Session I. Microbial Biotechnology

Opening Lectures

I.OL.1

Metabolic engineering of the thermotolerant yeast *Ogataea polymorpha* for efficient convertion of lignocellulosics and by-product glycerol to ethanol

O. Kurylenko[1], J. Ruchala[2], M. Semkiv[1], I. Kata[2], K. Dmytruk[1], A. Sibirny[1,2]

[1]Department of Molecular Genetics and Biotechnology, Institute of Cell Biology, NAS of Ukraine, Drahomanov Street, 14/16, Lviv 79005 Ukraine; [2]Department of Biotechnology and Microbiology, Rzeszow University, Zelwerowicza 4, 35-601 Rzeszow, Poland

e-mail: sibirny@yahoo.com

Methylotrophic yeast *Ogataea* (*Hansenula*) polymorpha belongs to the most thermotolerant yeast organisms known with maximal growth temperature 50ºC. Thermotolerance is important trait for alcoholic fermentation as process goes faster and energy could be saved due to smaller differences between fermentation and distillation temperatures. Thermotolerance could be especially important during 2nd generation ethanol production as allows using cellulases and hemicellulases (optimum temperature at 50ºC and more) in the same vessel with thermotolerant yeast cells which convert sugars liberated by the enzymes to ethanol. This process is known as simultaneous saccharification and fermentation or SSF. *O. polymorpha* has additional promising features for 2nd generation ethanol production due to its ability to ferment important sugars of lignocellulosics as glucose, cellobiose and xylose and to grow on glycerol as the sole carbon and energy source. However, efficiencies of xylose alcoholic fermentation and glycerol conversion to ethanol by the wild-type strain of *O. polymorpha* are very low. Using combination of metabolic engineering and classical selection, ethanol production from xylose was increased 30–40 times and reached 15–17 g of ethanol/L at 45ºC. Several new approached of metabolic engineering were developed and used. They include knock out of transcription activator CAT8 and overexpression of genes DAS1 and TAL2 coding for peroxisomal transketolase (dihydroxyacetone synthase) and transaldolase, respectively. It was also found that knock out of PEX3 gene involved in peroxisome biogenesis, similarly to knock out of DAS1 and TAL2, genes, practically totally blocked ethanol production from xylose (but not from glucose) though did not affect growth on xylose as sole carbon and energy source. Overexpression of TKL1 and TAL1 genes coding cytosolic transketolase and transaldolase, respectively, also increased ethanol production form xylose whereas knock out of these genes hampered growth on xylose with moderate effects on xylose alcoholic fermentation. New approach in classical selection was based on isolation of the mutants resistant to glycolysis inhibitor, anticancer drug 3-bromopyruvate. It was found that near 70% of 3-brompyruvate-resistant mutants are characterized by increase in ethanol production from xylose. Ethanol production from glycerol was improved due to overexpression of genes coding enzymes of the initial (GCY1, DAK1, GUT1 and GPD1, encoding glycerol dehydrogenase, dihydroxyacetone kinase, glycerol kinase and glycerol-3-phosphate dehydrogenase, respectively) and of the final steps of glycerol conversion to ethanol (PDC1 and ADH1 coding for pyruvate decarboxylase and alcoihol dehydrogenase, respectively). Perspectives of further improvements of *O. polymorpha* for 2nd generation ethanol production are discussed.

I.OL.2

The yeast *Yarrowia lipolytica* as a cell factory

Patrick Fickers

Microbial Processes and Interactions, TERRA Teaching and Research Center, University of Liège - Gembloux Agro-Bio Tech, Gembloux, Belgium
e-mail: pfickers@uliege.be

Y. lipolytica is a non-conventional yeast, well-known for its unusual metabolic properties. Based on its ability to secrete high amounts of proteins and metabolites of biotechnological interest, *Y. lipolytica* has several industrial applications, including heterologous protein synthesis or citric acid production. We will present here different tools; including vectors, promoters and selectable markers; that have been developed for metabolic engineering or synthetic biology in this yeast. Successful examples of their application will be also presented.

Keywords: *Yarrowia lipolytica*, promoter, selectable marker, erythrulose, CalB lipase

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I.OP.1

Synthesis of raffinose-derived potential prebiotics by bacterial levansucrase and their effects on composition and metabolism of fecal microbiota of normal- and overweight children

Triinu Visnapuu[1], Karin Ernits[1], Signe Adamberg[2], Madis Jaagura[3], Anneli Larionova[4], Tiia Voor[4], Kaarel Adamberg[2,3] and Tiina Alamäe[1]

[1]Department of Genetics, Institute of Molecular and Cell Biology, University of Tartu, Riia 23, 51010 Tartu, Estonia; [2]Department of Chemistry and Biotechnology, Tallinn University of Technology, Ehitajate tee 5, 19086 Tallinn, Estonia; [3]Center of Food and Fermentation Technologies, Akadeemia tee 15a, 12618 Tallinn, Estonia; [4]Department of Pediatrics, Institute of Clinical Medicine, University of Tartu, N. Lunini 6, 51014 Tartu, Estonia
e-mail: triinu.visnapuu@ut.ee

Obesity is an emerging problem in Western societies and it has a direct link to dietary habits. Therefore research on dietary modulation of the gut microbiota composition towards more beneficial patterns is essential. Prebiotics (e.g. fructans) specifically support the healthy balance of gut microbiota and exert health benefits.

The aim was to synthesize potentially prebiotic substrates from raffinose using bacterial levansucrase[1] and evaluate the effect of those oligo- and polyfructans on fecal pools of overweight (OW) and normal-weight (NW) children. Raffinose, melibiose and levan of plant origin[2] were tested alongside. Levan and raffinose mixture (Raf-mix) were produced from raffinose using Lsc3 from *Pseudomonas syringae* DC3000 as a catalyst. According to HPLC analysis, the Raf-mix contained 48% melibiose, 15% raffinose, 4% fructooligosaccharides and 16% levan. Growth experiments were carried out in isothermal microcalorimeter[3]. The composition of microbiota in inocula and enrichments was analyzed by 16SrRNA sequencing. Metabolites were analyzed by HPLC and GC.

Differences were observed in consortia shifts and metabolites during growth on provided substrates between fecal pools of OW and NW children. Lactate-producing bacteria (*Streptococcus*, *Enterococcus*) became enriched in the pool of OW children resulting in lactic acid as the signature metabolite. Acetic and butyric acids were prevalent at fermentation by NW pool coinciding with enrichment of *Catenibacterium*. Levan specifically increased the abundance of *Holdemanella*, *Eggerthella*, *Senegalimassilia* and *Bacteroides*.

In conclusion, potentially prebiotic oligo- and polyfructans were produced by Lsc3. Different fermentation patterns of non-digestible saccharides were observed by fecal microbiota of NW and OW children.

References:
1. Visnapuu T *et al.* (2015) *N Biotechnol* **32**: 597.
2. Mardo K, Visnapuu T *et al.* (2017) *PLoS One* **12**: e0169989.
3. Adamberg *et al.* (2015) *PLoS One* **10**: e0144042.

Keywords: levan, fructooligosaccharides, levansucrase, candidate prebiotic

Acknowledgements:

Funding was received from ERF (grant EU48667) and Estonian Ministry of Education and Research (grants PUT1050, IUT1927, ERC grant no 9180).

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I.OP.2

Microbial community structure and resistome during anaerobic co-digestion of food waste and commercial organic waste

Kärt Kanger[1], Nigel G.H. Guilford[2], HyunWoo Lee[2], Camilla L. Nesbø[2,3], Elizabeth A. Edwards[2]

[1]Institute of Ecology and Earth Sciences, University of Tartu, Vanemuise 46, 51003, Tartu, Estonia; [2]BioZone Centre for Applied Biosciences and Bioengineering, University of Toronto, 200 College Street, Toronto, ON, M5S 3E5, Canada; [3]Department of Biological Sciences, University of Alberta, 11455 Saskatchewan Drive, Edmonton, AB, T6G 2E9, Canada
e-mail: kart.kanger@ut.ee

Antimicrobial resistance is a globally recognized public health risk that has gained attention both in the medical and natural environments. High incidence of antibiotic resistant bacteria and antibiotic resistance genes (ARGs) in various types of organic wastes demands for the development of effective treatment strategies. The objective of this study was to analyse the effect of anaerobic co-digestion of food waste and commercial organic waste on microbial community structure and resistome.

A lab-scale anaerobic digester consisting of six sequentially fed leach-beds and an up-flow anaerobic sludge blanket (UASB) reactor was fed weekly with a mixture of food waste and fibres (cardboard, boxboard, newsprint, fine paper) resulting in stable biogas production. Seven samples including food waste, the mixture of food waste and fibres used as leach-bed feed, digestate after six weeks of digestion, and anaerobic granules from the UASB were subjected to DNA extraction and shotgun metagenomic sequencing using the Illumina NextSeq500 platform. The bioinformatic pipeline included quality control, metagenomic assembly, binning, detection of ARGs and taxonomic classification. Additionally, ARGs and mobile genetic elements were quantified using high-throughput qPCR.

Both metagenomic approach and qPCR identified ARGs in all samples with significantly higher levels in the substrates (food waste, leach-bed feed) compared to digestion products (digestate, UASB granules). Total ARG abundance in the substrates reached 0.8 copies/16S-rRNA-gene, while remaining below 0.05 copies/16S-rRNA-gene in digestion products based on metagenomic analysis. The most abundant bacterial phyla belonged to *Proteobacteria*, *Firmicutes* and *Bacteroidetes*, although the microbial community differed significantly between the substrates and products. Metagenomic binning identified four potential ARG hosts in the substrates (*Erwinia*, *Bifidobacteriaceae*, *Lactococcus lactis*, *Lactobacillus*) and one ARG-containing bin in digestion products (*Acidobacteria*).

In summary, we identified potential hosts of ARGs in commercial organic waste and showed that anaerobic digestion can significantly reduce ARG abundances and alter the microbial community structure.

Keywords: antimicrobial resistance, anaerobic digestion, food waste

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I.OP.3

Capsular exopolymer obtained from *Rhodococcus opacus* FCL1069 cells

Magdalena Czemierska[1], Aleksandra Szcześ[2], Anna Jarosz-Wilkołazka[1]

[1]Faculty of Biology and Biotechnology, Maria Curie-Sklodowska University, Akademicka 19, 20-033 Lublin, Poland; [2]Faculty of Chemistry, Maria Curie-Sklodowska University, Pl. Marii Curie-Skłodowskiej 2, 20-031 Lublin, Poland

e-mail: magdalena.czemierska@poczta.umcs.lublin.pl

Industrial processes generate multiple secondary products which can be accumulated in soil and water and very often cause irreversible damages. The degradation of such substances is usually expensive and time-consuming. The real problem is the accumulation of harmful substances such as heavy metals, dyes, inorganic compounds like polycyclic aromatic hydrocarbons and petroleum degradation products in water reservoirs. The main aim of investigations is to find the most effective methods of the treatment. Among many processes, very interesting and promising is bioflocculation, a physico-chemical phenomenon which is based on the interaction between the bioflocculant and particles in the solution. Substances involving in the bioflocculation process and improving its efficiency are called the bioflocculants. These substances are mostly macromolecular extracellular compounds (exopolymers), consisting of polysaccharides, proteins, nucleic acids and are produced by microorganisms. Depending on the nature of their interactions with microbial cells, exopolymers could be classified as slime and capsular. In case of capsular exopolymers several extraction methods have been reported e.g. extraction with formaldehyde and NaOH, addition of EDTA, sonication, and thermal treatment.

The aim of the studies was to select the most efficient extraction method of capsular exopolymer from *Rhodococcus opacus* FCL1069 cells. Both physical (sonication) and chemical (addition of EDTA) methods were tested. *R. opacus* culture broths were incubated on a rotary shaker for 11 days (28°C, 150 rpm). During that time, the samples of cultures broths were collected every 48 h. Bacterial cells were separated from culture broth by centrifugation and were washed with 1 M sodium chloride. The extraction method selection was based on the amount of exopolymer obtaining in each variant as well as polysaccharide and protein contents. Additionally, exopolymers were tested for flocculating activity in the presence of kaolin and calcium chloride suspension.

Keywords: bioflocculation, capsular exopolymer, bacteria

Acknowledgements:

This work was supported by the National Science Centre (2016/21/N/NZ9/02309).

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