

Introduction: Dietary supplements which containing lactic acid bacteria are becoming more and more popular on the Polish market. Effectiveness of probiotics depends on several factors such as: adequate selection of strains, procedure of their preparation and most important - number of live cells. Bacteria belonging to genera *Lactobacillus* and *Bifidobacterium* are the most often used in probiotic preparations.

The aim of the study was to evaluate the count of bacteria present in the different batches of several preparations, stored before distribution in different temperatures recommended by manufacturer.

Materials and methods: Indication of viability of probiotic bacteria was tested on 24 dietary supplements, from 2 or 3 different batches. Preparations were analyzed in order to determine the bacteria count in one dose of the preparation. Selected 13 probiotic products were tested three or four times to illustrate viability of bacteria strains during validity time. Samples were stored in room temperature or in refrigerator, as recommended.

The strains isolated from probiotic products were identify by two methods. The first was biochemical method using commercial tests API 50 CHL to identify strains from *Lactobacillus* genera and API 20 A for *Bifidobacterium*. The second method was MALDI-TOF technique which based on the protein profile of each species of bacteria.

Results: Analyze of viability of probiotic dietary supplements shows that not all of these preparations contain suitable number of lactic acid bacteria as declared on the label. Preparations stored in refrigerator characterized better survival than preparations kept in room temperature. Identity of lactic acid bacteria shows differences between two used methods. Not all results of identification confirm strains declared by manufacturer.

Conclusions: Microbial quality of probiotic dietary supplements should be controlled. Moreover, storage preparations in refrigerator should be recommended.

Fructan-containing exopolysaccharydes-producing cultures of lactic acid bacteria for functional foods

P. Semjonovs, A. Patetko, I. Denina, L. Auzina, D. Upite, R. Treimane, R. Linde, A. Fomina, L. Paegle, M. Ruklisa, I. Vina, A. Danilevichs

Laboratory of Industrial Microbiology and Food Biotechnology, Institute of Microbiology and Biotechnology, University of Latvia. Kronvalda boulv. 4, Riga LV -1586, Latvia
E-mail: bioarturs@gmail.com

P 13

Keywords: exopolysaccharides, fructan, lactic acid bacteria, synbiotic, probiotic, prebiotic, fermented foods

Exopolysaccharides (EPS) are extracellular microbial polysaccharides. EPS produced by lactic acid bacteria (LAB) are of great interest because of their GRAS (generally recognized as safe) status, their rheological properties in food and health-beneficial properties. Bacterial EPS are important for variety of functions including cell protection, adhesion of bacteria to solid surfaces and cell-cell interactions, thus playing an important role in the probiotic activity of LAB and bifidobacteria. Supplementation of food substrates with fructan-containing additives e.g. LAB EPS before the fermentation enhances the development of probiotic microorganisms, thus improving the functional quality of fermented synbiotic products. Use of EPS or EPS-producing LAB cultures can provide stabilizing, viscosifying and water-binding properties, also contributing to the texture, mouth-feel and taste perception of fermented products. Novel strains of fructan-containing EPS synthesizing LAB have been isolated from natural environment. Industrially prospective LAB strains synthesizing EPS are useful for increasing the beneficial properties of traditional fermented foods or novel products. The species of isolated EPS-synthesizing LAB are not only characteristic inhabitants of mammals incl. humans digestive system but also crucial constituents of the microflora of spontaneously fermented foods e.g. cheese, sourdough bread leavens, fermented sausages, milks and vegetables that makes them prospective in further studies for food application due to both reasons – health promoting and technological ones. Prebiotic, immunomodulating, cholesterol lowering and digestive system's health promoting properties of various fructans and LAB EPS have been widely studied.

Acknowledgements
This research was supported by ERAF 2.1.1.1. Contract's Nr 2010/0322/2DP/2.1.1.1.0/10/APIA/VIAA/108

The fermentation of buckwheat based substrates by *Saccharomyces sp.*

J. Lukjanenko, R.Scherbaka, R. Jonina, A.Vigants

Institute of Microbiology and Biotechnology, University of Latvia, Kronvalda Blvd. 4, Riga, Latvia LV-1586
E-mail: avigants.@lu.lv

P 14

Keywords: buckwheat, *Saccharomyces*, ethanol, saccharification, fermentation

The buckwheat seeds and other tissue contain lot of valuable substances for human diet. It is rich in carbohydrates and could be the appropriate substrate for alcohol fermentation. The buckwheat substrates can be used as raw material for the producing of beer like alcoholic beverage. The task of present study was to evaluate the saccharification and fermentation parameters of buckwheat mash by the different *Saccharomyces* strains.

The saccharification of buckwheat mash by the commercial enzyme preparations Termamyl 120 L and San Super 240 L (Novozyme), as well, by barley malt was done and the results were compared. The barley malt content of 10% (w/w) from total mash was sufficient for the complete saccharification of buckwheat starch.

The fermentations were done at various temperatures by three yeast strains: *S.cerevisiae*, *S.carlsbergensis* and *S.bayanus*. The fermentations were performed on the medium containing the different proportions of buckwheat and barley malt. The fermentation characteristics were measured and in the most cases *S.cerevisiae* strain showed the best results by ethanol yield and productivity, excepting the fermentations at the low temperatures where the better ethanol productivity was obtained by *S.bayanus*. The highest ethanol yield (about 87 % from theoretical) was obtained on the substrate containing 50% buckwheat and 50 % barley malt.

The simultaneous saccharification and fermentation of buckwheat mash was evaluated with various San Super 240 L concentrations. It was found that saccharification rate by this enzyme at 30°C was sufficient for co-fermentation process.

An impact of substrate content (buckwheat – barley malt ratio), the fermentation temperature and used yeast strain on the amount of higher alcohols and other by-products were studied.

Acknowledgements

This work was supported by ESF project No.2009/0207/1DP/1.1.1.2.0/09/APIA/VIAA/128 “Establishment of Latvian interdisciplinary interuniversity scientific group of systems biology"

Cultivation of different Lactobacillus species

J.Jermolajevs[1], L. Peshkova[1], Z. Rudevica[2] , G. Vitols[1]

[1] Riga Stradin's University A. Kirchenstein Institute of microbiology and virology Ratsupites 5, Riga, Latvia, LV-1067
[2] Latvian University Biological study and research centre, Ratsupites 1, Riga, Latvia, LV - 1067
E-mail: guntis@animalab.lv

Keywords: Lactobacillus, glycopeptides, immunomodulators, bifido bacteria

Rapid development of technology for production of lactic acid bacteria and its use as a food supplement for prevention of various diseases, created a growing interest in development of effective methods of isolation of natural glycopeptides from lactic acid bacteria. That is a reason why a group of Latvian scientists have developed a unique technology of isolation and purification of natural soluble glycopeptides containing main structural unit disaccharide-dipeptide from lactic acid bacteria's cell wall using natural food grade starting materials only. Different species of lactobacillus as L. reuteri, L. acidophilus, L. helveticus, L. bulgaricus, L. plantarum and Bifidobacterium lactis were cultivated for production of fermented hydrolysates and isolation of glycopeptides and other biology active compounds from them. Glycopeptides have immunomodulator activity which helps to strenghten immune system and rehabilitate organism after X-ray and drug therapy. Lactobacillus were cultivated on the standard media MRS broth, what contain peptone 10 g/L, beef extract 10g/L, yeast extract 5 g/L, dextrose, polysorbate 80, citrate sodium, acetate, Mg, Mn and phosphate. The titre was 10^{10}- 10^{11} CFU/g. The yield of total bacteria was 1- 1,5 g/L calculated for dry powder. During scientific investigations lactobacillus were cultivated on cheaper media, which contains local production skimmed milk powder 30 – 100 g/L and 3 – 5 g/L yeast extract. Lactobacillus which was cultivated on media 100g/L skimmed powder and 5 g/L yeast extract the titre was 10^{9}- 10^{10} CFU/g. The yield was 2 – 3 g/L calculated for dry powder. The yield of Lactobacillus species cultivated on cheaper culture media was higher than the same species cultivated on standard media. The explanation was that it needs more washing cycles of cultivated lactobacillus. Our opinion is that it is hard to get free from skim milk metabolites. Cultivated lactobacillus species on skimmed milk was fermented and hydrolysed by pepsin and bromelain and by that glycopeptides together with milk peptides, which have biology activity were received. Natural glycopeptides were tested for cytotoxicity and cytoprotector activity. The results showed that glycopeptides haven't citotoxicity and pacients can use them up to 3 g per day. Organism takes so many glycopeptides as necessary for the strength of immuno system, because glycopeptides are working on molecular level. Immunomodulators on base of natural glycopeptides from

different species of Lactobacillus and their combination with other biology active compounds as beta - glucans are used for treatment and prevention of different diseases as Hepatitis C, Herpes infection, Tuberculosis, Solid Cancer and other.

Acknowledgements

Glycopeptides isolation was supported by ERAF project Nr.2DP/2.1.1.1.0/10/APIA/VIAA/125.

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Physiological charactistics of yeast, immobilized on hydrogenated hydroxyapatite

D. Borovikova[1], **R. Scherbaka**[1], **A. Katashev**[2], **Yu. Dekhtyar**[2], **A. Patmalnieks**[1], **A. Rapoport**[1]

[1]*Institute of Microbiology and Biotechnology, University of Latvia, Kronvalda Blvd. 4, Riga, Latvia LV-1586*
[2] *Institute of Biomedical Engineering and Nanotechnology, Riga Technical University, Kalku str. 1, Riga, Latvia LV -1658*
E-mail: borovikovadiana@inbox.lv

Keywords: yeast, immobilization, hydroxyapatite

Natural adhesion of yeasts to the different surfaces gives the possibility for development of new approach for yeast immobilization. The immobilization of cells occurs in two steps: 1) incubation of cells with carrier and sedimentation of cells on the surface of carrier - adhesion and 2) stabilization of cells on carrier surface with dehydration to stabilize immobilization. The properties of the surface itself are crucial for the cell adhesion, too. In the previous works, the hydrogenation procedure was used to alter surface properties of the hydroxyapatite carrier. The present work demonstrated that hydrogenation of the hydroxyapatite carriers prior to yeast immobilization stimulates cell adhesion and has an influence on cells metabolic activity – glucose consumption, ethanol production and growth rate.

Acknowledgements
This research was supported by the European Regional Development Fund project Nr.2010/0288/2DP/2.1.1.1.0/10/APIA/VIAA/038 “Wasteless technology for obtaining bioethanol and other valuable products from deciduous wood”

New benzanthrone based fluorescent dyes for determination of the intracellular storage compounds in yeast *S. cerevisiae*

I.Krallish, S. Gonta, L.Savenkova

[1] *Institute of Microbiology and Biotechnology, University of Latvia, Riga, Latvia*

P 17

Trehalose and glycogen have been implicated as potential stress protectants that accumulated in yeasts during various stress conditions. We investigated the level of trehalose and glycogen in response of *Saccharomyces cerevisiae* to ethanol stress. The disaccharide trehalose, which accumulated dramatically in yeast cells during heat shock and stationary phase enhance thermotolerance and reduces the aggregation of denaturated proteins. Presented work report a new role for trehalose in protecting cells against oxygen radicals, which accumulated in yeast cells under ethanol stress. Resistance of the yeast *S. cerevisiae* to ethanol stress was studied under 10% (v/v) ethanol concentration for different time periods incubation in growth medium supplemented with ethanol after yeasts were reached stationary phase of growth.

Ethanol toxicity is associated with its ability to suppress the biosynthesis of macromolecules, denaturating the cytoplasmatic proteins, reduction in the activity of glycolytic enzymes included in the synthetic pathways for synthesis of both storage products – trehalose and glycogen. Role of trehalose in the ethanol stress tolerance is well studied, but there is not much information available about the trehalose and also glycogen, as another important storage product in yeast cells, in ethanol induced oxidative stress. Therefore the aim of this study was to investigate the correlation between oxidative stress and trehalose and glycogen level in ethanol stressed *S. cerevisiae* cells. The level of both storage compounds as well as ROS level in yeast cells was detected using the new benzanthrone based fluorescent dyes.

Acknowledgements

This research was supported by the European Structural Funds, project Nr. 2009/0205/1DP/1.1.1.2.0/09/APIA/VIAA/152

Effects of adenine limitation on morphology and physiology of yeast *S. cerevisiae* cells

J. Kibilds, A. Kokina, J. Liepins

Institute of Microbiology and Biotechnology, University of Latvia, Kronvalda Blvd. 4, Riga, Latvia LV-1586
E-mail: juris.kibilds@gmail.com

Keywords: adenine starvation, glucose consumption, trehalose accumulation, OD measurements, cell cycle arrest, survival rate, cell size

Recently it has been shown that metabolic response of yeast cells under starvation for different nutrients differs. Physiological responses to basic nutrient (C, N, P) limitations are different to limitations of specific purines or amino acids [1]. Underlying these effects could be the inability of cells to arrest cell cycle in phase G1 or phase G2 which results in inefficient use of other nutrients, leading to reduction in survival rate. As of now, there is very little information available regarding physiological responses to adenine limitation that is investigated here.

In this research, we have examined the influence of adenine limitation, that onsets in YPD media, on commonly used yeast *S. cerevisiae* strain W303. OD of the yeast culture, dry weight and cell number per unit of volume, rate of glucose consumption, accumulation of trehalose, survival rate of the population of yeast cells were measured. Also, arresting of cell cycle in different phases and changes in cell morphology were investigated. By comparing these results to data obtained from isogenic strain without adenine auxotrophy it is possible to speculate on possible adenine auxotrophy influence on yeast physiology.

Acknowledgements

Research was supported by the European Social Fund (ESF), project "Establishment of Latvian interdisciplinary interuniversity scientific group of systems biology", project Nr. 2009/0205/1DP/1.1.1.2.0/09/APIA/VIAA/128

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Anhydrobiosis in yeast: FTIR spectroscopy studies of yeast grown in anaerobic conditions

M. Grube, M.Gavare, L. Rozenfelde, A.Rapoport

Institute of Microbiology and Biotechnology, University of Latvia, Kronvald Blvd. 4, Riga, Latvia LV-1586
E-mail: marita.gavare@inbox.lv

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Keywords: yeast, FTIR spectroscopy, dehydration, anhydrobiosis

In spite yeasts belong to those microorganisms which are rather resistant to dehydration/rehydration treatments it was not possible to transfer into the state of anhydrobiosis those cells which were grown in anaerobic conditions. Recently we finally succeeded in the solution of this problem on the basis of our knowledge accumulated during studies of dehydration of aerobic yeasts. To understand the reasons of such extremely high instability of yeast grown in anaerobic conditions we performed Fourier transform infrared (FTIR) spectroscopy study. FT-IR spectroscopy is a non-destructive analytical method more and more often used for analysis of various bio-samples and on the whole in biotechnology. Two main advantages of IR-spectroscopy are - the analysed samples are not subjected to any chemical treatment thus avoiding secondary reactions, and all the compounds present in the sample are measured simultaneously, thereby simplifying and speeding up the analysis. Microbial cells incl. yeasts mainly are composed of carbohydrates, proteins, lipids and nucleic acids. It is known that the macromolecular composition of cells in most cases is influenced by various environmental conditions. In this study FT-IR spectra of yeasts *Saccharomyces cerevisiae* were registered on a mircoplate reader HTS-XT (Bruker, Germany) and data analyzed by OPUS 6.5. We compared native and desiccated yeast which were grown in aerobic (stable after dehydration) and anaerobic (unstable) conditions. Besides that we investigated also the same cultures after their preliminary treatment in polyols solutions which significantly increased the resistance of both yeast cultures to dehydration/rehydration.

Anhydrobiosis in yeasts: is it possible for yeast grown in anaerobic conditions?

L. Rozenfelde, A. Rapoport

Institute of Microbiology and Biotechnology, University of Latvia, Kronvalda Blvd. 4, Riga, Latvia LV-1586
E-mail: lindyr@inbox.lv

Keywords: yeast, anaerobic conditions of growth, dehydration, anhydrobiosis

Many of processes in biotechnology are linked with the use of yeast grown in anaerobic conditions. Nevertheless, at the moment there are no active dry preparations of such yeast produced by yeast industry. The reason is the impossibility to dehydrate successfully (with the maintenance of viability of dry cells) yeast grown in anaerobic conditions. So, till the moment it was not clear at all if such yeast can be transferred into the state of anhydrobiosis. This abstract is the first information on the possibility to reach such state also for anaerobically grown yeast culture. This research was performed on the basis of the knowledge on mechanisms of anhydrobiosis accumulated by our group during studies of dehydration effects upon aerobically grown cultures. It was shown by us earlier that incubation in hyperosmotic solutions can activate intracellular protection responses and alter cells survival after following dehydration. Also the incubation substance may play a key role. The present study was conducted to clarify the effect of hyperosmotic solutions made by addition of sugars and polyols on the viability of yeast *Saccharomyces cerevisiae* grown anaerobically. We have found that incubation in 1M polyol – xylitol and/or glycerol solutions increases the viability of the cells considerably. We observed that treatment with xylitol gives remarkable improvement in viability of yeast cells after both fast and slow rehydration. The treatment with glycerol improves the viability of cells after slow rehydration. As the result, we conclude that incubation in high molarity solution before dehydration can alter the viability of yeast grown in anaerobic conditions and transfer the significant part of cells population into the state of anhydrobiosis. At the same time it is shown that the choice of incubation substance is very important.

Global regulator Fis influences biofilm formation in *Pseudomonas putida*

H. Moor, M. Kivisaar, R. Teras

Department of Genetics, Institute of Molecular and Cell Biology, University of Tartu and Estonian Biocentre, Riia 23, Tartu, Estonia 51010
E-mail: hannamoor@gmail.com

P 21

Keywords: biofilm formation, motility, *Pseudomonas putida*

Pseudomonas putida is a soil bacterium often found in plant rhizosphere. *P. putida* is known to promote plant growth and have an antagonistic effect on pathogens. These activities and their potential use in agriculture have prompted an increasing interest in *P. putida* and other soil bacteria. It has been shown that bacteria must be highly motile for attaching to and colonizing the root. Our group has previously studied colonization of barley roots by *P. putida* and its regulation by Fis [1], an important global regulator. Fis has been shown to affect 5% of *E. coli* genes including motility genes, but very little is known about the function of this protein in *P. putida*.

We show that overexpression of Fis drastically reduces swimming motility. Observing the nonmotile Fis-overexpressing bacteria under microscope shows formation of bacterial aggregates. To see if those are early stages of biofilm formation, we conducted a biofilm formation assay. According to the assay Fis overexpression induces biofilm formation. It is possible that the elevated expression of Fis is important in the adaptation of *P. putida* during colonization of plant roots when the migration of bacteria is no longer favoured. Forming biofilm on the root must also be important for protecting the plant againts pathogens.

Acknowledgements
This work was supported by Targeted Financing Project TLOMR0031.

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Fis affects production of extracellular polysaccharides in *Pseudomonas putida*

A. Lahesaare[1], **J. Jakovleva**[1], **J. L. Ramos**[2], **M. Kivisaar**[1] **and R. Teras**[1]

[1] *Department of Genetics, Institute of Molecular and Cell Biology, Tartu University and Estonian Biocentre, Riia 23, Tartu, Estonia*
[2] *Department of Molecular and Cellular Biology of Plants, Granada, Spain*
E-mail: andrio@ut.ee

Keywords: *Pseudomonas putida*, EPS (extracellular polymeric substances), Fis (factor for inversion stimulation), biofilm

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Microorganisms are producing extracellular polymeric substances (EPS) which are a complex mixture of biopolymers primarily consisting of polysaccharides. EPSs are abundant extracellular products secreted to the surrounding media and accumulated on the cell surface. The main functions of EPS include the mediation of the initial attachment of cells to different substrates and protection against environmental stress and dehydration. EPSs form the structure and architecture of the biofilm matrix. However, in free-living bacteria the synthesis of EPSs is repressed. For switching from planktic life form to sessile, it is important to repress genes involved in motility and induce EPS synthesis for stronger fixation to surface. Indeed, the precise control of expression of genes involved in switching between one life form to another is regulated by many factors including the global transcription factors. One of the global regulators, Fis (factor for inversion stimulation), is well studied in *Escherichia coli*, but the role of this protein in pseudomonads has only been examined briefly.

In this study we show that artificial overexpression of Fis in *Pseudomonas putida* induces approximately 1.7 times the production of exopolysaccharides. Moreover, we predicted *in silico* binding sites for Fis upstream of the genes of that encode for the production of polysaccharides, e.g. *alg44* and genes responsible for the pili synthesis, e.g. PP1890. The effect of overexpression of Fis on the level of transcription of these genes appeared in microarray studies obtained in collaboration with prof Juan Luis Ramos from Granada, Spain. Also, using ß-galactosidase activity assay we observed the decreasing effect of an overexpression of Fis onto the transcription of PP1890 (2-fold change) and increasing effect on *alg44* (1.8-fold change), compared to wild type strain. Furthermore, using DNaseI footprint assay, we showed the binding of Fis to the upstream region of the PP_1890 gene *in vitro*. The results, obtained in our studies, indicate that Fis is involved in the control of production of EPSs in *P. putida* and thereby in biofilm formation and motility of bacteria.

Acknowledgements
This work was supported by funding from the Targeted Financing Project TLOMR0031.

The competitiveness of *Pseudomonas putida* on barley roots is regulated by Fis

J. Jakovleva[1], A. Teppo[1], A. Velts[2], S. Saumaa[1], H. Moor[1], M. Kivisaar[1], R. Teras[1]

[1]*Department of Genetics, Institute of Molecular and Cell Biology, Tartu University and Estonian Biocentre, Tartu, Estonia*
[2]*Competence Centre for Cancer Research, Tallinn, Estonia*
E-mail: annikate@ut.ee

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Keywords: *Pseudomonas putida*, Fis, rhizosphere, competitiveness

Pseudomonas putida has been considered to be an important rhizosphere microorganism being able to promote plant growth and efficiently protect plants against pathogens. Global transcription factors can be an important link between signals from the environment and the physiological state of bacteria. Fis (*factor for inversion stimulation*) is a global regulator, that is well studied in *Escherichia coli*, regulating genes from many different categories, but very little is known about the role of this protein in pseudomonads.

In pseudomonads, flagellar movement is an important trait for the colonization of plant roots. According to studies in *Enterobacteriaceae*, Fis regulates positively the flagellar movement of bacteria. Therefore, we were interested in the role of the Fis protein in *Pseudomonas putida*, especially the possible regulation of the colonization of plant roots.

According to our results overexpression of Fis significantly decreases swimming motility of *P. putida*. In addition, we found that the overexpression of Fis caused aggregation of cells. Since both the motility and aggregation of cells are known to affect biofilm development, we examined the effect of Fis overexpression on biofilm formation. We found that Fis overexpression facilitates biofilm formation of *Pseudomonas putida*. To study whether Fis could affect the colonization of plant roots, we chose barley (*Hordeum vulgare*) for experiments. Barley is an agriculturally important cereal plant in Northern Europe, where the cold climate makes it difficult to grow other cereal plants such as maize and wheat. Our results indicate that Fis affects the competitiveness of bacteria on plant roots by regulating swimming motility and biofilm formation. It is possible that the elevated expression of Fis is important for the adaptation of *P. putida* during the colonization of plant roots by encouraging the formation of biofilm when the migration of bacteria is no longer favoured [1].

Acknowledgements

This work was supported by the Howard Hughes Medical Institute International Research Scholar Program (grant 55005614) and by funding from the Targeted Financing Project TLOMR0031.

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Interplay between specialized DNA polymerases in *Pseudomonas putida*

T. Jatsenko, K. Tavita, M. Kivisaar

Department of Genetics, University of Tartu, Riia mnt. 23, Tartu, Estonia, 51010
E-mail: tanjajat@gmail.com

Keywords: TLS, specialized DNA polymerases, *Pseudomonas putida,* DNA damage tolerance

Genomic DNA is constantly damaged by endogenous metabolic compounds or by exogenous DNA damaging chemicals. Resulting DNA lesions block the replication process that may lead to cell death. To deal with it, nature has favoured versatile damage tolerance systems that allow completing the replication in the presence of DNA damage.

Specialized DNA polymerases play a central role in DNA damage tolerance by catalyzing proficient DNA synthesis past replication-blocking DNA lesions (process known as translesion synthesis TLS). *Pseudomonas putida* possesses four specialized DNA polymerases: Pol II, Pol IV and two damage inducible DNA polymerases encoded by "mutagenic operon" *imuAimuBdnaE2*: ImuB which is related to Y-family DNA polymerases and DnaE2, a homologue of the replicative Pol III catalytic subunit α. We have constructed a set of *P. putida* strains deficient in multiple TLS polymerases Pol II, Pol IV, ImuB and DnaE2, and studied their involvement in spontaneous and DNA damage-induced mutagenesis. The frequency of spontaneous mutation rates elevated about fivefold in triple and quadruple mutant background, which indicated the involvement of TLS DNA polymerases in the error free bypass of endogenous DNA lesions.

According to our studies on DNA damage tolerance caused by various mutagens, ImuB contributes to the tolerance of cytotoxic alkylating DNA lesions induced by methylmethane-sulfonate (MMS). Pol IV might be involved in TLS past oxidative DNA damage induced by paraquat and in TLS or repair of various DNA lesions caused by DNA crosslinking agent mitomycin C. However, the individual contribution of these TLS DNA polymerases to damage tolerance was seen only in the absence of Pol II and DnaE2, which suggests that the activity of ImuB, Pol IV and even replicative DNA polymerases in wild-type cells might be influenced by the presence of DnaE2 and Pol II polymerases. Our results imply the possibility that access of particular TLS polymerase to undamaged DNA or specific DNA lesion is highly regulated by the activity of other DNA polymerases probably by competition for binding to the β-clamp. Therefore, the mutation frequency might be highly dependent on the constant interplay between all DNA polymerases in *P. putida* cells.

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Homologous recombination in starving *Pseudomonas putida*: Impact of a local genomic structure and the presence of phenol

K. Tavita[1], K. Mikkel[1], M. Tark-Dame[1#], H. Jerabek[2, 3], R. Teras[1], J. Sidorenko[1], R. Tegova[1*], A. Tover[1*], R. T. Dame[4], M. Kivisaar[1]

[1]Department of Genetics, Institute of Molecular and Cell Biology, Tartu University and Estonian Biocentre, 23 Riia Street, 51010 Tartu, Estonia
[2]Institute for Theoretical Physics, Philosophenweg 19, D-69120 Heidelberg, Germany
[3]Interdisciplinary Center for Scientific Computing (IWR), Im Neuenheimer Feld 368, 69120 Heidelberg, Germany
[4]Leiden Institute of Chemistry, Gorlaeus Laboratories, Laboratory of Molecular Genetics and Cell Observatory, Leiden University, Leiden, The Netherlands
[#]Present address: Swammerdam Institute for Life Sciences, University of Amsterdam, P.O. Box 94215, 1090 GE Amsterdam, The Netherlands.
[*]Present address: Icosagen Cell Factory OÜ, Nooruse 9, 50411 Tartu, Estonia.
E-mail: sida@ut.ee

Keywords: Homologous recombination, stationary-phase bacteria, *Pseudomonas putida*, reactive oxygen species, phenol stress, nucleoid-associated proteins

Genetic recombination between homologous DNA sequences is a general strategy used by cells for repairing and tolerating DNA damage. Additionally, homologous recombination (HR) is involved in horizontal gene transfer between the same or related species which is considered as the primary reason for bacterial antibiotic resistance development and evolution of new catabolic pathways. In natural environment of bacteria accumulation of DNA damage induced by various stress factors and chemicals contributes to increased rates of mutations and homologous recombination (HR) to afford adaptive evolution of bacterial populations.

We have constructed an assay which enables to investigate HR in carbon-starved populations of soil bacterium *Pseudomonas putida* in the presence of an aromatic compound phenol. In this assay HR between the plasmidial and the chromosomal non-functional alleles of phenol degrading genes produces a functional allele and allows the growth of bacteria on phenol as a sole carbon source. Our results show that prolonged starvation of *P. putida* in the presence of phenol stimulates HR. The emergence of recombinants is facilitated by reactive oxygen species and suppressed by DNA mismatch repair (MMR) enzymes. The chromosomal location of the HR target also influences the frequency and dynamics of HR events. A bioinformatical screen of binding sites of nucleoid-associated proteins (NAPs) revealed that chromosomal DNA regions which flank the test system in bacteria exhibiting a lower HR frequency are enriched in binding sites for a subset of NAPs compared to those which express a higher frequency of HR. This implies that the binding of these proteins imposes differences in local structural organization of the genome that

could affect the accessibility of the chromosomal DNA to HR processes and thereby the frequency of HR.

Acknowledgements

This work was supported by grant 7376 from the Estonian Science Foundation, by funding of Targeted Financing Project SF0180031s08 from Estonian Ministry of Research and Education, and by grant #55005614 from the Howard Hughes Medical Institute International Research Scholars Program to MK.

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Antimicrobial activity of natural substances against oral pathogen microorganisms

L. Ratkevica[1], G. Krumina[2], D. Tamoshiuniene[3], A. Brinkmane[4], D. Babarykin[2]

[1] *Faculty of Biology, Latvia University of Latvia, Kronvalda Blvd. 4, Riga, Latvia LV-1586*
[2]*Institute of Innovative Biomedical Technology, Inchukalna Street 2, Riga, Latvia LV-1014*
[3]*Rūta Ltd., Siauliai, Lithuania*
[4]*Riga Stradins University, Dzirciema Street 16, Riga, Latvia LV-1007*
E-mail: linda.ratkevica@gmail.com

Keywords: oral pathogens, *Candida albicans*, *Streptococcus mutans*, plant extracts, antimicrobial activity

Wide variety of microorganisms forms human oral microflora. Changes in quantity and composition of microorganisms in the specific circumstances have not only local outcomes, but also can affect all organism. Nutrients, humidity and lack of oxygen can contribute to the specific microorganisms and it may have negative impact on healthy balance of oral microflora. The aim of the study was to investigate the effect of the plant extracts on oral pathogen microorganisms *Candida albicans* and *Streptococcus mutans* in vitro.

Water and ethanol extracts of 14 plants (*Acoraceae, Asteraceae, Lamiaceae, Myrtaceae*) with described biological activities and their combinations were analyzed by agar diffusion and minimum inhibitory concentration method on clinically isolated *C.albicans* and *S.mutans* strains.

Plant water extracts had no significant antifungal activity except cinnamon (12.7 mm) and clove (20.7 mm). Ethanol/water extracts showed different antifungal activity - strongest were clove (38.0 mm), cinnamon (37.7 mm), propolis (35 mm) and lavender (23.5 mm) extracts.

Antibacterial activity was shown only by ethanol/water extracts of natural substances. The most active was clove (29.0 mm), propolis (25.6 mm), cinnamon (23.6 mm) and lavender (21.0 mm) extracts. In addition antimicrobial activity enhancing synergy was found in their combinations with extracts which has lower or no effect.

MIC was determined for the extracts that showed highest activity detected by agar diffusion method. Although extracts concentration in medium was reduced to 0.3%, in this concentration *C.albicans* and *S.mutans* CFU/ml decreased by more than 80%, therefore MIC could not be defined, except for propolis and cinnamon (1.7%). Changing pH and other parameters with some additives also had impact on extracts activity.

The results of the study show good perspective to develop a variety of functional food for caries prevention as well as other oral health problems.

Exogenous isolation and molecular characterization of several β-lactamases encoding plasmids

M. Adamczuk, Ł. Dziewit, M. Nieckarz, A. Lis, J. Baj, D. Bartosik

Department of Bacterial Genetics, Institute of Microbiology, Faculty of Biology, University of Warsaw, Warsaw, Poland
E-mail: adamczuk@biol.uw.edu.pl

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Keywords: plasmid, antibiotic resistance, β-lactamase

Plasmids are extrachromosomal replicons perceived as natural vectors of the horizontal gene transfer. Although they are not essential for bacteria survival, their presence may provide many specific features which increase fitness of their hosts. Many of them: (i) enable bacteria to use hardly degradable compounds as a source of nutrients (catabolic plasmids), (ii) increase virulence and infectiousness of their hosts (virulence plasmids) and (iii) confer antibiotic resistance (resistance plasmids). In this study we have identified (by exogenous isolation) several plasmids encoding β-lactamases. The source of plasmids were bacteria living in activated sludge of the main Warsaw wastewater treatment plant (Czajka). For selection of the plasmid containing *Escherichia coli* transformants, various antibiotics were used, including penicillins, cephalosporins and carbapenems. Based on results of the RFLP analyses and resistance profiles, a pool of plasmids has been identified and subjected to DNA pyrosequencing. The bioinformatic analyses of the obtained nucleotide sequences revealed genetic structure of the replicons and enabled defining their resistance determinants. Functional analyses allowed to distinguish mobilizable and conjugative plasmids as well as to determine the resistance breakpoints. Small replicons were predominant among the identified plasmids. Apart from β-lactamases some of them encoded resistance to aminoglycosides, tetracyclines and trimethoprim. These determinants were parts of transposable elements (e.g. transposable module, TMo, generated by insertion sequence IS*Ecp*1) or class 3 integrons. Such variety implicate that bacteria residing beyond nosocomial environment constitute a rich reservoir of antibiotic resistance genes.

A suite of bacterial assays for profiling of toxicity mechanisms of engineered nanoparticles

O. Bondarenko, A. Ivask, A. Kahru

Laboratory of Molecular Genetics, National Institute of Chemical Physics and Biophysics, Akadeemia tee 23, Tallinn 12618, Estonia
E-mail: olesja.bondarenko@kbfi.ee

P 28

Keywords: synthetic nanoparticles, bioluminescent bacterial biosensors, oxidative stress

Engineered nanoparticles (eNPs) are tiny particles with the size between 1-100 nm, which defines their unique physicochemical properties. eNPs are increasingly produced and are already used in thousands of industrial and commercial products, including clothes, filters, food additives, cosmetics, sporting goods and many others [1]. The worldwide production of NPs and their release to the environment requires more efficient, rapid and cost-effective strategies for testing their potential adverse effects. The common property of all eNPs and is the ability to induce the production of reactive oxygen species (ROS), which results in deleterious state called oxidative stress. In the case of metal-containing eNPs – the main focus of our research – the oxidative stress may be induced by the surface of eNPs or by their dissolution (release of metal ions). The dissolution-related mechanism of toxicity can be easily described, whereas surface-specific toxic effects of eNPs may be unpredictable.

Since 2006, the research of our lab has been focused on developing of bio-analytical approaches for the profiling of (eco)toxicological properties of eNPs combining traditional (eco)toxicological tests, genetically engineered bacteria and analytical techniques. For that, we developed a suite of *in vitro* tests consisting of (i) non-mutated Gram-positive and Gram-negative bacteria, including pathogens such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*, (ii) bioluminescent *Escherichia coli* strains with step-wise decreased capacity to resist ROS and decreasing their bioluminescence in presence of oxidative stress-inducing chemicals, (iii) various recombinant bacterial biosensors that sense the sub-toxic levels of intracellular superoxide radicals, hydrogen peroxide, DNA damage or Cu/Ag/Zn/Cd ions by increasing their bioluminescence. This suite of bacteria was used for the high-throughput *in vitro* toxicological profiling of various metal-containing eNPs and the results will be presented.

Acknowledgements
The support from SF0690063s08, ETF8561, European Social Fund and FP7 project NanoValid (contract No 263147) is acknowledged.

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Microbial Strain Collection of Latvia (MSCL) for life sciences in Latvia

D. Eze, V. Nikolajeva, Z. Petrina

Microbial Strain Collection of Latvia, Faculty of Biology, University of Latvia, Kronvalda Blvd. 4, Riga, Latvia LV-1586

E-mail: daina.eze@lu.lv

Keywords: collection, strains, deposition, cooperation

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MSCL was founded in 1993, it is a member of World Federation for culture collections (WFCC) and of European culture collections' organization (ECCO). Status of International Depositary Authority (IDA) since 1997.

MSCL currently holds more than 1300 strains of bacteria, filamentous fungi and yeasts. The great part of them has been isolated in Latvia.

Safe and sure maintenance of microbial cultures in MSCL has enabled good cooperation with research institutions and private companies. MSCL is involved in several joint projects funded both by European Regional Development Fund (ERDF) and by National research programme.

In cooperation with the Latvian Institute of Organic Synthesis the synergistic action of new designed antimicrobial agents with penicillins against ß-lactamase producing strains was determined at MSCL.

The developed biological method against *H. annosum,* which causes root rot of conifers elaborated in cooperation with the Latvian State forest research institute "Silava" seems to be a good alternative to chemical treatment.

Several associations and strains of bacteria for efficient bio-hydrogen production have been tested and some wild type hydrogen producers from nature have been isolated in the frame of collaborative project with Institute of Solid State Physics University of Latvia.

The possibility to develop new anti-aging and skin regeneration promoting products derived from plants, fungi and therapeutic mud deposits found in Latvia has attracted attention of biotechnologists and local manufacturers. Isolation, identification and characterization of biologically active substances from flamentous fungi and identification of therapeutic mud's natural microorganisms also are performed at MSCL.

MSCL maintains the greatest part of microbial cultures included in the composition of biological products of Ltd "Bioefekts". Upon request, viability tests of commercially important strains are carried out at MSCL.

Inspection tools for biocorrosion

D. Kalnina[1], D. Jakovlevs[1], G. Krieke[1], V. Nikolajeva[2], G. Tupe[3], O. Valcina[3]

[1] Institute of General Chemical Engineering, Riga Technical University, Kalku 1, Riga, Latvia LV-1658
[2] Microbial Strain Collection of Latvia, University of Latvia, Kronvalda Blvd. 4, Riga, Latvia, LV-1586
[3] Institute of Food Safety, Animal Health and Environment, Lejupes Street-3, Riga, Latvia, LV-1076
E-mail: daina.kalnina@rtu.lv

Keywords: biocorrosion, microbiological analysis, chemical analysis, corrosion management, decision making

Biocorrosion or microbially influenced corrosion is a large and complex issue that is critical for different industries and for infrastructure: in storage tanks, buildings, bridges, process equipment and piping (water, gas and oil). Microorganisms in the corrosion process were ignored in the past, but is now acknowledged and take the focus of present and future research activities.

The objective was to study the biocorrosion at the bitumen - metal interface. The interface is variable leading to different microbiological and chemical events. Corrosion process can be influenced by microbial biofilms. The biocorrosion is governed by different acid-producing and sulphate-reducing bacteria as well as hydrocarbon utilizers, breaking down bitumen coatings.

Endospore producing and sulphite-reducing bacterium *Clostridium perfringens* was detected and identified in investigated corrosion products. The number of colony-forming units of aerobic and facultatively anaerobic bacteria was estimated.

Scanning electron microscopy revealed closely adherent corrosion products that were characterized by X-ray diffraction as mainly $FeCO_3$, Fe_2O_3, $Fe(OH)_2$ with minor phases of FeO(OH) and Fe_3O_4.

Siderite ($FeCO_3$) exhibitet the highest crystallinity but other phases appeared less crystalline (amorphous). By using several solvents it was possible to extract chemically different components from the bitumen that could then analyzed. Bitumens biocorrosion was characterized using FTIR after extraction and cleanup procedures using solvents with different polarity. The use of bitumen isolation in the past with the unfavourable geophysical conditions has a high risk for biocorrosion and could be a high risk in the deterioration of pipe lines studied.

This study has shown the importance of analysing the microbial degradation of both the steel and the bitumen using a collection of analytical tools for a better understanding on the entire system.

Acknowledgements
The authors gratefully acknowledge Mg.sc.ing. E.Dauksta (Institute of Technical Physics) for measurement of FTIR spectra.

Detection of methanogenic bacteria using fluorescence *in situ* hybridization

S. Valucka

Department of Microbiology and Biotechnology, Faculty of Biology, University of Latvia, Kronvalda Blvd. 4, Riga, Latvia LV-1586
E-mail: sv08073@lu.lv

Keywords: FISH, fluorescence *in situ* hybridization, methanogens, auto-fluorescence

Fluorescence *in situ* hybridization (FISH) is a method used to detect microorganisms with specific nucleic acid sequence that is complementary to the sequence of the fluorescently labeled probe. This method was used to assess its expediency to detect presence of methanogenic bacteria in the sample with the probe ARC915.

In the experimental part representatives of two genera were used: acetotrophic methanogens *Methanosaeta harundinacea* DSM 17206 (bacteria contain smaller amount of the coenzyme F_{420} that is necessary for the auto-fluorescence than bacteria of other genera) and hydrogenotrophic methanogens *Methanobacterium bryantii* DSM 863. Sample containing various bacteria species from bioreactor also was used. Looking at the samples of both genera under ultraviolet illumination separately, clearly visible auto-fluorescence was observed only in the sample containing bacteria of *Methanobacterium*. After applying FISH technique to bacteria, clearly observable fluorescence under ultraviolet illumination was observed in both cases. Fluorescence was observed also in the sample from bioreactor.

During experimental part many modifications were applied to the method to get the highest possible intensity of fluorescence. The most influential factors were concentration of probe, method of fixation on the glass slide and the time of the hybridization. Taking into account that some methanogenic bacteria cannot be detected on the bases of their auto-fluorescence under ultraviolet illumination due to the insufficient amount of the coenzyme F_{420}, FISH is a valid method to detect all methanogens in the sample.

Acknowledgements

This work was supported by the National Research Programm LATENERGI project "Research of hydrogen extraction, storage and energy production methods and elaboration of prototypes". The author thanks Dr. biol. Vizma Nikolajeva for her help and support.

Detection and quantification of antibiotic resistance genes from the bacterial community of the Baltic Sea

K. Tiirik[1], H. Nõlvak[1], J.-K. Preem[1], K. Oopkaup[1], A. Heinaru[2], J. Truu[1]

[1]Institute of Ecology and Earth Sciences, Faculty of Science and Technology, University of Tartu, 46 Vanemuise St, 51014, Tartu, Estonia

2 Institute of Molecular and Cell Biology, Faculty of Science and Technology, University of Tartu, 23 Riia St, 51010, Tartu, Estonia

E-mail: kertu.tiirik@gmail.com

Keywords: antibiotic resistance, Baltic Sea, next-generation sequencing, resistance genes, qPCR

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Antibiotics are one the most successful drugs used for human therapy but they must be considered as important pollutants as well. Residues from human environments may contain antibiotics and antibiotic resistance genes that can contaminate natural environments and the clearest consequence of that is the selection of resistant bacteria. The Baltic Sea is the second largest isolated brackish water reservoir on Earth, serving as a drainage area for people in nine different countries which differ from one another in antibiotic use and sewage treatment politics. The aim of this study was to quantify antibiotic resistance genes (*tetA*, *tetB*, *tetM*, *ermB*, *sul1*, *blaSHV*, *ampC*) from the bacterial community of the Baltic Sea. We used qPCR to quantify resistance genes from four different sample sites of the Baltic Sea over two years and sequencing after 16S rRNA gene to characterize the bacterial communities. The results revealed that all the resistance genes targeted in the study were detectable from the Baltic Sea. Percentage of *tetA, tetB, ermB ja blaSHV* genes in sea bacterial community varied between 0,004–0,05%, 0,0001–0,0005%, 0–0,0005% and 0–0,002%, respectively. The most numerous antibiotic resistance gene was *tetA* gene and it was also with the highest percentage compared to the 16S rRNA genes of the whole microbial community. The most similar microbial communities were obtained in year 2008 from Gulf of Tallinn and Gulf of Finland. The structure of the bacterial community of the Baltic Sea varied over time and in space.

Acknowledgements

This research was financially supported by the Ministry of Education and Science of Estonia (grants SF0180127s08 and SF0180026s08).

Microbial uptake of diesel oil sorbed on modified peat PeatOs

O. Muter[1], K. Potapova[1], D. Porsnovs[2], M.Klavins[2]

[1] Institute of Microbiology and Biotechnology, University of Latvia, 4 Kronvalda Blvd., Riga, Latvia, LV-1010
[2]Department of Environmental Science, University of Latvia, 19 Raina blvd., Riga, Latvia, LV-1586
E-mail: olga.muter@inbox.lv

Keywords: bacteria consortium**,** hydrocarbons, nutrient amendments, peat sorbent.

Peat sorbent is an oil organic adsorbent produced from modified peat. Among the benefits of the use of peat sorbent are the effectivenes on land and water; it is non-toxic, non-leaching, lightweight, non-abrasive and vapour suppressive [1]. Thermally treated peat was shown to be a prospective sorbent for oil removal during spills [2-3]. The further reuse or utilization of the used peat sorbents is one of the important issues related to the efficiency and ecological safety of this approach [4].

The aim of these experiments was to determine the capacity of bacteria consortium MDK.EKO-7 to clean up the spent peat sorbent.

Peat was modified via low temperature pyrolysis. The obtained sorbent has following characteristics: C 64.8 %, H 4.3 %, N 0.4 %, specific surface area 34 m^2/g, oil sorption capacity 7 g oil/g sorbent. The modified peat (Peat based oil sorbent - PeatOS) was further used in biodegradation experiments. 10 g PeatOS was spiked with 200 mg of diesel oil. The process occurred in a slurry model system was carried out during 36 days at 28 °C and was evaluated by microbial enzymatic activity, as well as by a decrease of hydrocarbons concentration in dynamics.

In this study, the tested peat was specifically treated with the aim to enhance the oil sorption capacity. Therefore, activity of microorganisms onto a modified peat was not obvious, for example, due to an increased hydrophobicity of peat.

Degradation of the diesel fuel sorbed onto peat sorbent (PeatOS) (diesel concentration 200 mg/g dw), was shown to be possible in a slurry system (diesel oil : modified peat : nutrient amendments : bacteria consortium). A decrease of hydrocarbons concentration up to 90 % was detected after 36 days incubation [5]. Taking into consideration the effect of the diesel loading rate to biodegradation activity, the testing of higher concentrations of diesel is necessary in future.

Acknowledgements

State Research program Nr. 2010.10-4/VPP-5 NatRes, the 1st Project „Mineral Resources" is a supporter of CBM 2012. This study was financed by the projects Y3-26492 and 26493 of this Program.

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The effect of elevated air humidity on soil bacterial community in silver birch stand growing in an experimental free air humidity manipulation facility

J-K.Preem, J. Truu, M. Truu, K. Rosenvald, Ü. Mander, K. Lõhmus

Institute of Ecology and Earth Sciences, University of Tartu, Vanemuise 46, Tartu, Estonia
E-mail: jpreem@ut.ee

Keywords: elevated air humidity, silver birch, soil microbial community

A free air humidity manipulation facility (FAHM) was established in 2007 to study the effect of increased air humidity on plants (silver birch, hybrid aspen and two types of understory vegetation) performance and functioning with respect to rising air humidity predicted for Northern Europe. To evaluate the effects of increasing air humidity to the bulk and rhizosphere soil bacterial communities of silver birch (*Betula pendula Roth.*) stands, massively parallel sequencing of 16S rDNA V6 variable region was used. Soil samples from 0-10 cm soil layer of control and fumigated plots in two replicates were collected in October 2009 and 2011.

Analysis of molecular variance (AMOVA) showed significant differences in bacterial community structure between control and fumigated plots both in bulk soil and rhizosphere soil. Significant differences also in temporal dynamics of microbial communities of fumigated and control plots were found. Abundance *Acidobacteria* and *Planctomycestes* decreased only in soils of fumigated plots, and this change was more pronounced in rhizosphere soil samples. In comparison with fumigated plots overall temporal changes in bacterial community structure were larger in control plots indicating stabilization effect of fumigation on soil microbial communities during the study period.

Acknowledgements
This study was supported by Target Projects No SF0180127s08 and SF0182732s06 of the Ministry of Education and Research of the Republic of Estonia, Estonian Science Foundation grant 7527, and by the European Regional Development Fund through the Excellence Centre FIBIR (Frontiers in Biodiversity Research) and ENVIRON (Centre of Excellence in Environmental Adaptation).

Effect of soil chemical characteristics and water regime on denitrification gene (*nirS*, *nirK,* and *nosZ*) abundances in created riverine wetland complex

T. Sildvee[1], M. Truu[1], J. Truu[1], H. Nõlvak[1], A. Kaasik[2], W.J. Mitsch[3], Ü. Mander[1]

[1]Department of Geography, Institute of Ecology and Earth Sciences, University of Tartu, Vanemuise 46, 51014 Tartu, Estonia
[2]Department of Zoology, Institute of Ecology and Earth Sciences, University of Tartu, 46 Vanemuise Street, Tartu 51014, Estonia
[3]Wilma H. Schiermeier Olentangy River Wetland Research Park, The Ohio State University, 352 W. Dodridge Street, Columbus, OH 43202, USA
E-mail: teelesildvee@gmail.com

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Keywords: denitrification potential, *nirK*, *nirS*, *nosZ* , soil chemical characteristics, treatment wetland, water regime

Constructed wetlands have been proposed as solution to protect aquatic ecosystems from excessive nitrogen. The effect of site specific characteristics such as soil chemical parameters (pH, C/N, total C and N, lactate soluble P and K, NH_4-N, NO_3-N, Ca, Mg, and organic matter content), water regime, and soil and wetland type on the abundance of denitrifying genes encoding nitrite (*nirS* and *nirK*) and nitrous oxide (*nosZ*) reductase and their proportions in bacterial community was investigated in the river diversion wetland complex in Olentangy River Wetland Research Park, Ohio, USA. All together, 33 soil samples from freshwater marshes, a river diversion oxbow, a riverside, and an upland area were collected from 0-15cm topsoil layer (except for the open areas of kidney shaped wetlands where organic and mineral layers were taken separately) according to longitudinal and transverse gradients. The size of the denitrifiers community was characterized by using quantitative PCR.

The results revealed highest *nosZ/nirS+nirK* ratio in transitional and upland areas referring to the greater N_2O reduction potential in those zones compared to the permanently flooded open areas. The proportion of *nirS* genes in total bacterial community was greatest in open water zones, while *nirK* was highest in upland areas. The directions of the relationships between gene abundances and chemical variables indicated that *nirS* and *nirK* type denitrifiers preferred different environmental conditions and *nirK* denitrifiers possessed *nosZ* gene more often than *nirS* denitrifiers. pH showed a stronger positive effect on *nirS* gene abundances in freshwater marshes ($p<0.01$) compared to the oxbow ($p<0.05$) referring that the effect of pH on *nirS* gene differ in different environments. *nirS* gene abundance was also positively affected by Ca content in open ($p<0.01$) and transitional ($p<0.05$) water regime area soils.

Acknowledgements

This study was supported by Target Project No SF0180127s08 of the Ministry of Education and Research of the Republic of Estonia, Estonian Science Foundation grant 9387, and by the European Regional Development Fund through ENVIRON (Centre of Excellence in Environmental Adaptation).

Soil microbial activity affected by crop rotation

V. Šteinberga[1], O. Mutere[2], L. Dubova[1], I. Alsiņa[1], I. Jansone[3]

[1] Institute of Soil and Plant Sciences, Latvia University of Agriculture, 2 Liela street, Jelgava ,Latvia LV-3001
[2]Institute of Microbiology and Biotechnology, University of Latvia, Kronvalda Blvd. 4, Riga, Latvia LV-1586
[3] State Stende Cereal Breeding Institute, Dizstende, Talsu district, Latvia LV-3258,
E-mail: Vilhelmine.Steinberga@llu.lv

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Keywords: forecrop, soil respiration, urease, dehydrogenase, FDA hydrolysis, bacteria, actinomycetes, fungi

The field experiments were carried out at the experimental cereal breeding fields in Stende, Talsu district, Latvia. Soil had been under short-term organic or conventional crop management. Buckwheat (*Fagopyrum esculentum* Moench.) and wheat (*Triticum aestivum* L.) was grown as the forecrops for barley (*Hordeum vulgare* L.). In the organic management system buckwheat or wheat was ploughed as green manure. In the conventional management system fertilizers (NPK 16:16:16) 500 kg ha^{-1} were used, dressing ones with ammonium nitrate 120 kg ha^{-1}. Herbicide Mustang was used as recommended by producers.

The soil quality was characterised by physico-chemical (pH value, organic matter, N, P, K) and biological properties, i.e. plate count of different physiological groups of microorganisms, soil microbial respiration, as well as enzymatic activity of soil microorganisms, including urease, dehydrogenase and fluoresceine diacetate hydrolysis activity. Soil sampling was done three times during vegetation period.

Results showed that buchweat as forecrop increased number of bacteria at all sampling times, but number of actinomycetes and fungi increased in July. Soil microbial respiration activity was higher in the organic management system in comparison with conventional one. The tendency of increased soil respiration intensity at the end of crop vegetation was observed in the organic management system after wheat cultivation as forecrop. The reverse regularity was observed in conventional management system. Significantly higher activity of urease was observed in the organic management system after wheat cultivation as forecrop. Soil enzymatic activity fluctuated due to climate seasonality. Decrease of dehydrogenase activity was observed at the end of crop vegetation period.

Acknowledgements
Work was supported by Latvian Council of Sciences, project 09.1530.

Applying humus and peat extracts to manage soil microbial activity

L. Dubova[1] , V. Šteinberga[1], I. Alsiņa[1], G. Bremanis[2], S. Maļecka[2] , M. Kožokaru[1], J. Bārtuļs[1]

[1] Institute of Soil and Plant Sciences, Latvia University of Agriculture, 2 Liela street, Jelgava ,Latvia LV-3001
[2] State Stende Cereal Breeding Institute, Dizstende, Talsu district, Latvia LV-3258
E-mail: Laila.Dubova@llu.lv

Keywords: soil respiration, urease, dehydrogenase, FDA hydrolysis, rape, oat

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The experiments was designed to clarify the effects of the commercial products made from peat and vermicompost on soil microbial activity. Both preparations contain humic substances and are manufactured by ZPRF "Intellectual resources" Ltd in Latvia. Vegetation experiments were placed in pots with peat substratum or soil in greenhouses of the Latvia University of Agriculture. Field experiments were carried out in State Stende Cereal Breeding Institute. Oats (*Avena sativa* L.) and spring rape (*Brassica napus* L.) were grown at both locations. In vegetation pot experiments the plants were treated with preparations twice a month. The first treatment was done at sowing. Concentrations of 100, 10, 1 mL L^{-1} in each treatment time were used. Control was without peat or vermicompost preparation. In field experiments plants were treated once a month with preparation concentration 10 mL L^{-1}. Plant growth, biochemical parameters and soil (substratum) biological activity (number of bacteria, actinomycetes and fungi, soil respiration and enzymatic activity) were tested.

Results showed that the effect of preparations on depends on the plant species and the type of used organic preparation. The dose of peat preparations correlate with soil respiration intensity, but large doses of vermicompost preparation might decrease intensity of respiration. The effect depends of plant species and cultivation conditions. The number of bacteria, actinomyces and fungi; soil respiration and enzymatic activity fluctuated due to plant developmental stage. Impact of peat or vermicompost preparations on soil biological activity depends not only of used concentration but is influenced by soil or growth media type too.

Acknowledgements
This work was supported by ERAF research project No 2010/0313/2DP/2.1.1.1.0./10/APIA/VIAA/082

Bacterial community composition, structure and succession in subsurface flow constructed wetland

K. Oopkaup[1], J. Truu[1], M. Truu[1], I. Talpsep[2], T. Sildvee[1], J-K. Preem[1], Ü. Mander[1]

[1] *Institute of Ecology and Earth Sciences, University of Tartu, Vanemuise 46, 51014 Tartu, Estonia*
[2]*Estonian Fund for Nature, Lai 29, Tartu, Estonia*
E-mail: kristjan.oopkaup@ut.ee

Keywords: next-generation sequencing, bacterial community, denitrifying bacteria, network analysis, treatment wetland

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This study is focused on the diversity and dynamics of bacterial communities in horizontal subsurface flow constructed wetlands (CW) units treating municipal wastewater. Bacterial community was profiled using Illumina HiSeq2000 sequencing of the V6 region of 16S rRNA gene. We obtained in average 142088 sequences per sample (total of 2131325 sequences) and the amount of operational taxonomic units (OTUs) varied between 877 - 984 OTUs in biofilm samples, at 5% cutoff level. The most abundant bacterial groups were Betaproteobacteria (20.4%), Gammaproteobacteria (14.7%), Sphingobacteria (8.2%), Flavobacteria (6.5%), and Alphaproteobacteria (5.9%). Among 100 OTUs, assigned to genera, 50 were shared by all 15 samples. Multivariate analysis indicated similar bacterial community succession pattern in CW units. Molecular ecological networks approach was applied to determine the network structure of bacterial communities during community succession in CW units. The analysis identified three bacterial modules, and further analysis will be performed to relate network interactions with environmental traits and treatment efficiency. Most of the key module members are near the line between peripheral nodes and connectors, which from ecological perspective represent specialists and generalists, respectively. Denitrifying microbial community structure analysis that was based on *nosZ* gene indicated as well homogeneous succession over time; however its relation with treatment capacity was low. The abundance of bacteria that was estimated using quantitative PCR using *nosZ* gene for denitrifying bacteria and *16S rRNA* gene for total abundance showed rapid increase over time after one month period. Taken together, the information obtained in this study will be a base for further studies relating to the bacterial community structure and function in constructed wetlands for wastewater treatment.

Acknowledgements
This study was supported by Target Project No SF0180127s08 of the Ministry of Education and Research of the Republic of Estonia, and by the European Regional Development Fund through ENVIRON (Centre of Excellence in Environmental Adaptation).

Estimation of bio-hydrogen lactose fermentation by various *E. coli* strains using FT-IR spectroscopy

M. Grube[1,2], I. Dimanta[3], M. Gavare[1,2], I, Strazdina[1,2], U. Kalnenieks[1,2]

[1]*Institute of Microbiology and Biotechnology, University of Latvia, 4 Kronvalda blvd., Riga, LV 1010, Latvia*
[2]*Department of Water Engineering and Technology, Riga Technical University, 16/20 Azenes str., Riga, LV1048, Latvia*
[3]*Institute of Solid State Physics, University of Latvia, 8 Kengaraga str.*
E-mail: grube@lu.lv

Keywords: *E.coli,* bio-hydrogen, lactose, fermentation

Bio-hydrogen production is advantageous and environment-friendly process, especially when renewable, organic resources are used as substrates. In this study lactose or cheese whey were used as a growth media component for production of bio-hydrogen by *E. coli.* FT-IR spectroscopy, Unisense Clark-type hydrogen microsensor and experimental test-system (prototype bioreactor) connected to the RGAPro-100 mass-spectrometer, were used to determine the concentration of lactose, analyze the macromolecular composition *of E. coli* biomass, evaluate the conversion of lactose and production of bio- hydrogen.

Escherichia coli BW25113 *hyaB hybC hycA fdoG frdC ldhA* aceE::kan (from prof. T.K. Wood, USA) was used in our experiments. This strain was used as the host strain for modifications in order to enhance the hydrogen production yield from lactose. The obtained strains were: *Escherichia coli* BW25113, carrying plasmid pTrc:lacY and *Escherichia coli* BW25113 pTrc:lacY, subjected to selection for the ability to grow on elevated lactose concentrations. Strains were tested for the hydrogen gas production using lactose or cheese whey as a substrate. Hydrogen concentrations in liquid and gaseous environments were measured with two experimental systems for quantitative measurements of hydrogen yield and rate.

HCA of FT-IR spectra of biomass and fermentation supernatants were useful to indicate the variable cell maclomolecular composition and conversion of lactose. An assessment was developed to establish whether *E. coli* BW25113 *hyaB hybC hycA fdoG frdC ldhA aceE* lactose consumption is a viable possibility for bio-hydrogen production. Results in the report will be discussed in detail.

Acknowledgements
This study was supported by ERAF project No. 2010//0298/2DP/2.1.1.1.0/10/APIA/VIAA/157 “Technologies for biogas production from agricultural and dairy wastes”. We thank prof. T. K. Wood (USA) for *E. coli* BW2511 strain.

New approaches for waste-less bioethanol production from hardwood

N. Vedernikov[1], M. Puke[1], I. Kruma[1], L. Rozenfelde[2], G. Khroustalyova[2], D. Borovikova[2], A. Patmalnieks[2], N. Matyushkova[3], A. Katashev[4], Yu. Dekhtyar[4] , A. Rapoport[2]

[1]Laboratory of Polysaccharides, Latvian State Institute of Wood Chemistry, Riga, Latvia; [2]Laboratory of Cell Biology, Institute of Microbiology and Biotechnology, University of Latvia, Riga, Latvia;
[3]Department of Microbiology and Biotechnology, Faculty of Biology, University of Latvia, Riga, Latvia;
[4]Institute of Biomedical Engineering and Nanotechnologies, Riga Technical University, Riga, Latvia
E-mail: maris.puke@inbox.lv

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Keywords: furfural, bioethanol, wood, waste-less technology

It is a known fact that about 70% of all oil reserves in the world have been already used and 3% of remaining amount is used every year now. That's why alternative ways of fuel production are searched for. The main opportunity for the fuel production has wood biomass. It was shown by Brazil and later USA and other countries that petrol can be fully or partly replaced with bioethanol. The basic advantage of bioethanol production is that wood lignocellulosic biomass is a renewable resource. Bioethanol production from wood biomass has been researched for over than 30 years however still there is no solution for the use of pentoses which can be rather significant part of wood biomass. Attempts to find yeast strains which can utilise pentoses still have not reached the industrial levels. Another possibility is to use C5-sugars for the obtaining of rather valuable chemical compound. Unfortunately till the moment this approach always led to unreversible destruction of remaining part of lignocelluloses. In our work new technology is proposed for coincident furfural and bioethanol production. This technology consists from unique method of obtaining of furfural which does not damage the cellulose. Second stage of this technology is special enzymatic treatment of lignocelluloses which leads to the formation of glucose. Next stage is bioethanol synthesis from C6 sugars by microorganisms is a rather well known process. We are trying to improve this stage by selection of yeast strains with higher productivity and by the use of immobilized yeast systems. Final stage of this technology is linked with the use of remaining lignin for the growth of medicinal mushrooms. It is expected that this new waste-less technology will significantly change industrial production of bioethanol.

Acknowledgements
The work has been financed by European Regional Development Fund, contract Nr. 2010/0289/2DP/ 2.1.1.1.0/10/APIA/VIAA/038

Model-based cultivation of *Escherichia coli* RB791 and BL21 strains: process parameter comparison from laboratory bioreactor and shake flask scale fermentations

O. Grigs[1], **V. Galvanauskas**[2], **J. Vanags**[3], **K. Dubencovs**[1], **V. Stepanova**[1], **A. Trubaca**[1]

[1]*Riga Technical University, Faculty of Materials Science and Applied Chemistry, Riga, Latvia*
[2]*Kaunas University of Technology, Process Control Department, Kaunas, Lithuania*
[3]*Latvian State Institute of Wood Chemistry, Riga, Latvia*
E-mail: oskars.grigs@rtu.lv

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Model-based design of bioreactor and flask-scale cultivations of two different Escherichia coli strains RB791 and BL21 was performed and the results were discussed. Aim of this research was to utilize mathematical modeling approach to calculate optimal substrate feeding rates corresponding to desired biomass specific growth rates (μ) and to evaluate and compare the most commonly used process model parameters, such as yield coefficients (Y), substrate specific consumption rate (q), Monod saturation/inhibition constants (K), etc. for both strains and both scales. In the fed-batch experiments run under substrate limitation conditions, biomass growth processes with specific growth rates of 0.15, 0.25, and 0.35 h-1 with value shift of ± 0.05 h-1 were performed. Acetate measurement results show that under these conditions an undesirable accumulation of this by-product was avoided. In such a way, smooth and controlled biomass growth according to the predictions of the process model was maintained. E. coli biomass concentrations of up to 60 g/l were achieved using defined fed-batch media.

Acknowledgements
The authors and Biosystems group (LLU) are acknowledging the financial support received from the State Education Development Agency of Latvia and European Social Fund within the scientific research project “Establishment of Latvian interdisciplinary interuniversity scientific group of systems biology” (ref. no. 2009/0207/1DP/1.1.1.2.0/09/APIA/VIAA/128).

In silico simulations of heterologously expressed glycerol dehydrogenase in yeast *Saccharomyces cerevisiae*

V. Brusbardis[1], J. Liepins[2]

[1] *Faculty of Information Technologies, Latvia University of Agriculture, Liela iela 2, Jelgava, Latvia LV-3001*
[2] *Institute of Microbiology and Biotechnology, University of Latvia, Kronvalda Blvd. 4, Riga, Latvia LV-1586*
E-mail: valters.brusbardis@gmail.com

Keywords: Yeast, NADPH, glycerol cycle, kinetic model

In bakers' yeast *Saccharomyces cerevisiae* cytoplasmic NADPH is mainly produced in pentose phosphate pathway (PPP). Thus NADPH production is linked with carbon flow and engineering of PPP might have unwanted effects on yeast growth or desired product formation.

We introduced *Hypocrea jecorina* NADP dependent glycerol dehydrogenase (gld2) [2] in *S. cerevisiae* in order to increase production of cytoplasmic NADPH.

In order to predict possible impact of gld2 expression to cytoplasmic NADPH concentration and ratio between NADPH and NADP+ - we developed quantitative kinetic model of the system. It was composed of the main reactions of glycolysis and PPP. Kinetic model of glycolysis published by F. Hynne [1] was used as basis of the model. The main reactions and kinetics of PPP were derived from M. Ralser [3]. gld2 and dak1 (*S. cerevisiae* dihydroxoacetone kinase) reactions were added to the model. Enzyme kinetics of gld2 was experimentally obtained. Following kinetic parameters were used for gld2 – KM for NADP+ was 0.14 mM and for glycerol it was 121 mM, Vmax for reaction was 2008 mM/min. Finally deterministic simulations of the model were performed in Copasi 4.6B32 software.

In silico simulations of mathematical model suggested that expression of gld2 could increase concentration of NADPH and ratio between NADPH and NADP+ in a cytoplasm of yeast. Without expression of gld2 NADPH concentration was 0.141 mM and NADP+ was 0.028 mM resulting in ratio up to 5 between these two species. gld2 expression promoted NADPH/NADP ratio increase up to 13 and concentration of NADPH reached 0.158 mM. Results achieved were in accordance with results published by M. Ralser [3].

In vivo results are pending to approve *in silico* results.

Acknowledgements

Author is acknowledging the financial support received from the State Education Development Agency of Latvia and European Social Fund within the scientific research project "Establishment of Latvian interdisciplinary

interuniversity scientific group of systems biology" (ref. no. 2009/0207/1DP/1.1.1.2.0/09/APIA/VIAA/128).

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Variability of convergence duration optimizing dynamic biochemical networks

N. Bulipopa, I. Mozga, E. Stalidzans

Biosystems group, Department of Computer Systems, Faculty of Information Technologies, Latvia University of Agriculture, Jelgava, Latvia LV-3001
E-mail: cvetkova.natalja@gmail.com

Keywords: optimization, biochemical networks, automatic termination of optimization, COPASI

Optimization of steady states of dynamic biochemical networks usually is done numerically applying global stochastic optimization methods. Correct selection of optimization duration and therefore the automatic termination of optimization is unsolved task in development of industrially efficient strains of microorganisms. The properties of convergence dynamics of some global stochastic optimization methods depending on complexity of kinetic equations within the same model are studied. The optimization task is the improvement of yeast glycolysis performance for ethanol production. COPASI software is used. Results indicate significant differences in the convergence dynamics between different combinations. The choice of optimization method and duration of optimization runs should be based on number of tests on the convergence quality, speed and repeatability.

Acknowledgements
We acknowledge financial support of European Structural Fund - Project “Establishment of Latvia interdisciplinary interuniversity scientific group of systems biology” - realized by Latvia University of Agriculture (contract no. 2009/0207/1DP/1.1.1.2.0/09/IPIA/VIAA/128).

A software tool for automatic comparison of genome-scale reconstructions

M. Mednis[1], Z. Rove[1], V. Galvanauskas[2]

[1] Latvia University of Argiculture, Biosystems group, Liela iela 2, Jelgava, Latvia, LV-3001
[2]Kaunas University of Technology, Studentu g. 50-162, Kaunas, Lithuania, LT-51368
E-mail: martins.mednis@llu.lv

Keywords: reconstruction, model comparison, inconsistencies detection

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A software tool ModeRator 1.0 is proposed. We have developed an online software tool for comparison of genome-scale reconstructions stored in COBRA-compatible format. While COBRA toolbox can run only in Matlab environment, ModeRator 1.0 can detect inconsistencies in complex reconstructions and to compare the reconstructions even without having Matlab and COBRA installed on a computer.

The tool can be used online at http://biosystems.lv/moderator/ or hosted on a local server. ModeRator 1.0 is implemented in pure PHP, and its source code is available for download at http://sourceforge.net. The application and installation instructions are available. ModeRator has been successfully tested on representative genome-scale metabolic network models of *Z. mobilis*, *E. coli* and *S. cerevisiae*.

Acknowledgements

The authors and Biosystems group (LLU) are acknowledging the financial support received from the State Education Development Agency of Latvia and European Social Fund within the scientific research project “Establishment of Latvian interdisciplinary interuniversity scientific group of systems biology” (ref. no. 2009/0207/1DP/1.1.1.2.0/09/APIA/VIAA/128).

The use of various natural sources in isolation of hydrogen producers

M. Neibergs
Department of Microbiology and Biotechnology, University of Latvia, Kronvalda Blvd. 4, Riga, Latvia LV-1586
E-mail: Miks_neibergs@inbox.lv

Keywords: Hydrogen, bacteria, fermentation, peat, sapropel

Hydrogen is the future of energy production for our everyday life and work, because of high conversion efficiency, recyclable properties and available access. Bacteria are able to produce this precious gas using multiple biochemical reactions [1]. Most studied form of those transformations is dark fermentation, where microscopic organisms use carbon rich natural resources to breakdown them into acetate, butyrate or other simple organic compounds [2]. Hydrogen gas is usual by-product of those reactions and it can be relatively easily extracted and stored for human purposes.

For the purposes of this work naturally occurring substrates like peat, sapropel and silage were studied for the possibility of obtaining hydrogen producers from them. Samples of substrates were placed in anaerobic culture media in order to test the capacity of resident microorganisms to produce hydrogen gas into environment. Soil and test samples were chosen randomly without conducting analysis of their composition beforehand. The impact of nutrition source was also observed by using different food sources in the media i.e. glucose and glycerol.

With the use of Crystal™ ID kits both anaerobic and facultative anaerobic hydrogen producers were identified in silage and sapropel, while peat proved to be less efficient source of raw material for such microorganisms. By determining the amounts of produced gas it was also concluded that glucose is more suitable as food source than glycerol for these kinds of bacteria.

Acknowledgements
Many thanks to my supervisor Dr. biol. Vizma Nikolajeva for help in realization of this work. This work was supported by the grant No. L-2746-ZR-N-090 University of Latvia.

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Algorithm of Genome-scale metabolic implementation in metabolic engineering

I. Odzina

Latvia University of Agriculture, Faculty of IT, Biosystems group, Liela iela – 2, Jelgava, Latvia, LV-3001
E-mail: ilona.odzina@gmail.com

Keywords: Stoichiometric analysis, model analysis, algorithm.

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Systems biology is a rapidly growing field that is based on building and validating in silico models of biological systems using a wealth of experimental data. These models can be applied to generate novel, testable and often quantitative predictions of cellular behavior [1]. Building these models is required stoichiometric model analysis, followed by the addition of dynamic model parameters. To make it easier to resolve this task is necessary to develop an algorithm that will facilitate the metabolic engineering problems solution. The algorithm describes the metabolic engineering task progress from stoichiometric analysis of the dynamic parameters of the addition and analysis. In generating these models need stoichiometric model analysis, followed by the addition of dynamic model parameters. For an easier to resolve this task is necessary to develop an algorithm that will facilitate the metabolic engineering solutions to the task.

Acknowledgements

This study is supported by ESF project 'Latvian Interdisciplinary Interuniversity Scientific Group of Systems Biology' (Ref.2009/0207/1DP/1.1.1.2.0/09/APIA/VIAA/128)

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Comparative study on the attachment of bacteria consortium MDK.EKO-7 onto different carriers

A. Berzins[1], K. Potapova[1], S. Strikauska[2], O. Muter[1]

[1]Institute of Microbiology and Biotechnology, University of Latvia, 4 Kronvalda Blvd., Riga, Latvia, LV-1010
[2]Latvia University of Agriculture, 2 Liela Str., Jelgava, Latvia, LV-3001
E-mail: andrejs54@inbox.lv

Keywords: attachment, bacteria consortium, carrier, microbial enzymatic activity.

Processes of the bacteria immobilization are widely used in environmental biotechnologies. A choice of the carrier for biodegradation process is an important factor in the high degree biodegradation of pollution, and efficient maintenance of the system over a long period.

The aim of this work was to compare the process of bacteria attachment onto different types of carrier. 23 carriers, including peat, ceramic beads, straw, - were characterized by measuring the biofilm enzymatic activity, as well as by counting the number of colony forming units and by microscopy.

The two-columns solid-phase / submerged biofiltration model system with inorganic carrier for diesel volatile hydrocarbons biodegradation was worked out. A stable biofilm formation onto ceramic beads was formed during 20 days experiment.

The five-columns model system was designed for removal of diesel volatile hydrocarbons with the use of dual composed carrier material, i.e. ceramic beads and rape straw. The highest removal efficiency for volatile hydrocarbons among biofiltration columns tested, i.e., 46.7 %, was found to be in the column containing rape stalks. The most intensive compaction of packing material was detected in the columns with ceramic beads and rape pods, i.e., 8.7 and 10.6 %, respectively. Compaction in other columns varied in the range of 3.3 ÷ 4.0 %. The same columns were characterized by the most considerable decrease of cellulose in the rape pods after 44 days experiment. Activity and concentration of microorganisms in the columns were estimated by FDA hydrolysis microbial activity and plate count [1]. The results of this study indicate to the significance of the composition of packing material. Further experiments should be performed at the bigger scale.

Acknowledgements

State Research program Nr. 2010.10-4/VPP-5 NatRes, the 1st Project „Mineral Resources" is a supporter of CBM 2012. This study was financed by the projects Y3-26493 of this Program.

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Potential applications of porous ceramic materials from Devonian and Quaternary clay deposits of Latvia for biotechnological purposes

Z. Petriņa[1], T. Griba[1], V. Nikolajeva[2]

[1] Institute of Microbiology and Biotechnology, University of Latvia, Kronvalda Blvd. 4, Riga, Latvia LV-1586
[2] Department of Microbiology and Biotechnology, Faculty of Biology, University of Latvia, Kronvalda Blvd. 4, Riga, Latvia, LV-1586
E-mail: zaiga.petrina@lu.lv

Keywords: Devonian clay, Quaternary clay, porous ceramic, microorganisms

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Clay is the most common sedimentary rock, which form the largest part of the Earth upper crust. After treatment it is widely used in industry [1]. Porous clay carriers can be used in wastewater treatment [2], in environmental bioremediation [3] and in other fields of biotechnology.

Ceramic granules sintered at different temperatures and sieved particles obtained from grounded granules were prepared at the Institute of Silicate Materials, Riga Technical University. The main raw material was clay obtained from Latvian clay deposits. Gram-negative saprophytic bacterium *Pseudomonas putida* MSCL 650 was chosen as a model. Adhesion of *P. putida* was carried out in the controlled batch experiments. Total number of bacteria and the number of viable bacteria in the suspension recovered from surface of granules and grounded granules were monitored throughout incubation 28 °C. The results were expressed as colony-forming units (CFU) per gram. Then granules were dehydrated at 22 °C during 16 days and bacterial viability was monitored. Bacteria were stained with DAPI or with Gram Stain and Viability Kit and observed under a fluorescence microscope.

Experimental data analysis showed that bacterial adhesion has taken place in all of the investigated expanded clay granules sintered at temperature of 1100-1200 °C and afterward incubated at temperature of 20 °C. The amount of viable adhered and detached *P. putida* cells reached 10^3-10^5 CFU per gram of Quaternary clay granules and about 10^6 CFU per gram of Devonian clay granules. Nevertheless, similar experiments with increased temperature to 30 °C brought the number of CFU to zero value for Quaternary clay granules but did not have significant influence on the number of adhered bacteria for Devonian clay granules. It was demonstrated that Quaternary clay ceramic materials increased the pH value of the liquid that could indicate on the bactericidal properties.

Acknowledgements

This study was supported by the National Research Programme of Latvia (programme No. 2010.10-4/VPP-5 NatRes, the 1st project "Mineral Resources"). Authors are grateful to Dr.sc.ing. R. Švinka and Dr.habil.sc.ing. V. Švinka for providing ceramic materials.

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Influence of environmental factors on the attachment of *Pseudomonas putida* on Devonian clay porous ceramic granules

T. Griba[1], Z. Petriņa[1], V. Nikolajeva[2]

[1] *Institute of Microbiology and Biotechnology, University of Latvia, Kronvalda Blvd. 4, Riga, Latvia LV-1586*
[2] *Department of Microbiology and Biotechnology, Faculty of Biology, University of Latvia, Kronvalda Blvd. 4, Riga, Latvia, LV-1586*
E-mail: mrs.griba@gmail.com

Keywords: Devonian clay, porous clay ceramic, *Pseudomonas putida*, adhesion

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Adhesion of microorganisms is a common ecological feature. Surface adhesion and biofilm development is a survival strategy employed by virtually all bacteria and refined over millions of years [1].

Nowadays, the study and application of adhesion have taken great importance for biotechnology as one of the methods of cell immobilization for practical needs. Investigations show that porous ceramic carriers can be used in wastewater treatment [2], in environmental bioremediation [3] and other biotechnological applications.

The aim of studies was to detect factors affecting adhesion of bacteria *Pseudomonas putida* on porous ceramic granules. Ceramic granules were prepared and characterized in the Institute of Silicate Materials, Riga Technical University. The main raw material was clay obtained from Latvian clay deposits. Adhesion of *P. putida* MSCL 650 was carried out in the controlled batch experiments. The number of colony-forming units (CFU) of *P. putida* on the Devonian clay granules sintered at temperature of 1100 °C after 4 h long incubation was estimated. The level of the adhesion significantly ($P < 0.05$) depended on temperature, pH value and ionic strength of the medium as well on the initial bacterial concentration. The optimum pH for bacterial adhesion was about 6. Temperature of 30 °C was more appropriate for the adhesion than temperature of 20 °C. Negative correlation was observed between the number of adhered bacteria and ionic strength ($r = -0.84$) in the medium and between the number of bacterial CFU in the suspension and ionic strength ($r = -0.77$) in the medium over the range from 0 to 200 mM NaCl. Investigated concentrations of bacterial suspension up to 11 log CFU mL^{-1} did not reach a saturation of bacteria on the surface of granules. It could be suggested that the adhesion intensity is sufficient for subsequent development of bacterial colonies in adequate environmental conditions.

Acknowledgements

This study was supported by the National Research Programme of Latvia (programme No. 2010.10-4/VPP-5 NatRes, the 1st project "Mineral Resources"). Authors are grateful to Dr.sc.ing. R. Švinka and Dr.habil.sc.ing. V. Švinka for providing ceramic granules.

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Microbiological studies- substantial part of State research program NatRes project "Mineral Resources"

V. Segliņš
University of Latvia, Raina blvd. 19, Riga, Latvia LV-1586
E-mail: Valdis.Seglins@lu.lv

Keywords: State research program, scientific cooperation, value added technologies and products

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State research program NatRes project „Mineral Resources" specific attention to biotechnologies is based on scientific potential regarding novel and outstanding biotechnological products and technologies with particular utilisation of local mineral resources and derivates. Project in general supported and promoted the collaboration of scientists from various scientific fields from the exploration of the basic mineral resources, raw material processing and new product development. Besides traditional localized in separate laboratories and research groups, the new technologies and multidisciplinary innovations are result of efficient scientific collaboration and implementation of engineering solutions. Therefore the project is a multidisciplinary scientific project, comprising six deeply interconnected and synergic research directions with high application potential for national economy. The project is being implemented in a close cooperation of the research groups from University of Latvia and Riga Technical University, focusing on 17 research themes involving scientists in geology, chemistry, biology, as well as different scientific areas of environmental and material studies. This is the key of success and project know-how.

Mentioned above themes of scientific research are from evaluation of clay resources in Latvia to novel biotechnological products and technologies based on ceramsite with high potential of commercial development in the future. There are two major issues to be stressed as value added- new technologies and products, both as measurable scientific results regarding clay ceramics, peat extracts and biotechnology.

Acknowledgements
State Research program Nr. 2010.10-4/VPP-5 NatRes the 1st Project „Mineral Resources" is supporter of CBM 2012.

Biodegradation of treated softwood and hardwood species by brown rot fungi

A. Janberga[1], **I. Irbe**[1], **V. Biziks**[1], **N. Kurnosova**[1], **B. Andersons**[1], **I. Andersone**[1]

[1]*Latvian State Institute of Wood Chemistry, 27 Dzerbenes street, Riga, Latvia LV-1006*
E-mail: aj07019@lanet.lv

Keywords: brown rot fungi, enzymatic activity, CEN/TS 15083-1, EN 84.

Brown rot fungi degrade wood cellulose and hemicelluloses, and modify lignin (Jin et al. 1990). In the temperate climate zone, 80% of wood construction damages is caused by brown rot fungi (Green and Highley 1995; Irbe et al. 2009).

For enzymatic activity measurements, brown rot fungi *C. puteana*, *P. placenta* and *G. trabeum* were grown in submerged fermentation of wheat bran-containing medium. The enzymatic activity of xylanase and endoglucanase, and total cellulolytic activity were measured.

The highest enzymatic activity of all enzymes was detected for *C. puteana* produced xylanase, i.e. 8.3 U ml^{-1}. The highest activity of endoglucanase was detected for *P. placenta,* i.e. 4.4 U ml^{-1}. The total cellulolytic activity showed the lowest value, i.e. 0.1 U ml^{-1} for all the fungi under study.

In accordance with EN 350-1, sapwood of softwood (pine and spruce), and hardwood (grey alder, aspen and birch) are classified as not durable. To improve the durability of softwoods, they were impregnated with wood preservatives - *Celcure AC 500* and *Dikants* and hydrothermally modified in two regimes - 150°C/3h and 160°C/1h. Hardwoods were hydrothermally modified at 160°C/1h. The efficiency against softwood degradation by *C. puteana, G. trabeum* and *P. placenta* was determined according to the standards CEN/TS 15083-1 and EN 84. The efficiency against hardwood degradation by *C. puteana* was determined according to the standards CEN/TS 15083-1 and EN 84.

The best results according to CEN/TS 15083-1 were obtained for spruce, hydrothermally modified at 150°C/3h, and the *Celcure AC 500* vacuum treated pine (amount of the chemical preservative 3.3 kg/m^3) - both showed a mass loss of 1.0%. The best results according to EN 84 were obtained for spruce, hydrothermally modified at 160°C/1h; mass loss was 0.0%.

According to CEN/TS 15083-1, the mass loss of hydrothermally modified hardwood at 160°C/1h was 7.1%, 7.1% and 13.3% for aspen, birch and grey alder, respectively, but after leaching according to EN 84, mass loss was 17.2%, 20.0% and 26.2% for aspen, birch and grey alder, respectively.

In accordance with CEN/TS 15083-1, the wood is estimated as very durable, if the mass loss ≤ 5%.

Heat treatment was more effective than chemical treatment, and better results for all the three fungi were determined for pine. Hydrothermal modification by the same conditions (temperature/time) was more effective for softwoods than hardwoods.

Investigation of the microbial storage flora of birch and spruce sawdust

J. Blomqvist[1], S. Hedén[1], M. Sandgren[2], T. Lestander[3], V. Passoth[1]

[1] Swedish University of Agricultural Sciences (SLU), Uppsala BioCenter, Dept. of Microbiology, Box 7025, 750 07 Uppsala, Sweden
[2] Swedish University of Agricultural Sciences (SLU), Uppsala BioCenter, Dept. of Molecular Biology, Box 590, SE-751 24 Uppsala, Sweden
[3] Swedish University of Agricultural Sciences (SLU), Unit of Biomass Technology and Chemistry, Linnaeus väg 6 901 83 Umeå, Sweden
E-mail: johanna.blomqvist@slu.se

Keywords: sawdust, storage, birch, spruce, yeast, mould, bacteria

Sawdust is a rest product from forest industry that holds a great potential for industrial application both as raw material for building and furniture production, for heating and electricity production and for biofuel production. Before utilising sawdust for the different purposes the material has to be stored for varying time periods. During those periods, occasions of microbial infections have been reported by the production personnel, e.g. moulding or occurrence of bad smells.

Up to now, no investigations of the microbial storage flora on sawdust have been performed. We investigated the microfungi and bacterial population on birch and spruce sawdust stored for twelve weeks at 2, 20 and 37ºC.

Rather high numbers of bacteria were found in the storage systems that were higher than those of moulds and yeasts. Yeasts were only found in sawdust stored at 2ºC. Many of them were cryophilic and have earlier been associated with insects living on wood. At higher temperatures, moulds of the genera *Penicillium* and *Aspergillus* were dominating. The occurrence of the pathogenic species *Aspergillus fumigatus* in 37ºC-storages demonstrated the demand for conserving measures. However, biopreservation with yeasts that have proven anti-mould activities at other materials was not successful as the tested strains were not able to grow in the 20 and 37ºC storages. The impact of the microflora on the stored sawdust on its further application will be discussed.

Detection and identification of silver birch *Betula pendula* Roth wood inhibiting microorganisms for determination of potential wood discoloration initiators

K. Bitenieks[1], A. Korica[1], Ž. Bacāns[2], V. Nikolajeva[3], I. Baumanis[1], I. Veinberga[1], D. E. Ruņģis[1]

[1] Genetic Resource Centre, Latvian State Forestry Research Institute "Silava", Rigas street 111, Salaspils, Latvia, LV-2169
[2] Latvijas Finieris, Bauskas Street 59, Riga, Latvia, LV-1004
[3] Microbial Strain Collection of Latvia, Faculty of Biology, University of Latvia, Kronvalda Blvd. 4, Riga, Latvia, LV-1586
E-mail: kriss.bitenieks@gmail.com

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Keywords: PCR-DGGE, ribosomal DNA, defective wood, *Betula pendula* Roth, fungi, bacteria, wood, colour defects.

Discoloration of birch wood creates significant economical losses, especially in veneer production. Wood discoloration does not affect its mechanical properties but discoloration is undesirable due to visual imperfection (defects). A single agent (factor) causing discoloration has not yet been determined. Nevertheless research indicates possible connection with presence of microorganisms.

The aim of this thesis was to study fungal and bacterial communities on visually defective heartwood of Silver birch (*Betula pendula* Roth) and processes which can cause discoloration.

Furthermore, possible influence of microorganisms on discoloration was examined. The objects of this study were birch trees with unaffected wood and trees with wood discoloration from birch plantation in Rembate and other sites in Kurzeme and Latgale. Three differential methods for detecting microorganisms were used, BBL Crystal biochemical identification system for bacteria, mycelial isolation and PCR-DGGE. Pure fungal and bacterial cultures were used to inoculate birch wood blocks, in order to determine the ability of the isolated microorganisms to induce birch wood discoloration. DNA isolated from fungal cultures was amplified using universal fungal primers and sequenced. PCR-DGGE was used to compare profiles of fungi and bacteria isolated from wood samples by their ribosomal DNA gene fragments. The results didn't identify species which may be connected with birch wood discoloration but indicate that microorganisms are related to discolored birch wood.

Acknowledgements
Financial support was provided by MNKC (ERAF 2DP/2.1.2.1.1/10/APIA/LIAA/003) and Latvijas finieris.

Impact of *Glomus mosseae* and *Rhizobium phaseoli* on the yield formation of garden beans

I. Alsiņa[1], L. Dubova[1] , V.Šteinberga[1], L. Liepiņa[2], L. Strauta[3]

[1] *Institute of Soil and Plant Sciences, Latvia University of Agriculture, 2 Liela street, Jelgava ,Latvia LV-3001*
[2] *Institute of Biology, University of Latvia, Miera Str 3, Salaspils LV-2169*
[3] *Laboratory of Agronomical Research, , Latvia University of Agriculture, 2 Liela street, Jelgava, Latvia LV-3001*
E-mail: Ina.Alsina@llu.lv

Keywords: garden beans, mycorrhiza, rhizobia

The aim of this research is to access the influence *Glomus mosseae* isolates and *Rhizobium phaseoli* strain on the growth and yield quality of garden beans grown in different soil types.

Glomus mosseae isolates (BEG and LL1) were obtained from Institute of Biology, University of Latvia, *Rhizobium phaseoli* strain – from Rhizobium Collection of Institute of Soil and Plant Sciences, Latvia University of Agriculture. Two bean cultivars 'Saxa' and 'Goldtime' were grown in 0.5 L vegetation pots with peat substratum. Seeds were inoculated with *Rhizobium phaseoli* strain (P9M2) before sowing. *Glomus mosseae* was added to peat substratum during sowing. Uninoculated plants were used as control. At the stage of 3rd true leaf garden beans were transplanted in the four different soil types.

Plant yield, dry matter and protein content were tested. Soil respiration intensity, activity of dehydrogenase and fluoresceine diacetate hydrolysis activity were tested.

Results showed that the effect of both preparations depends on soil features. Higher yield was obtained from plants treated with combination of *Glomus mosseae* and *Rhizobium phaseoli.* Higher yield, higher soil respiration intensity and enzymes activity in sandy soil was observed in samples were combination of *G.mosseae* isolate LL1 and *R. phaseoli* was used. There are no significant differences of used isolates in peat soil, but in clayey soil the best results were obtained with BEG isolate and *R. phaseoli* strain. Higher content of nitrogen was detected in the leaves and pods of beans treated before sowing with *G.mosseae* LL1 isolate and *R. phaseoli.* Correlation between frequency of mycorisation and garden bean pod number and weight was detected.

Acknowledgements
Work was supported by Latvian Council of Sciences, project 09.1456

Lack of RsmA-mediated control results in constant hypervirulence, cell elongation, and hyperflagellation in *Pectobacterium wasabiae*

V. Kõiv[1], L. Andresen[1], M. Broberg[2]

[1] *University of Tartu, Institute of Molecular and Cell Biology, 23 Riia Street, Tartu 51010, Estonia*
[2] *University of Helsinki, Department of Biosciences, Division of Genetics, P.O. Box 56 (Viikinkaari 5), FIN-00014, Helsinki 00014, Finland*
E-mail: pontu55@hot.ee

Keywords: plant pathogen, RsmA, Erwinia, flagella, swarming

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Members of the *Pectobacterium* family are plant pathogens responsible for causing soft rot in numerous types of plants, including economically important carrot and potato crops. The main factors of pathogenicity involve bacterial motility and production of plant cell wall degrading enzymes (PCWDE). The latter include pectinases, cellulases, and proteases, and these are produced in large amounts following contact with a host plant . The expression of virulence factors is tightly controlled by environmental factors *via* a cellular regulatory network. Final regulatory step of virulence factor synthesis involves the posttranscriptional repressor RsmA .

In this study the physiological role of gene regulation by RsmA is under investigation. Disruption of *rsmA* gene of the *Pectobacterium wasabiae* strain, SCC3193 results in 3-fold decrease in growth rate and increased virulence. The comparison mRNA levels of the *rsmA*$^-$ mutant and wild-type using a genome-wide microarray shows that genes for known virulence factors and flagella genes are upregulated, while genes responsible for cell division and peptidoglycan synthesis are downregulated in the *rsmA*$^-$ strain. The enzymatic activity of PCDWE is very high throughout the growth curve, without induction by plant components. The *rsmA*$^-$ strain exhibits a higher propensity to swarm compared to the wild-type strain. Virulence experiments conducted in potato tubers demonstrate that although the *rsmA*$^-$ strain is more efficient at tissue maceration, its ability to compete with the natural bacterial community in a host is reduced. Taken together, in the absence of RsmA, cells revert to a constitutively infective phenotype characterized by expression of virulence factors and swarming. We hypothesize that lack of control over these costly energetic processes results in decreased growth rate and fitness.

Acknowledgements
This work was funded by grants SF0180088s08 and SF0180166s08 from the Estonian Ministry of Education and Research

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Isolation and identification of *Bacillus thuringiensis* (Bt) from natural sources, the Leningrad region

V.P. Ermolova

All-Russia Research Institute for Agricultural Microbiology, Podbelsky chausse 3, St-Peterburg, Pushkin-8, 196608. Russia.
E-mail: svetagrishechkina@mail.ru

Keywords: *Bacillus thuringiensis* (Bt), identification, insecticidal activity, larvicidal activity.

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In our country, as well as all over the world pilot studies are carried out, widely and successfully on the allocation of the original group of bacteria *Bacillus thuringiensis* (Bt) and the development of microbial preparations based on it. In the Institute of Agricultural Microbiology, a series of biologicals with entomopathogenic activity was developed. They are based on Bt variety aimed at different insect targets and are widely used in agricultural practice: bitoksibatsillin, baktokulitsid, batsikol etc.

In 2009 in St. Petersburg, its suburbs and the Leningrad region samples from different natural sources (soil, litter, water, mud, sick and dead insects) were collected to isolate the bacteria Bt as promising as producers of biological of insecticidal action.

Standard approaches were used in our stadies: screening test sample by dwindling smear on agar, microscopic analysis of the presence of crystalline endotoxin, intraspecies identification scheme proposed by H. De Barjac and A. Bonnfoi (1968) and O. Lysenko (1985). Biochemical properties of isolates were studied, using the system of indicator paper SIP-drive instead of liquid chromatography paper differential diagnostic environments. Exotoxin content was estimated for *Muska domestica* larvae, insecticidal activity for *Leptinotarsa decemlineata* Say, larvicidal activity for *Aedes aegypti* mosquito larvae.

Over 3,000 colonies of microorganisms were studied. 62 microbial culture were selected by morphological characteristics. The microscopic analysis showed that 12 of 62 isolates form crystalline endotoxin of different shapes. Due to physiological, biochemical and serological studies the bacilli isolated were classified as *Bac. thuringiensis* H_1 H_{14}; H_{3a3b} and were united into three serovariants. 4 isolates were referred to serovariant BtH_1, 5 isolates - to serovariant BtH_{14}, 3 isolate - to serovariant BtH_{3a3b}. Productivity of the bacilli isolated varied from 2.4-3.2 billion spores / ml QOL. Biological properties and the practical significance of the selected strains of BtH_1, BtH_{14} and BtH_{3a3b} are similar to the typical strains, they will be included into the Bt bank collection, as perspective producers of insecticidal biologicals.

BtH_{10}- perspective biological preparation with polyfunctional action for plant protection from pests and diseases

S. D. Grishechkina

All-Russia Research Institute for Agricultural Microbiology, Podbelsky chausse 3, St-Peterburg, Pushkin-8, 196608. Russia
E-mail: svetagrishechkina@mail.ru

Keywords: *Bacillus thuringiensis*, antifungal activity, polyfunctional properties, phythophathogenic fungi.

Among microbiological preparations the most widely used are biologicals based on entomopathogenic bacterium *Bacillus thuringiensis* (Bt), which also possesses antifungal activity. Bt is the leader of bioinsecticidal producers which can be concidered as a good alternative to plant protection chemicals. Like other bacilli it has a multiplicity of physiological and biochemical features that ensure the assimilation of nutrient substrates and antibiosis against partners in the ecological community, that enables to develop polyfunctional properties against insect pests and pathogenic fungi.

Biological preparation BtH_{10}, which producer is *Bac. thuringiensis (darmstadiensis)*, contains components of the liguid, spores, insecticidal and fungicidal endo and ekzotoksins. Due to the fact that the preparation has a complex effect on pests and phytopathogenic fungi it can be used as a universal unit of cultivation and crop protection. The main peculiar feature of the preparation BtH_{10} compared with similar biologicals is its selective action against harmful beetles, including imago (*Leptinotarsa desemlineata*, genus *Phyllotreta. Oulema melanopus, Meligetes aeneus, Colaphellus hufti, Phaedon armoraciae, Anthonomus rubi, Rhinoncus sibiricus*).

Under laboratory condition in vitro fungicidal BtH_{10} properties were identified for a number of pathogenic fungi. Afte adding the preparation into nutrient medium inhibition of growth of fungi colonies was observed as following: *Botrytis cinerea* by 80%, *Bipolaris sorokiniana* -78%, *Fusarium oxysporum* - 52%, *Rhizoctonia sp.* - 42%, *Alternaria alternate*-36%.

Under field condition a complex effect of the preparation was revealed. The efficacy of the treatment plant treatment with BtH_{10} liquid formulation at rates BtH_{10} 15 l /ga was accordingly: against *Anthonomus rubi* - 68% and *B. Cinerea* – 60%.

A reserve strengthening fungicidal effect of the preparation is BtH_{10} application technology which considers biological characteristics of relationships phytopathogenic fungi and plant. The relationship specificy in each pair of a fungi-BtH_{10} will be unigue in accordance with the characteristics of the fungi life strategy that determines the technological approaches for the

biological preparation application in everry case. For instans, to suppress *B. cinerea* appropriate spraying strawberry plants is effective. To control *F. oxysporum* the soil watering with the preparation is efficient, to reduce *B. sorokiniana* harmfulness seed dressing treatment is effective.

The analysis of experimental data allows to characterise BtH_{10} as a perspective biopreparation with polyfunctional action that possesses both insecticidal and antifungal effects.

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Microorganisms for rodents control

G.N. Minina, E.V. Bologova

All-Russia Research Institute for Agricultural Microbiology, Podbelsky chausse 3, St-Peterburg, Pushkin-8, 196608. Russia
E-mail: svetagrishechkina@mail.ru

Keywords: *B. issatchenko*, rodents, productivity, virulence, nutrient media

The only biological preparation which is effective and safe is used in Russia to control harmful rodents. The producers of the preparation are *Issatchenko bacteria* (*Salmonella enteritidis var.Issatchenko*) that were identified as early as 1897 by B.L.Isachenko out of rodents corpses during epizootics of grey rats in St. Petersburg. The long-term practice of these organisms application showed their safety for humans, animals and birds. Pathogenity of the bacteria was tested on the most common and harmful species of rodents. The analysis of the data obtained made it clear that different species of rodents have specifical susceptibility to the bacteria. For example, small rodents such as *Microtus arvalis, Microtus socialis, Mus musculus,* and others are particularly susceptible to the bacteria.

A significant deal of research for latest years has involved the work on preservation of useful propeties of b.*Issatchenko* strains (productivity and virulence) which are key indicators of the biological product quality.

Our research was aimed to make up new nutrient media allowing to improve the preparation quality and reduce its cost.

The following media were tested: wheat-peptone broth, protein-meat medium and meat-fish broth. Meat-peptone broth was used as a control variant.

The titer microbial alls in 1 ml b. *Issatchenko* medium after day-growth at 37^0C was accordingly: in wheat-peptone broth - $0,59 \times 10^9$, in the protein-meat medium - $0,48 \times 10^9$, meat-fish broth - $0,43 \times 10^9$, in the control variant - $0,48 \times 10^9$.

The virulence data (Ld_{50}) of b. *Issatchenko* strain were as following: under cultivation in wheat-peptone broth - 381,000, in protein-meat medium -560,000, in meat-fish broth -350,000, in the control variant – 500,000.

To conclude, the new nutrient media tested were not inferior in terms of productivity and virulence compared with the control variant. These new media can be recommended to prepare the uterine culture for microbial cells accumulation in biological preparations production.

Obtaining *Bacillus thuringiensis* on hydrolisates of brown algae

I.A. Naumov, V.P. Ermolova

All-Russia Research Institute for Agricultural Microbiology, Podbelsky chausse 3, St-Peterburg, Pushkin-8, 196608. Russia
E-mail: Igornaumov@bk.ru

Keywords: *Bacillus thuringiensis var.darmstadiensis* H_{10} *(*BtH_{10}*),* sodium alginate, brown algae.

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At present time, technologies for complex processing of algal feedstock to produce mannitol and sodium alginate are developed. Including the electrochemical technology for improved quality of sodium alginate. But insoluble fractions consisting of cell-wall polysaccharides (cellulose, fucoidan, laminaran) hydrolysates which can form the basis of culture media in the biotechnology industry do not find practical application.

Processing of algae - the process of large-tonnage, therefore, microbiological preparations from waste formed in such productions may be produced in large volume. It is well known that the priority for the protection of plants are eco-friendly products based on the bacteria *Bacillus thuringiensis*.

The aim of the study was to explore the possibility of cultivating *Bacillus thuringiensis var.darmstadiensis (*BtH_{10}*)* on carbohydrate waste generated after centrifugation a tenderized algal biomass formed on the stage of separation of solution of sodium alginate.

In optimized BtH_{10} yeast and corn starch polysaccharide medium was replaced by this waste with humidity of 95%. Adding 20, 30, 40 and 50% by weight of nutrient carbohydrate waste was studied. BtH10 cultured in Erlenmeyer flasks containing 50 ml of culture medium at 220 rev / min at 30 ° C. The titer of the culture fluid, formation of spores and crystalline endotoxin was evaluated in 72 -96 hours after the start of cultivation. As a result the best option was to use a 40% waste additive. It provided titer 2.0 x 10^9 spores / ml. After 72 hours the bacterial culture synchronously morphed to 95% spores and formed crystals. In other cases, the titer varied in the range (0.9-1.5) x 10^9 spores / ml.

The proposed method can reduce the cost of obtaining a bacterial preparation by replacing corn starch in a culture medium by the waste. Thus solving the problem of carbohydrate utilization of waste generated from obtaining of sodium alginate. More specifically the production of 1 ton of bacterial preparation with a titer of 2 x 10^9 spores / ml recycled 400 kg wet waste.

Screening for ligninolytic enzymes produced by *Lentinula edodes* (Berk.) Pegler

N. Matyushkova, I. Poppele, E. Azena

Department of Microbiology and Biotechnology, Faculty of Biology, University of Latvia,

Kronvalda Blvd. 4, Riga, Latvia, LV-1586

E-mail: natalja.matjuskova@lu.lv

Keywords: ligninolytic enzymes, screening, shiitake

Shiitake *Lentinula edodes* is edible, worldwide cultivated basidiomycete with medicinal properties and biotechnological and environmental applications. *L. edodes* belongs to white-rot fungi, which are able to degrade lignin. There are three major types of ligninolytic enzymes – lignin peroxidase LiP, manganese-dependent peroxidase MnP and laccase. Some white-rot fungi synthesize only one or two types of lignolytic enzymes. *L. edodes* strains differ in their morphological, physiological properties, incl. ability to produce ligninolytic enzymes. In the present study we report about the screening of fifteen *L edodes* strains for ligninolytic enzymes production. Laccase acivity in solid malt extract media was detected by ABTS reagent. Malt extract AzurB agar was used for LiP screening. Malt extract phenol red agar and Brilliant Green Agar were used for MnP activity measurements. Laccase and MnP activity were revealed for all *L. edodes* strains. Contrary to laccase and MnP, LiP production by current strains wasn't found. *L. edodes* strains differ in their growth rates and in MnP and laccase production amount. The correlation between mycelium growth rate and ligninolytic enzymes activity wasn't observed.

Acknowledgements

This work has been supported by the European Regional Development Fund project Nr. 2010/0288/2DP/2.1.1.1.0/10/APIA/VIAA/038

The effect of chlorination on *Escherichia coli* viability in drinking water

V. Denisova, L. Mezule, T. Juhna

Riga Technical University, Department of Water Engineering and Technology, Azenes 16/20, Riga, Latvia, LV-1048
E-mail: viktorija_denisova@inbox.lv

Keywords: Chlorination, electrochemical disinfection, *Escherichia coli*, CT value

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Chlorination is the most often used final drinking water disinfection technique in developed countries. The assessment of water quality and all engineering calculations on disinfection effectivity are based on microbial ability to form colonies on nutrient rich medium. However, it is now widely accepted that many microorganisms when subjected to oligotrophic conditions, e.g. drinking water, can attain a state of non-cultivability, often referred as viable but not culturable (VBNC) when cells retain all their metabolic activity, e.g., respiration, enzymatic activity, but are unable to form visible colonies on nutrient rich media [1]. Thus, the aim of this study was to analyse the disinfection effectivity based on cell ability to divide and metabolic activity.

Besides chemical based chlorination, the effect of electrochemical disinfection where active disinfectant is generated from ions naturally found in water was assessed. Batch scale disinfection studies involved various treatment regimes to neutralize faecal indicator organism *Escherichia coli*. In addition to classical cultivation, cell ability to divide and cell metabolic activity measurements (respiratory activity) were performed.

The results of this study showed that irrespective of the treatment method, *E. coli* first lost its cultivability, then the ability to divide and finally metabolic activity. For chlorination the CT values were more than ten times higher when loss in metabolic activity was assessed. For electrochemical disinfection the results showed that at low current intensity and low chloride ion concentration (0,1 A, 6,8 mg/l), no cultivable *E. coli* cells were obtained after 15 minutes, whereas less than one log decrease was observed for metabolic activity.

Thus, the results showed that the traditional faecal indicator – *Escherichia coli* which is regarded as very susceptible to chlorination can survive for much longer periods when its metabolic activity, not cultivability is evaluated.

Acknowledgements

This work has been partly supported by the European regional development fund within the project “Development of innovative water procession technology using nanostructured ceramics” No. 2010/0257/2DP/2.1.1.1.0/10/APIA/VIAA/012.

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Application of functional state modelling approach for yeast fed-batch fermentation modelling

S. Vilums

Biosystems Group, Faculty of Information Technology, Latvia University of Agriculture,
Lielā iela 2, LV-3001 Jelgava, Latvia
E-mail: sandis.vilums@gmail.com

Keywords: Bioprocess modelling, fed-batch fermentation, parameter estimation.

The yeast *Saccharomyces cerevisiae* is one of the most relevant microorganisms in biotechnology industry. In view of increasing importance of ethanol as an alternative source for chemicals and liquid fuel a great deal of research interest in ethanol fermentation has been generated [1].

Continuous evaluation of process parameters during the fermentation is essential for obtaining a mathematical model of the fermentation that can simulate the process. Global process models are generally used for bioprocess modelling. The main disadvantage of such approach is complexity of model structure and a large number of model parameters, which complicates model simulation and parameter estimation.

The functional state modelling approach [2-3] is an alternative concept of bioprocess modelling. In functional state the process is described by local model that is valid in actual state only. A set of local model together with functional state equations can be used to describe, monitor and control the yeast growth process. In case of high coincidence of model and experimental data this approach can be used not only for process predictions but also for early stabilization of process using fermenter control program. This approach shows substantial benefits for using it as foundation for the yeast fed-batch fermentation model based control system development. The yeast growth functional state estimation is valuable for clarifying appropriate parameters that can adequately describe the actual physiological state of yeast growth process.

This approach makes it easier to perform model simulation and parameter estimation compared with the complex global model. The main advantage of the local modelling approach over global model with complex structure is that the parameters of each local model can be estimated separately from the other local model parameters.

Acknowledgements

This paper was written by financial support of European Structural Fund – Project "Establishment of Latvia interdisciplinary interuniversity scientific group of systems biology" – realized by Latvia University of Agriculture (contract no. 2009/0207/1DP/1.1.1.2.0/09/IPIA/VIAA/128).

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LIST OF PARTICIPANTS

Name	Surname	e-mail	
Marcin	Adamczuk	adamczuk@biol.uw.edu.pl	P 27
Ave	Ahelik	aveahelik@gmail.com	P 02
Tiina	Alemäe	talamae@ebc.ee	I 19, O 03, P 26
Laura	Alksne	lauruxla@inbox.lv	O 17
Ina	Alsina	ina.alsina@llu.lv	P 55
Arta	Balode	arta.balode@stradini.lv	I 06, P 09
Vasily	Bankovsky	science@biosan.lv	I 13
Egija	Berga	egijaberga@gmail.com	P 07
Natalja	Berza	natalija.berza@gmail.com	P 05
Agnese	Berzina	agnese.berzina@hotmail.lv	P 09
Andrejs	Berzins	andrejs54@inbox.lv	P 48
Marianna	Bessmeltseva	marianna@tftak.eu	O 09, O 07
Kriss	Bitenieks	kriss.bitenieks@gmail.com	P 54
Liisi	Blank	liisiblank@gmail.com	O 08
Johanna	Blomqvist	johanna.blomqvist@slu.se	P 53
Olesja	Bondarenko	olesja.bondarenko@kbfi.ee	P 28, I 05
Diana	Borovikova	borovikovadiana@inbox.lv	O 24, P 16, I 17, P40
Valters	Brusbardis	valters.brusbardis@gmail.com	P 43
Michal	Bukowski	m.bukowski@uj.edu.pl	O 06
Natalja	Bulipopa	cvetkova.natalja@gmail.com	P 44
Rimantas	Daugelavicius	r.daugelavicius@gmf.vdu.lt	I 02
Viktorija	Denisova	viktorija_denisova@inbox.lv	
Ilze	Dimanta	ilze.dimanta@gmail.com	O 18, O 22, P 39, P 47
Laila	Dubova	laila.dubova@llu.lv	P 37, P 36, P 55
V.P.	Ermolova	svetagrishechkina@mail.ru	P 57, P 60
Daina	Eze	daina.eze@lu.lv	P 29, O 17
Marita	Gavare	marita.gavare@inbox.lv	P 19, P 39, O 05
Lelde	Grantiņa-Ieviņa	lelde.grantina@lu.lv	O11
Tatjana	Griba	mrs.griba@gmail.com	P 50, P 49
Oskars	Grigs	oskars.grigs@rtu.lv	P 42
Sveta	Grishechkina	svetagrishechkina@mail.ru	P 58
Mara	Grube	grube@lu.lv	P 39, P 19, O 05
Julija	Halimona	julija_halimona@inbox.lv	O12
Kaisa	Haukka	kaisa.haukka@helsinki.fi	I07

LIST OF PARTICIPANTS

Hermann	Heipieper	hermann.heipieper@ufz.de	I04
Ilze	Irbe	ilzeirbe@edi.lv	P 52
Merja Rita Christine	Itävaara	merja.itavaara@vtt.fi	I 08
Anna	Janberga	aj07019@lanet.lv	P 52
Janis	Jasko	janis.jasko@gmail.com	O 21
Tatjana	Jatsenko	tanjajat@gmail.com	P 24
Jevgenijs	Jermolajevs	animalab@animalab.lv	P 15, P 01
Natalja	Kabanova	natalja@tftak.eu	O 20, O 19
Anne	Kahru	anne.kahru@kbfi.ee	I 05, O 04, P 28
Lilija	Kalediene	lilija.kalediene@gf.vu.lt	I 10
Uldis	Kalnenieks	uldis.kalnenieks@lu.lv	I 20, P 39
Daina	Kalnina	dkkalnin@latnet.lv	P 30
Rudolfs	Kalnins	rudolfs.ka@gmail.com	O 13
Kaja	Kasemets	kaja.kasemets@kbfi.ee	O 04, I 05
Juris	Ķibilds	juris.kibilds@gmail.com	P 18
Maia	Kivisaar	maia.kivisaar@ut.ee	I 01, P 21, P 22, P23, P 24, P 25
Kristjan	Kogerman	Kristjan.kogerman@bruker.se	I 21
Vila	Kõiv	pontu55@hot.ee	P 56
Agnese	Kokina	agnese.kokina@lu.lv	O 25, O 05, P 18
Emils	Kozlinskis	emils.kozlinskis@gmail.com	P 10
Irina	Krallish	krallish@latnet.lv	P 17
Juta	Kroiča	juta.kroica@rsu.lv	P 05
Ivo	Krustok	ivo.krustok@mdh.se	O 16
Andrio	Lahesaare	andrio@ut.ee	P 22
Eleri	Lapp	elerilapp@gmail.com	P 02
Eglė	Lastauskienė	egle.lastauskiene@gf.vu.lt	I 18
Lauma	Laurinovica	lauma.laurinovica@gmail.com	O 22
Jānis	Liepins	janis.liepins@lu.lv	O 05, O 25, P18, P 43
Mindaugas	Malakauskas	mindaugas@lva.lt	O 10
Natalja	Matjuskova	natalja.matjuskova@lu.lv	P 61
Linda	Mezule	linda.mezule@rtu.lv	P 62
Martins	Mednis	martins.mednis@llu.lv	P 45
Edvīns	Miklasevics	Edvins.Miklasevics@rsu.lv	O 26
G.	Minina	svetagrishechkina@mail.ru	P 59
Hanna	Moor	hannamoor@gmail.com	P 21, P 23

LIST OF PARTICIPANTS

Ivars	Mozga	ivars.m@gmail.com	P 44, O 23
Indrikis	Muiznieks	indrikis.muiznieks@lu.lv	I 22
Olga	Muter	olga.muter@inbox.lv	I 11, P 33, P 48
Ranno	Nahku	ranno@tftak.eu	O 01, O 02
Igor	Naumov	igornaumov@bk.ru,	P 60
Miks	Neibergs	miks_neibergs@inbox.lv	P 46
Vizma	Nikolajeva	collect@lu.lv	O 11, O17, O 18, O 22, P 09, P 29, P 30, P 49, P 50, P 54
Hiie	Nõlvak	hiie.nolvak@gmail.com	O 15, P 32, P 35
Ilona	Odzina	odzina@inbox.lv	P 47
Kristjan	Oopkaup	kristjan.oopkaup@ut.ee	P 38, P 02, P 32
Volkmar	Passoth	volkmar.passoth@slu.se	I 15, P 41, P 53
Arturs	Patetko	bioarturs@gmail.com	P 13
Karl	Peebo	karl@tftak.eu	O 02, O 01
Zaiga	Petrina	zaiga.petrina@lu.lv	P 49, O 17, P 29
Milda	Pleckaityte	mildap@ibt.lt	P 04
Jens-Konrad	Preem	jpreem@ut.ee	P 34, O 16, P32, P 38
Vija	Ramniece	vija.ramniece@inbox.lv	P 11
Sigita	Ramonaite	ramonaite@lva.lt	O 10
Renate	Ranka	renate_r@biomed.lu.lv	P 06
Alexander	Rapoport	rapoport@mail.eunet.lv	I 17, O 24, P 16, P 19, P 20, P 40
Linda	Ratkevica	office@akd.apollo.lv	P 26
Aigars	Reinis	aigars.reinis@rsu.lv	P 05
Linda	Rozenfelde	lindyr@inbox.lv	P 20, P 40, P 19
Zane	Rutkovska	rutkovska.zane@gmail.com	O 18
Bernhard	Schink	bernhard.schink@uni-konstanz.de	I 14
Valdis	Seglins	valdis.seglins@lu.lv	P 51
Julia	Sidorenko	muss.musculus@gmail.com	P 25
Virginijus	Siksnys	siksnys@ibt.lt	I 03
Teele	Sildvee	teelesildvee@gmail.com	P 35, P 38, O 15
Ingus	Skadins	ingus.skadins@rsu.lv	O 5
Egils	Stalidzans	egils.stalidzans@gmail.com	O 23, I 20, P 44
Vilhelmine	Šteinberga	vilhelmine.steinberga@llu.lv	P 36, P 37, P 55
Valerija	Stepanova	valerija.stepanova@gmail.com	P 42

LIST OF PARTICIPANTS

Irina	Stulova	irinastulova@yahoo.com	O 19, O 20
Annika	Teppo	annikate@ut.ee	P 23, I 01
Kertu	Tiirik	kertu.tiirik@gmail.com	P 32, O 15
Ievgeniia	Tiukova	ievgeniia.tiukova@slu.se	P 41
Tone	Tonjum	tone.tonjum@medisin.uio.no	I 17
Jaak	Truu	jaak.truu@ut.ee	I 09, I12, O 15, O 16, P 32, P 34, P 35, P 38
Anda	Valdovska	anda.valdovska@llu.lv	P 03
Sintija	Valucka	sv08073@lu.lv	P 31
Ruta	Veinalde	ruuta@biomed.lu.lv	P 08
Armands	Vigants	armands.vigants@lu.lv	P 14
Ene	Viiard	ene@tftak.eu	O 07, O 09
Sandis	Vilums	sandis.vilums@gmail.com	P 63
Triinu	Visnapuu	visnapuu@ut.ee	O 03, P 26
Guntis	Vītols	guntis@animalab.lv	P 01
Veiko	Voolaid	veiko.voolaid@gmail.com	O 14
Anna	Zawistowska	azawistowska@il.waw.pl	P 12
Peteris	Zikmanis	peteris.zikmanis@lu.lv	