LBA.1

Sequence differentiation and expression among genes of the folic acid biosynthesis pathway in industrial *Saccharomyces cerevisiae* strains

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Folic acid is important vitamin in human nutrition and its deficiency in pregnant women's diets results in neural tube defects and other neurological damage to the fetus. Additionally, DNA synthesis, cell division and intestinal absorption are inhibited in adults. Since this discovery, governments and health organizations worldwide have made recommendations concerning folic acid supplementation of food for women intending to become pregnant. This has led to the introduction in many countries of fortification, where synthetic folic acid is added to flour. It is known that *Saccharomyces cerevisiae* (brewers' and bakers' yeast) is one of the main producers of folic acid and they can be used as natural source of this vitamin. Proper selection of the most efficient strains may enhance the folate content in bread, fermented vegetables, dairy products and beer by 100%.
The aim of this study was to select the optimal producing yeast strain by determining differences in a nucleotide sequences in the *FOL2*, *FOL3* and *DFR* genes of folic acid biosynthesis pathway. The Multitemperature Single Strand Conformation Polymorphism (MSSCP) method and further nucleotide sequencing for selected strains were applied to indicate SNPs in selected gene fragments. Real Time PCR technique were also applied to examine relative expression of *FOL3* gene.
It was observed that correlation exists between the folic acid amount produced by industrial strains and changes in the nucleotide sequence of adequate genes. The study revealed correlations between nucleotide sequence changes and amino acid composition in fitting genes. The most significant changes occur in the *DFR* gene, mostly in the promoter part, which causes major protein structure modifications in KKP 232 and KKP 277 strains.

Key words: folic acid, *Saccharomyces cerevisiae*, gene polymorphisms

LBA.2

Molecular and physiological comparison of four *Saccharomyces cerevisiae* wine yeast strains

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Yeasts belonging to genus *Saccharomyces* are widely used for commercial applications, especially in wine and brewery industry. These industrial strains are selected due to their specific properties including transformation of carbohydrates into alcohol during the fermentation process or tolerance to high levels of ethanol and sugar.
Our research is based on physiological properties of four various *Saccharomyces cerevisiae* strains and molecular detection of genes responsible for tolerance of technological stress factors.
Aim: Differentiation of four industrial wine *Saccharomyces cerevisiae* strains using analysis of δ sequences. Physiological properties of these strains and detection of genes responsible for tolerance of high osmotic pressure and high levels of alcohol.
Methods: Strains were obtained from Culture Collection of Industrial Microorganisms of prof. Wacław Dąbrowski Institute of Food and Agriculture Biotechnology. Assimilation tests performed for carbon and nitrogen sources. Genomic DNA isolation conducted using yeast cells from liquid culture in line with a phenol-chloroform method. PCR amplification was carried for fragments of *GPD1*, *TPS1*, *TPS2* genes and δ sequences.

Key words: *Saccharomyces*; δ sequences; ethanol stress; osmotic pressure

LBA.3

Omics approach in analysis of *Pseudomonas mandelii* ssp. capable of degrading hexachlorocyclohexane

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Background and Aims: Upon intensive agriculture, pollutants like pesticides have tendency to accumulate in soil which is the greatest reservoir of microbes which are potential source of genes encoding biotechnologically relevant traits. Knowledge about majority of soil microorganisms and their metabolism of toxic compounds still remains elusive. As prokaryotes are known for their high adaptability to different environmental conditions, there is very likely that soil bacteria poses ability for production of vast number of bioactive molecules involved in pesticide decomposition. Our aim was to identify microorganisms capable of degrading hexachlorocyclohexane (γHCH) and investigate this metabolic process by bioinformatic analysis of deeply sequenced bacterial genome coupled with transcriptomic data from RNA-seq and proteomic data from mass spectrometry.
Methods: Bacteria were isolated from heavily contaminated soil samples, derived from expired pesticide storage infrastructure in Poland. Isolated microorganisms were tested for growth abilities on media with pesticide as a main carbon source. Genomic DNA and mRNA were isolated from pure cultures and sequenced using Illumina HiSeq and MiSeq platforms. Transcriptomic and proteomic profiles were compared for bacteria living on a medium with and without γHCH.
Results: Screening of isolated bacteria resulted in identification of *Pseudomonas mandelii* ssp. exhibiting ability to form a clear zone around the colony, what indicated its capability for γHCH degradation. Reads assembly form genome sequencing resulted in nearly complete 6Mb genome. Intriguingly, analysis of assembled genome did not revealed presence of known genes coding proteins involved in γHCH degradation. Comparison of RNA-seq of bacteria grown in presence of γHCH against the ones from control conditions revealed that mRNA coding 5 specific proteins is elevated 25 to 60 times. Further studies showed that four of identified genes are localized in the same locus possibly creating an operon. Most of the identified mRNAs encode proteins from the family of quinohemoproteins, however, with very low identity in aminoacid sequences to known and previously tested enzymes. Exact function of proteins described above in γHCH degradation well be investigated in close future by proteomic and biochemical analysis.
Keywords: pesticide, hexachlorocyclohexane, genomics, transcriptomics

LBA.4

Identification of selected microorganisms from activated sludge able to benzothiazole and benzotriazole transformation

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Benzothiazole (BT) and benzotriazole (BTA) are present in the environment — especially in urban and industrial areas, usually as anthropogenic micropollutants. The presence of BT and BTA has been found in surface water, groundwater, seawater, lake water, in industrial and municipal wastewater, as well as in soils and sediments. The origin of presence those substances in environment are various industry branches (e.g. food, chemical, metallurgical or electrical industry), households and surface runoff from industrial areas. Increasingly strict regulations on water quality and the fact that discussed compounds are poorly biodegradable make that they become a serious problem in the environment. Considering this, is important to look for environmentally friendly and socially acceptable ways to remove BT and BTA. The aim of the study is to identify microorganisms capable of benzothiazole (BT) and benzotriazole (BTA) transformation or/and degradation in aquatic environment. Selected microorganisms were isolated from activated sludge. The identification of microorganisms capable of BT and BTA removal was possible using molecular biology techniques (PCR, DNA sequencing). Among isolated microorganisms of activated sludge are bacteria potentially capable of BT and BTA biotransformation and/or removal. The 12 bacteria species able to benzothiazole and benzothiazole were identified. They can grow in medium with BT and BTA as a only source of carbon. Microorganisms previously adapted to the presence of studied substances at a concentration of 10 mg/l showed a greater rate of growth of colonies on media than microorganisms unsuitable for the presence of such compounds.

Key words: biotransformation, benzothiazole, benzotriazole, DNA sequencing, PCR

Acknowledgements: Katarzyna Kowalska is scholar of DoktoRIS — Scholarship Program for Innovative Silesia, co-financed by the European Union under the European Social Fund.