Session II. Molecular Microbiology

Opening Lecture

II.OL.1

Lipids from non-conventional feedstocks by oleaginous yeasts for sustainable biodiesel and fish feed

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Microbial oil may represent a sustainable alternative to vegetable oil, both in terms of biodiesel and food production. We investigated oil production by oleaginous yeasts grown on lignocellulose hydrolysate or crude glycerol, a waste-product of biodiesel production. Screening experiments showed that basidiomyceteous yeasts (*Rhodotorula* spec.) produce lipids more rapidly and to higher concentrations than ascomycetes (*Lipomyces* spec.). Moreover, basidiomycetes co-produce carotenes and poly-unsaturated fatty acids, which can be used as high-value chemicals. We established novel in situ methods of lipid quantification in yeast cells and extraction methods for carotenoids. Fermentation techniques were introduced to test lipid production under reproducible conditions (e.g. [1]). We were able to produce ethanol and lipids from residues of furfural extraction [2]. In an analysis of a biorefinery approach, i.e. biogas and electricity production from fermentation residues, the energy output from biolipid production from lignocellulose had an energy balance similar to ethanol production (41% of the total energy in the biomass) and resulted in substantial greenhouse gas savings [3, 4]. We have also tested lignocellulose-based yeast oil as ingredient in fish feed, and did not find any negative impact on the cultivated fish.

References:
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2. Brandenburg *et al.* (2018) Bioethanol and lipid production from the enzymatic hydrolysate of wheat straw after furfural extraction. *Appl Microbiol Biotechnol* (published online)
3. Karlsson *et al.* (2017) Greenhouse gas performance of biochemical biodiesel production from straw- soil organic carbon changes and time-dependent climate impact. *Biotechnol Biofuels* **10**: 273
4. Karlsson *et al.* (2016) A systems analysis of biodiesel production from wheat straw using oleaginous yeast: process design, mass and energy balances. *Biotechnol Biofuels* **9**: 229.

Keywords: Oleaginous yeasts, biofuels, fish feed, carotenoids

II.OP.1

Sequence and phylogenetic analyses of Dobrava-Belgrade virus in *Apodemus agrarius* from southwestern Poland

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Hantaviruses (Genus *Orthohantavirus*, Family *Hantaviridae*, Order *Bunyavirales*) are enveloped negative-sense single-stranded RNA viruses, containing large (L), medium (M) and small (S) genomic segments. Reservoir hosts belong to three taxonomic orders (Rodentia, Eulipotyphla and Chiroptera). Rodent-borne hantaviruses pose a critical worldwide threat due to outbreaks of hemorrhagic fever with renal syndrome (HFRS) and lack of effective therapeutics. In Europe, Dobrava-Belgrade virus (DOBV) and Puumala virus (PUUV) are the principal causes of HFRS. In Poland, a serologically confirmed HFRS case was first reported in 2006. Subsequently, the first HFRS outbreak, occurring in southeastern Poland, included 10 patients infected with DOBV and three with PUUV. IgG antibodies against DOBV and PUUV have also been demonstrated among forestry workers in northeastern, eastern and southern Poland. Molecular evidence of DOBV and PUUV has been reported in *Apodemus flavicollis* and *Myodes glareolus*, respectively, from southeastern Poland. To further clarify the distribution of rodent-borne hantaviruses in southwestern and northern Poland, we analyzed total RNA, extracted from lung tissues of 94 small mammals (42 *A. agrarius*, 25 *A. flavicollis* and 27 *M. glareolus*) captured in Lower Silesia (Wrocław, Milicz Ponds, and Sleza Landscape Park) and Pomerania (Gdańsk) during 2009–2015, for hantavirus RNA by nested PCR using hantavirus-specific oligonucleotide primers. DOBV was detected in two *A. agrarius* (4.8%), while *A. flavicollis* and *M. glareolus* were negative. Analysis of the L segment (coordinates 2.997–3.312) and S segment (coordinates 36–1.325) of the newfound DOBV strains showed high sequence homology at the nucleotide and amino acid levels, respectively, with DOBV strains in Europe. Phylogenetic trees generated by the maximum-likelihood method showed a shared lineage with DOBV from *A. agrarius* in Slovakia, Russia and Hungary. The molecular detection of DOBV in *A. agrarius* in southwestern Poland should alert physicians to be vigilant for HFRS cases.

Keywords: hantavirus, Dobrava, *Apodemus*, Poland

II.OP.2

Transcriptome analysis of cells carrying Type II Csp231I restriction-modification system reveals the unexpected link between its C regulatory protein and *rac* prophage

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Restriction-Modification (R-M) systems, widely spread among bacteria and archaea, represent both a mechanism of defence against infecting bacteriophages and of modulation of horizontal gene transfer. Of the four classes of R-M systems, the type II is described to be the simplest in mode of action. It is composed of a restriction-endonuclease (REase), which recognizes and cleaves specific target DNA, and of a methyltransferase (MTase), which methylates the same sequence preventing DNA from cleavage by cognate REase. Their counteracting activities, as toxin and antitoxin, need to be finely balanced *in vivo* in order to protect host DNA from damage. The molecular basis of this process are still unclear. However, current search of regulatory elements for R-M system operons is mainly focused at the stage of transcription.

Within many of the studied R-M systems, an important mode of regulation relies on the Controller (C) protein, which is a specialized transcription factor. Generally, C protein binds to a specific palindromic DNA sequence (C-box) embedded in its own promoter region to regulate its own transcription along with following REase gene.

Our study, which investigates the C protein of Csp231I R-M system, revealed the appearance of unexpected bacterial cell phenotype, which manifests as enormous cell elongation, loss of fitness and viability. Screen of several variants of R-M system showed this phenomenon to be correlated with the production of active C protein. Transcriptome analysis of cells carrying wild-type and C-deleted R-M systems brought to light a link between the presence of C.Csp231I and altered transcription of *rac* prophage-related genes. Our study aims to define the precise targets responsible for the phenomenon induced by C.Csp231I.

Keywords: R-M system, transcriptome analysis, *E. coli*, *rac* prophage.

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II.OP.3

Dual role of TonB3-PocAB system of *Pseudomonas putida*

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TonB3-PocAB system is a membrane-associated complex consisting of three proteins: TonB3, PocA and PocB. In *Pseudomonas aeruginosa* the TonB3-PocAB complex regulates motility mechanisms by assuring the right placement of FlhF which will determine the location of flagellum formation. Therefore, the absence of TonB3-PocAB results in random localization of flagella. For unknown reasons the complex is also essential for the polar placement of pili. TonB3-PocAB system's homologue in *Escherichia coli*, the TonB-ExbB-ExbD complex, is an energy transduction complex that contributes to active transport across the outer membrane by harnessing proton motive force.
We found that in *Pseudomonas putida* the TonB3-PocAB complex not only regulates the right placement of flagella but is also necessary for the maintenance of membrane integrity. Our results indicate that similarly to *P. aeruginosa* the TonB3-PocAB complex regulates the localization of flagella through FlhF but in addition to that the cells lacking intact TonB3-PocAB complex are, foremost in stationary phase, more sensitive to several stresses. There are multiple changes in the proteome of *tonB3* deficient strain in both exponential and stationary growth phase but the differences are far more extensive in exponential phase. A large portion of the changed proteins indicate that the TonB3-PocAB deficient cells have a problem with membrane integrity which could explain the increased stress sensitivity. However, the lowered stress tolerance is not related to the misplacement of flagella suggesting that the TonB3-PocAB system has a dual role in the cells of *P. putida*.

Keywords: TonB3-PocAB, membrane integrity, *P. putida*

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II.OP.4

Discovery of novel bacterial genes encoding the enzymes acting on modified uracil/uridine derivatives and their use for gene therapy in cancer treatment

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Modified nucleotides are present in several RNA species in all Domains of Life. While the biosynthetic pathways of these nucleotides were well studied in recent years, much less attention was drawn to the degradation of different RNAs and the return of modified nucleotides or their constitutents into the metabolism. Using an *Escherichia coli* uracil auxotroph strain, we screened the metagenomic libraries for genes, which would allow the conversion of either 2-thiouracil, isocytosine' or 2'-O-methyluridine into uracil and thereby lead to the growth on a defined synthetic medium. We have demonstrated that Domain of Unknown Function 523 (DUF523) containing protein is involved in the conversion of 2-thiouracil into uracil in *vivo*. We have also purified several recombinant isocytosine deaminases and a nucleoside hydrolase and demonstrated their enzymatic activities *in vitro*. These enzymes are also capable of converting the potential prodrugs 5-fluoroisocytosine, 5-fluorouridine, 5-fluoro-2'-O-methyluridine, and 5-fluoro-2'-deoxyuridine into a well-known anticancer drug 5-fluorouracil. The human glioblastoma U87MG and colorectal adenocarcinoma Caco-2 cell lines were transfected with the recoded isocytosine deaminase genes, and their citotoxicity together with 5-fluoroisoicytosine was demonstrated. The therapeutic potential of the isocytosine deaminase/5-fluoroisocytosine pair has been demonstrated *in vivo*, where the co-injection of the isocytosine deaminase-encoding mesenchymal stem cells and 5-fluoroisocytosine have been shown to increase longevity of tumorized mice by 50%.

References:
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2. Urbonavičius J, Tauraitė D, Aučynaitė A, Rutkienė R, Meškys R (2017) A pair of the isocytosine deaminases and the prodrug 5-fluoroisocytosine. *Patent Application LT2017 533*.
3. Aučynaitė A, Rutkienė R, Tauraitė D, Meškys R, Urbonavičius J. Novel bacterial deaminases convert the prodrug 5-fluoroisocytosine into the active drug 5-fluorouracil. *Submitted*.

Keywords: modified nucleosides; metagenomics; gene therapy; cancer treatment

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II.OP.5

A strange case of GreA protein and GraL sRNA

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Transcription is one of the key processes in the bacterial cell. It is performed, controlled and aided by many different proteins of varying roles. GreA is one of such proteins-it is a transcription elongation factor. It rescues backtrackedRNA polymerase complex and cleavage of the nascent RNA strandafter pausing or arresting of the transcription complex, which may result from transcription errors.As such, it improves transcription fidelity.
Despite playing an important role in the bacterial cell, control of the expression of *greA* gene is still mostly shrouded in mystery. We know that the *greA* gene is autoregulated-GreA protein inhibits its own gene expression.This is especially exciting, considering that the *greA* leader region encodes a small RNA named GraL.Only about 1/3 of transcripts reaches full length, the rest is terminated at the GraL terminator. This suggests that GraL plays a crucial, yet undetermined role in the *greA* gene regulation. Indeed, *greA* autoregulation is independent of the promoter, instead GraL sequence seems to be necessary for this process.Here we explorethe role of the small RNA GraL in the *greA* gene autoregulation- to determine if its production is necessary for autoregulation or if it is merely a by-product of such regulation.

Keywords: *greA*, transcription, GraL

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II.OP.6

A β-*N*-acetylhexosaminidase *Ph*Nah20A of the marine bacterium *Paraglaciecola hydrolytica* degrades chito-oligosaccharides and transfers GlcNAc to acceptors

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Genomes of terrestrial bacteria have been intensively investigated enabling discovery of numerous enzymes representing a large diversity of activities. By contrast, the degradation capacity of glycoside hydrolases (GHs) from cold-tolerant marine bacteria has received far less attention and characteristics of marine GHs remain understudied. Recently the isolated and genome-sequenced marine bacterium *Paraglaciecola hydrolytica* S66^T exhibits a vast potential to degrade numerous saccharides as 113 GHs were predicted in its genome [1].

β-*N*-acetylhexosaminidases (β-NAHAs; EC 3.2.1.52) are key hydrolases in degradation and modification of *N*-acetylhexosamine-containing compounds, and classified into GH families 3, 20, 84, 109 and 116.

We showed that *P. hydrolytica* effectively degrades chito-oligosaccharides (COSs) which are abundant in marine environments by β-NAHA activity. A total of four EC 3.2.1.52 β-NAHAs were identified in the genome sequence of *P. hydrolytica*: *Ph*Nah3A, *Ph*Nah3B, *Ph*Nah20A and *Ph*Nah20B. The β-NAHA sequences shared only approx. 30% identity with characterized β-NAHAs. *Ph*Nah20A was predicted to belong to an operon of six genes with conserved organization in related marine bacteria. *Ph*Nah20A was successfully produced in *E. coli*. Purified enzyme was unstable, but stabilized by BSA or Triton X-100. The enzyme presented similar catalytic efficiencies for *p*NPGlcNAc and *p*NPGalNAc. *Ph*Nah20A was also able to form lacto-*N*-triose II (LNT2), a core structure of human milk oligosaccharides, by transglycosylation using chitobiose or *N*-acetylglucosamine-oxazoline as donor and lactose as acceptor. A series of mono- and disaccharides also worked as acceptors. NMR identified LNT2, β-Gal-1,4-β-Glc-1,1-β-GlcNAc and β-Gal-1,4-(β-GlcNAc)-1,2/3-Glc as main transglycosylation products from lactose and *N*-acetylglucosamine-oxazoline. *Ph*Nah20A is thus biochemically different from previously characterized β-NAHAs and revealed additional features not shown for marine GH20 before.

References:

1. Schultz-Johansen M *et al.* (2016) *Genome Announc* **4**: e00304.

Keywords: chitobiose degradation, *N*-acetylhexosamine specificity, stability enhancer, lacto-*N*-triose II

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