Session IV. Biotechnology

IV.P.1

Esters and higher alcohols determination in lager beer during primary fermentation performed at industrial scale

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Production of secondary metabolites such as volatiles compounds depends mainly on viability and vitality of yeast used in fermentation process. The esters and higher alcohols produced by yeasts during fermentation were reported to have large effects on the final sensorial quality of fermented beverages. This work aimed to assess the fluctuations in beer esters and higher alcohols content due to process parameters variation during primary fermentation. Therefore, fermentation trails were conducted at industrial scale using a commercial lager brewing yeast. Esters and higher alcohols profiles were determined following fermentation performance by monitoring yeast growth, ethanol synthesis, original wort gravity, pH and tank pressure throughout the fermentative process. Results showed increase in yeast cells ($58\pm6.55\times10^6$ cells/ml) due to the high yeast multiplication resulting in intense metabolic activity. Indeed, increased ethanol yield ($3.83\pm1.07\%$) recorded after 5 days of fermentation demonstrated higher yeast activity. Yeast grow was probably enhanced by wort fermentable sugars (glucose, fructose, maltose and maltotriose) and free amino acid content. Additionally, interactive effects of process parameters (temperatures, yeast pitching rates and wort specific gravity) could influence positively the yeast physiology and enable secondary metabolites production. Interestingly, the production of 2-methyl-1-butanol (44.82 ± 4.12 mg/l), and ethyl acetate (14.89 ± 4.58 mg/l) was significantly improved during the primary fermentation. However, acetaldehyde content increased simultaneously only within 3 fermentation days (10.51 ± 0.012 mg/l). These flavor-active compounds contribute to the overall sensorial properties of the final beer.

Key words: esters, higher alcohols, beer

IV.P.2

The effect of dietary fibre preparations from maize starch on the growth and activity of bacterial strains belonging to the phyla *Firmicutes*, *Bacteroidetes*, and *Actinobacteria*

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Over the past few decades there has been a significant increase in the prevalence of obesity in both children and adults. Obesity is a disease that has reached epidemic levels on a global scale. The development of obesity is associated with both environmental and genetic factors. Recent studies indicate that intestinal microorganisms play an important function in maintaining normal body weight.
The objective of the present study was to determine whether dietary fibre preparations from maize starch can stimulate the growth of *Bacteroidetes* strains while inhibiting growth of *Firmicutes* strains.
Dietary fibre were prepared from maize starch by simultaneous thermolysis and chemical modification in the presence of a volatile inorganic acid (hydrochloric acid) as a catalyst of dextrinization and an organic acid (citric acid in the fibre preparation K1 and tartaric acid in the fibre preparation K2) as a modifying agent. The study material consisted of faeces from 7 obese children aged 5 to 15 who were patients of the Children's Memorial Health Institute in Warsaw. The study group was selected based on the criteria of the International Obesity Task Force (IOTF), developed by Cole *et al.*
Fluorescent *in situ* hybridization (FISH) and determination of short-chain fatty acids SCFA and branched fatty acids BCFA using high-performance liquid chromatography.
Dietary fibre preparations from maize starch stimulated the growth of *Bacteroides* and *Actinobacteria* bacterial strains, while reducing the population of *Firmicutes* bacteria. The dietary fibre preparation produced with tartaric acid was more selective than the preparation obtained with citric acid. These preliminary results are currently being tested *in vivo*.

Key words: *Firmicutes, Bacteroidetes*, obesity, dietary fibre, prebiotics

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IV.P.3

Assesment of the oil contaminated soil and oil sludge bioremediation effectiveness

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In connection to significant intensification of oil and petroleum products process of exclusion of land from agricultural use have a large scale. In case of pipeline damage and due to leakage of equipment, a local soil is contaminated by oil and oil products. It is more rational to use biotechnological methods of bioremediation due to the action of microorganisms in the soil; the cleaning process runs smoothly as possible.
The aim of this study was to assess the efficacy of biological product's prototype using for cleaning of oil-contaminated soil and sludge from the "UMG" landfill. The content of petroleum hydrocarbons in contaminated soil samples ranged from 40 g/kg to 152 g/kg and oil sludge 230 g/kg soil.
Contamination of polluted soil at a low dose did not cause significant changes in the number of saprotrophic bacteria, the abundant development of microorganisms resistant to high concentrations of pollutant were observed but the normal soil bacteria were suppressed. The high concentration of hydrocarbons in contaminated soil samples caused a significant decrease in the number of saprotrophic bacteria. Low numbers of saprotrophic bacteria were also observed in the oil sludge.
In model studies, samples with minimal contamination, product had no significant influence on the content of hydrocarbons. With a high degree of contamination, the product was more effective speeding up the process of purification by 30–50% compared with the samples of contaminated soil and sludge without bacterial preparation.
During the pilot industrial tests it was founded that the biological product showed high activity against destructive petroleum hydrocarbons in this field, and can be successfully used for the reclamation of large areas and volumes of oily waste, the percentage of utilization for 30 day was 80% of the mass of the hydrocarbons in the soil and soil sludge.

Key words: bioremediation, petroleum hydrocarbons, soil sludge, biological product

IV.P.4

Phylogenetic analysis of HP0231 and crystal structure of its truncated form (HP0231m)

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In many Gram-negative bacteria, the periplasmic space is the major site for the maturation of proteins that enter this compartment. The three-dimensional structure of proteins is crucial for their function and stability. The proper folding of proteins containing cysteine residues, especially those with an even number of cysteines, often requires the oxidation of thiol groups between two cysteine residues and the formation of a covalent bond.
The *H. pylori* Dsb system is potentially a novel Dsb oxidizing system that is different from the Dsb systems operating in *E. coli* or in phylogenetically related *Campylobacter* sp.
H. pylori does not encode the classical DsbA/DsbB oxido reductases that are crucial for oxidative folding of extracytoplasmic proteins. Instead, it possesses an unusual pair of proteins involved in disulfide bond formation – periplasmic HP0231 with a dimeric structure resembling EcDsbG/C, and its membrane partner HpDsbI (HP0595), a protein with a Beta-propeller structure very rarely present in bacteria. We showed that HP0231 is most closely related evolutionarily to the catalytic domains of DsbG, even though it possesses catalytic motifs typical for canonical DsbA proteins. Similarly, the highly diverged N-terminal dimerization domain is homologous to the dimerization domain of DsbG. Additionally, we constructed a truncated HP0231 lacking the dimerization domain, denoted HP0231m. The HP0231m structure was solved and compared to the structures of EcDsbA and MtDsbA. The structure can be superimposed onto the full-length HP0231 structure (PDB: 3tdg) and we do not observe any significant differences between them. The strand Beta-1 forms hydrogen bonds to Beta-3, a feature observable in structures of catalytic domains of DsbG, DsbC, and also in class II DsbA-like proteins. This is in agreement with our phylogenetic analysis, which showed that the DsbG/C/HP0231 branch is more closely related to class II DsbA-like proteins than to canonical class I DsbA.

Key words: *Helicobacter pylori*, disulfide bonds, Dsb proteins

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IV.P.5

The role of stringent response in polyhydroxyalkanoates synthesis by *Pseudomonas putida* KT2440

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Polyhydroxyalkanoates (PHAs) are a class of biological polyesters naturally synthesized by bacteria as discrete granules and used as a storage material for carbon and reducing equivalents. Generally, PHAs are produced when the carbon source is present in excess and essential nutrient such as nitrogen, phosphate or oxygen is available in limited concentrations. Generally, PHAs can be divided into two groups according to the number of carbon atoms in their monomeric units: short-chain-length hydroxyalkanoic acids (3–5 carbon atoms, scl-PHA) and medium-chain-length (6–15 carbon atoms, mcl-PHA). Mcl-PHAs have gained much interest in research on biopolymers because of their ease of chemical modification. In view of the useful properties, mcl-PHAs may be promising materials in various spheres, especially in biomedical applications such as tissue engineering and drug delivery. Although various aspects of PHAs production, including bacterial fermentation, isolation and physicochemical characterization of the polymer have been studied extensively during the past few decades, knowledge on the molecular mechanism of this polymer biosynthesis is still unclear. In particular, little is known about the regulation of gene expression associated with the synthesis of PHAs.

Therefore, the main goal of this work is to analyse the role of stringent response during mcl-PHAs biosynthesis. In order to uncover the relation between these two processes the cultivations of *Pseudomonas putida* KT2440 and its *relA* mutant towards mcl-PHAs production were carried out. Sodium gluconate and oleic acid were used as external carbon sources. It was shown that the wild strain accumulated 12% and 19% of mcl-PHAs when the medium was supplemented with sodium gluconate and oleic acid, respectively. The results clearly indicate that the *relA* mutant synthesised from 2% to 8% of biopolymers depending on the substrate. The obtained results suggest that synthesis of ppGpp and thereby stringent response could be involved in the process of mcl-PHAs synthesis.

Key words: polyhydroxyalkanoates, *Pseudomonas putida,* stringent response

IV.P.6

Functional characterization of the *Helicobacter pylori* HP0377– determination of isomerase activity

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Helicobacter pylori is the second most common human pathogen in the world. *H. pylori* infection induces both acute and chronic gastritis and peptic ulcers. It is also considered to be a high-risk factor for the development of mucosa-associated lymphoid tissue lymphoma and adenocarcinoma of the stomach. HP0377 is a thioredoxin-fold protein containing the CSYC motif, which indicates that it functions as a disulfide oxidoreductase. Although there is no evidence that HP0377 is involved in cytochrome *c* assembly *in vivo*, that is the likely case because its resolved structure is similar to that of other CcmG (*cytochrome c maturation*) proteins, and because it is able to reduce the oxidized form of apocytochrome *c in vitro*. Additionally, *hp0377* gene is co-transcribed with the *ccsBA* (*hp0378*) involved in heme transport and its ligation to apocytochrome *c*.

HP0377, like other CcmGs, does not reduce insulin. This finding is consistent with a generally accepted view that CcmG proteins are specific thiol-oxidoreductases involved only in the cytochrome *c* maturation pathway. Therefore, HP0377 likely cooperates with a specific substrate, which is typical of CcmG proteins. However, these data are in contrast to some previously published data.

To clarify this inconsistency, and because there is a similarity between the active site of HP0377 (CSYC motif and Thr in *cis*-Pro loop) and the disulfide isomerase EcDsbC (CPYC motif and Thr in *cis*-Pro loop), we performed a disulfide isomerase assay by evaluating the ability of HP0377 to reactivate oxidized, scrambled RNase (scRNaseA). In this assay, the refolding efficiency of HP0377 was almost as high as EcDsbC. It is unique among CcmGs. What is more, when compared to another CcmGs amino-acid sequences, HP0377 lacks highly conservative glutamic acid in 95 position and has instead of it phenylalanine. Thus, we decided to generate mutated HP0377 (F95AandF95E) by site-directed mutagenesis and evaluate whether these changes influence on HP0377 isomerase activity and its ability to reduce apocytochrome *in vitro*.

Keywords: *Helicobacter pylori*, HP0377, CcmG, isomerase activity

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IV.P.7

Effect of application of micromycetes on plant growth and crop yield

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Previously selected active strains of micromycetes with plant growth-promoting activity (*Penicillium bilaiae* Pb14, *Aureobasidium pullulans* YA05 and *Rhodotorula mucilaginosa* YR07) were taken for micro field experiments. These strains were used as a basis for various compositions of preparations – application of each strain separately and application of complex of micromycetes.
Microfield experiments were conducted in small plots (1×1 m) to test the effect of micromycetes strains as bio-fertilizers. Soybean seeds (*Glycine max* cv. Almaty) were used in the experiment. The structure of soybean yield in the phenological phase of seed ripening was studied.
It was shown that the soybean yield ranged from 26.4±0.5 quintal/ha to 33.8±0.5 quintal/ha depending on the variant of the experiment. The yield increase in experiments ranged from 4.5 to 7.4 quintal/ha (17–28%). The highest yield was obtained in the variant with mixed composition – 33.8±0.5 quintal/ha.
Analysis of the data revealed a positive effect of micromycetes strains on the growth characteristics of soybean, which is reflected in increasing of plant height at 21–27% increase in the number of pods and seeds per plant at 19–25% and 21–29%, respectively. There was a significant increase in the mass of 1000 seeds at 18–26% in the experimental variants compared to the control. The highest mass of 1000 seeds was obtained in variant with complex of micromycetes where the value of this parameter reached 219.7±5.5 g.
It was found that increase of soybean yield and various parameters of structure of the yield was higher in variant with complex of micromycetes compared to the separate application of each strain. This suggests the possibility of synergistic effect between the components of micromycetes composition.

Key words: micromycetes, plant growth-promotion, crop yield

IV.P.8

The effect of polyphenols on *Bifidobacterium* growth

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Polyphenols are common group of plant based bioactive compounds, which can affect on human health because of their antioxidant and antimicrobial properties as well free-radical scavenging activity. An increasing interest is observed in the interaction between polyphenols and microbiotao ccurring in food and human gut. The aim of the presented work was evaluation the effect of some polyphenolic compounds on the growth of two strains of *Bifidobacterium*: *B. adolescentis* and *B. bifidum*.
The influence of some flavonoids: naringenin, hesperidin, rutin, quercetin, catechin as well as phenolic acids: gallic, caffeic, *p*-coumaric, ferulic, chlorogenic and syringic was determined by 96-well microtitre plate assay. MRS broth containing polyphenols in the range of concentration 8 μg/ml to 1 mg/ml was inoculated with bacterial cultures. The medium without addition of polyphenols with the same amount of bacteria was a positive control. Microplates were incubated at 37°C for 48 h and the absorbance was measured at 600 nm in a microplates reader after 24 and 48 h. Kinetics of growth was also carried out for chosen compounds.
All tested compounds influenced on the growth of examined bacteria, however both inhibitory and stimulatory effect was observed. In the higher concentration all polyphenols inhibited the growth of *Bifidobacterium* strains, but most of them showed stimulating effect in small amounts. Moreover, positive influence of some polyphenols was noticed after longer time of cultivation.

Key words: polyphenol, probiotic, inhibition

IV.P.9

A fusion of streptavidin with listeriolysin O as a universal cytotoxic component of an immunotoxin

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Immunotoxins (IT) have been developed over past 40 years as a promising therapeutics for potential use in the targeted cancer therapy. IT consists of two main fragments, cytotoxic agent (i.e. bacterial or plant toxins) and carrier molecule (monoclonal antibodies) which identifies and binds to target cells. Listeriolysin O (LLO) is the main virulence factor of *Listeria monocytogenes* and belongs to the cholesterol-dependent-cytolysin (CDC) protein family. LLO unique properties, which allows *L. monocytogenes* to survive inside host cells, suggest that it may be a good candidate for an immunotoxin construction.
A *sav* gene (encoding streptavidin) was fused to *hly* gene (encoding LLO) using OE-PCR method and resulting *sav-hly* fusion gene was cloned into *E. coli* expression vector. The resulting *E. coli* strain was used for production of fusion protein, which was then purified from bacteria lysate by affinity chromatography and gel filtration. Afterwards purified SAV-LLO protein was assayed for hemolytic activity and compared to the *wt* LLO activity. The binding of biotinylated monoclonal antibody (anti CD-20) and SAV-LLO was performed *via* streptavidin-biotin interactions. Obtained immunotoxin (mAb-SL) was assayed for hemolytic and cytotoxic activity. Direct comparison showed that SAV-LLO displayed approximately 28-fold lower activity than *wt* LLO, while mAb-SL had no hemolytic activity. The *in vitro* cytotoxicity assays were performed on Raji (CD20+) and Jurkat (CD20-) cell lines. The experiments showed that mAb-SL immunotoxin had reduced the population of Raji cells to approximately 30%, while Jurkat cells remained unaffected.
The differences in cytotoxic activity of mAb-SL on Raji and Jurkat cells and the total lack of hemolytic activity may suggest that this molecule potentially may become a potent immunotoxin, but further research is necessary in order to confirm the specificity of this molecule.

Key words: listeriolysin O, immunotoxin, streptavidin

IV.P.10

Antibiofilm activity of some essential oils against *Escherichia coli* and *Staphylococcus aureus*

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Essential oils, generally assumed to be more acceptable than synthetic substances, could be alternative for antibacterial and antifungal compounds. An increasing interest in the control of biofilm formation is also observed. In the presented work antimicrobial and antibiofilm activity of some commercial essential oils towards *Escherichia coli* and *Staphylococcus aureus* were examined. Twelve oils were used: cinnamon, orange, oregano, mint, lemon balm, clove, clove bud, grapefruit, sage, rose, lavender and anise.
Antibiofilm activity of tested essential oils was determined by 96-well microtitre plate assay. Bacteria were grown overnight at 37°C in nutrient broth and cultures were dispensed into wells containing medium with differentiated concentrations of essential oils. The microplates were incubated at 37°C for 48 h, washed three times with sterile water to remove loosely associated cells and dried at 60°C for 45 min. After drying, 100 µl of 0.4% crystal violet solution was added to all the wells. The biofilms formed in the bottom of the wells were washed with distilled water and immediately distained with 125 µl of 95% ethanol. Next, 100 µl of well solution were transferred to a well in a new plate and the absorbance measured at 600 nm in a microplates reader. The amount of biofilm formed was calculated by subtracting the absorbance values from control well.
All tested essential oils decreased biofilm formation by examined bacteria, however the results depended on the used compound and its concentration. Both *E. coli* and *S. aureus* were sensitive for at least eight of twelve oils, even in small concentration. The best results were obtained with clove and cinnamon essential oils.

Key words: essential oils, biofilm, antimicrobial activity

IV.P.11

Alternative substrates for microbial production of fumaric acid

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Biotechnological production of fumaric acid is one of the key elements of innovative technological processes collectively referred to as "green chemistry". Past efforts have focused on the search for microorganisms with the best production characteristics and to find ways to increase their productivity using media based mostly on glucose and yeast extract. Cost-effectiveness of biotechnological processes is associated with the search for alternative sources of carbon and nitrogen in both organic and mineral origin. The alternative for pure and expensive substrates may be crude glycerol, which is the waste byproduct of biodiesel production process. There are also innovative installations, where glycerol is obtained trough hydrolysis of animal fatty waste to stearin. Its microbiological conversion mainly to organic acids brings significant economic benefits. Crude glycerol contains lots of inorganic salts and MONG (matter organic non glycerol) which can be used for enhancing biomass growth and modify metabolic profiles.
Selected strains belonging to the genus *Rhizopus* sp. were tested for their ability to produce fumaric acid in media containing crude glycerol as a sole carbon source and as a co-substrate for wheat syrup. The second option proved to be more efficient. The other part of the experiment consisted of screening for choosing the best organic and mineral sources of nitrogen for growth of mycelia and, what is connected, enhance production of fumaric acid. Supplementation with potassium nitrate, ammonium nitrate, soybean/rapeseed and alfalfa meal were investigated. The use of some nitrogen sources revealed significant differences in the production of fumaric acid.

Key words: fumaric acid, crude glycerol, nitrogen source

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IV.P.12

Method of the measurement of the activity of polyketide synthase Cpk in *Streptomyces coelicolor* A3(2) mutants

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Streptomyces coelicolor A3(2) is a model organism for the genetic studies of the bacterial genus comprising producers of the majority of currently known antibiotics and other natural compounds of medical and industrial importance. Modular type I polyketide synthase Cpk from *S. coelicolor* A3(2) is responsible for the production of an unstable polyketide (coelimycin A) with antibacterial activity which is released to the medium. Coelimycin A posesses two epoxide rings which react with components of the culture medium and this gives rise to a mixture of products. Despite of the visible yellow pigmentation of the culture medium, absorbance measurements are not precise due to the lack of a clear peak in the spectrum. Cpk synthase products have also fluorescent properties, however both absorbance and fluorescence spectra change strongly with time. Moreover the compounds are highly water soluble and can not be extracted with organic solvents. Two yellow compounds arising from Cpk synthase activity have been identified so far – coelimycin P1 and P2 which are, respectively, N-acetylcysteine and glutamate adducts of coelimycin A.
Here we report a method of detection and relative quantification of coelimycin P2 based on HPLC analysis. The use of the minimal medium instead of rich organic media allowed to obtain a simple chromatogram in a direct measurement of culture medium. Thanks to supplementation with glutamate, the double epoxide is transformed mainly into coelimycin P2. This allowed us to compare coelimycin P2 production by *S. coelicolor* A3(2) strains bearing mutations within *cpk* gene cluster. To our knowledge, this is currently the most effective method available for measurement of the activity of Cpk synthase.

Key words: polyketide synthase, coelimycin, yCPK

IV.P.13

Glance of *Halobacterium salinarum* metabolism: bacteriorhodopsin and siderophore

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Bacterial rhodopsins are a family of bacterial opsins and retinal-binding proteins that provide light-dependent ion transport and sensory functions. Siderophores are low molecular weight produced microorganism. These mechanisms are important to know extremophilic bacteria ion-metabolism. For this reason, in this study we searched that *Halobacterium salinarum* siderophore and bacteriorhodopsin existences. Detection of siderophore existence, we were used Chrome azurol sulphonate (CAS) assay. Also, we were determinated siderophores chemical characterization by tetrazolium and Arnow‘s test. In order to determination of bacteriorhodopsin were analyzed bacterial rhodopsin gene sequence conventionally by Polymer chain reaction (Pcr). As a result, we showed that *Halobacterium salinarum* xeno-siderophores and molecular analysis results showed bacteriorhodopsin. We have worked on *Halobacterium salinarum* iron-uptake mechanism and rhodopsin existence,that can provide to understand ion transport metabolism.

Key words: bacteriorhodopsin, siderophore, bacterial opsin

IV.P.14

Role of the protein acetylation in processing of proteins aggregated as inclusion bodies

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Acetylation of lysine residues is conserved post-translational modification and occurs in all three kingdoms. In eukaryotes protein lysine acetylation regulates diverse physiological processes (cell cycle, cell morphology, protein synthesis, mRNA splicing, central metabolism). The role of acetylation in bacteria is still unknown however global proteomic studies revealed the existence of a few hundred acetylated proteins.
Here we investigated the influence of protein lysine acetylation and deacetylation on formation of inclusion bodies (IBs) of aggregation-prone VP1GFP protein, overproduced in *Escherichia coli*. In cells with decreased deacetylation (the *cob* mutant) IB production was delayed however the amount of VP1GFP was similar in all tested strains. Protein acetylation influenced also the resolubilization and reactivation of the aggregated VP1GFP. Our results indicate an essential role of protein acetylation in controlling protein aggregation and inclusion body formation in bacteria.

Key words: lysine acetylation, inclusion bodies

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IV.P.15

Molecular characterization of lytic enzymes from bacteria belonging to the genus *Clostridium*

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The phenomenon of increasing antibiotic resistance forced the need to find new ways to prevent and treat bacterial diseases, especially those caused by multi-drug-resistant bacteria. Before widespread use of antibiotics, physicians successfully treated bacterial infections with bacteriophages. Clinical use of phages and their lytic enzymes declined with increased use of antibiotics but now again attracts attention.
Lytic enzymes form a diverse group of proteins consisting of endolysins of bacteriophages and autolysins of bacterial origin. Endolysins at the end of bacteriophage lytic cycle cause lysis of the infected cell and the release of progeny virions. Bacterial autolysins are involved in numerous cellular processes including cell growth, cell-wall turnover, peptidoglycan maturation, cell division, separation, differentiation and pathogenicity.
Depending on the type of bond targeted in the structure of peptidoglycan, lytic enzymes may be divided into five classes: muramidases, glycosaminidases, endopeptidases, amidases, and lytic transglycosylases. The lytic enzymes presented in this work belong to group N-acetyl-muramyl-L-alanine amidases. Amidases are the most universal lytic enzymes – as the amide bond is the most conserved in the peptidoglycan.
The sources of enzymes used in this study are anaerobic pathogens: *Clostridium botulinum* and *Clostridium perfringens*. At the amino acid level, studied enzymes are similar to eukaryotic Peptidoglycan Recognition Proteins (PGRPs). In case of amidases of *Clostridium perfringens* we will examine two proteins: the larger enzyme includes additional N-terminal 65 amino acids most likely responsible for transport of the lytic enzyme across the cytoplasmic membrane. The aim of my project is to experimentally define the role of this putative signal peptide. The gene of the third investigated protein is derived from *Clostridium botulinum*, and is probably encoded by the prophage. The genes of these three amidases have been cloned, and recombinant proteins were overproduced in *E. coli* BL21(DE3). Purified proteins show activity against bacterial substrate *Clostridium sporogenes* ATCC7955.

Key words: peptidoglycan hydrolase, cell lysis, PGRP, endolysin

IV.P.16

Endophytic bacteria of Trans-Ili Alatau plants – promising components of microbial product for agricultural use

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Nowadays, the association of plants with useful microorganisms takes attention of scientists in terms of their possible use in the practice of adaptive crop research related to the environment. World practice has been developed a number of biological products, which are based on useful strains of endophytic bacteria.
From the tissues of plants grown in the foothills and piedmont plains of Trans-Ili Alatau, more than 382 isolates of endophytic bacteria cultured forms were isolated. The criteria for screening of strains with economically valuable properties, was the ability to produce the plant hormones and stimulate crop growth. Using the visible evaluation of the colorimetric method's results with Salkovskij reagent it was founded that some strains produce fairly active IAA on a medium with L-tryptophan. Among the strains studied 26 strains (66%) showed a positive response to high-quality production of IAA. Bacteria synthesized IAA from 18.6±1.1 to 82.4±2.3 mcg per ml. Such strains as *Streptosporangium sp.* KK1 (44.1 mkg/ml), *Rhodococcus sp.* KK2 (42.5 μg/ml), *Streptomyces sp.* KK3 (44.9 mg/ml) produced IAA more intensely but especially active producer of auxin is a *Jeotgalicoccus sp.* VAK1 strain, whose total production level of IAA reached to 82.4 mcg/mL.
The assessment of growth-stimulating effect during the interaction of bacterial culture with seedlings of agricultural plants is an important criterion. Thus, the phytotest conducted on soybean and barley seedlings showed that diluted cultures of the test bacteria strains are capable to stimulate the growth of plants *in vitro*. Especially expressed stimulatory effect was showed by *Streptomyces griseorubiginosis* KK4, *Streptomyces sp.* KK5 and *Jeotgalicoccus sp.* BAK1 strains, which was manifested in a significant increase of the length of the soybean's and barley's roots of and barley in comparison to the control without treatment.

Keywords: endophytic bacteria, plant growth-promotion, indole-3-acetic acid, barley, soybean

IV.P.17

Antimicrobial properties of montmorrilonite and soft elastomers containing lysozyme-functionalized montmorillonite

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Silicone-based elastomers are technologically-relevant materials used, amongst others, in medicine, civil engineering, and automotive applications. Here we report a study on blends of a commercially available silicone resin based on hydroxyl-terminated poly(dimethylsiloxane) (PDMS), Nanofil 116 (MMT) and lyophilized lysozyme (LYS) with activity 100 000 units/mg.
Static mechanical testing revealed that the elastomers containing 5 wt. % of protein-functionalized montmorillonite (LMMT) have interesting mechanical properties. Comparison PDMS and the PDMS/LMMT blend leads to a conclusion that the addition of LMMT reduces Young moduli by approx. 30%. In other words, the elastomer containing 5% of LMMT is by 30% softer than the unmodified PDMS resin. This observation is in contrast to what is typically observed when montmorillonite is added to a polymer. Addition of LMMT to PDMS renders also the elastomer selectively antimicrobial as revealed by tests performed according to National Committee for Clinical Laboratory Standards (NCCLS, Performance Standards for Antimicrobial Disk Susceptibility Tests; 11th edn, 2012; Approved Standard M02-A11). We tested 3 strains: *Escherichia coli* ATCC 1129, *Staphylococcus aureus* ATCC 6538 and *Klebsiella pneumoniae* ATCC 4352. Silicone-LMMT elastomer showed antimicrobial action only against one of tested strains: *K. pneumoniae* ATCC 4352. The inhibitory zone diameter was 11 mm. The results suggest that capsular polysaccharide may be the target of Silicone-LMMT blend antimicrobial action.
We have also decided to verify the antimicrobial properties of pure MMT, using slightly modified method described before [1, 2]. We tested strains: *S. aureus* ATCC 6538, *K. pneumoniae* ATCC 4352 and *E. coli* O24 (Polish Collection of Microorganisms). All tested strains incubated in liquid YP medium with 0.035 g of MMT (Nanofil 116) and in physiological saline with 0.035 g of MMT for an hour were sensitive to MMT bactericidal action. The results were quite surprising, as bactericidal action of pure MMT was not described before in the literature.

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Keywords: montmorillonite, bactericidal activity of lysozyme, polymers

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IV.P.18

SynKeD – Synthetic polyKetide Designer, a synthetic biology tool for computer aided designing of new polyketide products

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Polyketides are a large group of secondary metabolites, which are produced by certain microorganism and fulfill a number of different biological activities. They are structurally complex and are synthesized by several rounds of condensation of short building blocks – carboxylic acids. The chain elongation is accomplished by multienzyme complexes called polyketide synthases (PKS). Polyketides play a big role in biotechnology, medicine and drug discovery. Many current pharmaceuticals and medicaments were derived from these compounds. Because of the modular structure, new polyketides can be easily designed and synthetized. To facilitate the designing the new synthetic polyketides and their pathways, we worked out an informatics tool called Synthetic polyKetide Designer (SynKeD). The idea of the SynKeD comes directly from founding of synthetic biology, which aims at synthesis of new chemical compounds by genetically engineered organisms. The genetic engineering combines known natural metabolic pathways in a way to get new products.
SynKeD implements the idea of using well-known and described modules of modular type IPKS's, which can be considered as a "bricks" for construction of new polyketide synthesis pathways. SynKeD will be linked with the module collecting data on microbial genetic polymorphisms. The microbial module will also include nucleotide sequences of modules from known PKS's assigned for particular carboxylic acids units, which are incorporated into a polyketide chain. Based on this data, SynKeD will respond to the query containing a chemical formula of a polyketide chain. As a result, the application will return a nucleotide sequence encoding PKS modules involving in the synthesis of the desired polyketide chain.

Key words: database, polyketide synthases, polyketides, synthesis pathway, synthetic biology

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IV.P.19

A microbiological module of MultiGenBank database

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MultiGenBank is an bioinformatics project carried out at the Institute of Immunology and Experimental Therapy funded under action 2.3: Investments in development of science infrastructure, IEOP2007-2013. The aim of this project is to create an online platform containing two database modules. The first one will contain human genetic polymorphisms data associated with diseases,whereas the second module is design as a microbial data base including genetic data of microorganisms harbouring potentially useful genes.

The microbiological module will contain records corresponding to microorganism strains, to a great extent derived from Polish Collection of Microorganisms (PCM). For particular strains, the database will gather information about its genotypes, taxonomy, sequences of main molecular markers (16S rRNA, ITS-1 and ITS-2), additional markers (e.g. dnaJ, sod A, rpoB, gyrB, recA, groEL, dnaK) and results of RFLP, RAPD or other methods. Data, both newly submitted and present in the database, obtained with molecular techniques based on differences in lengths of DNA fragments (e.g. RFLP and RAPD) can be compared and analysed by GelCompar or GelQuest/ClusterVis. This function is dedicated for comparison of microorganisms on the strain or isolate level. The database can be searched for homologous sequences using BLAST algorithm. The found sequences can be aligned using Clustal Omega and simple phylogenetic trees will be constructed. An interesting feature of the module will be data about antibiotic resistance genes, genes encoding useful enzymes and involved in secondary metabolism. The enzymes may be of industrial or medical interest. The microbial module will be associated with a synthetic biology tool which will be useful in designing new synthetic polyketides and gene sequences encoding their synthesis pathways.

Key words: database, gene, genetic markers, microorganisms, RAPD, RFLP, secondary metabolism

IV.P.20

Production of chitinase and β-1,3-glucanase by selected species of yeast with potential importance in *Fusarium* biocontrol

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Presently the role of biological plant protection agents in agriculture is increasingly significant. Biological control proposes an environmentally friendly alternative to the use of chemical pesticides for controlling plant diseases. Yeasts can be used for bio control of plants fungal phytopathogens. Competition for nutrients and space is believed to be the major mode of this microorganism action. The involvement of fungal cell wall-degrading enzymes is also suggested to play a significant role in biocontrol. The present study indicates that the selected species of yeast are capable of producing and secreting cell wall-degrading enzymes, including chitinase and β-1,3-glucanase. Yeast obtained from the yeast collection of the Department of Biotechnology, Human Nutrition and Science of Food Commodities from the University of Life Sciences in Lublin, Poland. *In vitro* assays showed that selected yeast demonstrated antagonistic activity against fungal pathogens such as *Fusarium graminearum, F. poae* and *F. culmorum*. These pathogens cause diseases of field crops, especially cereals, and they are difficult to control. Furthermore, many species of *Fusarium* produce a number of secondary metabolites toxic to humans and animals.

Key words: biocontrol, yeast, phytopathogens, *Fusarium*

IV.P.21

Response of the microbial methanotrophic consortia isolated from various environments to osmotic stress

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Methanotrophic bacteria are a unique group of microorganisms capable of growth on methane as a sole carbon and energy source. They comprise an important element of the global carbon cycle, effectively utilizing atmospheric methane and abating emission of that greenhouse gas from anaerobic environments. Methanotrophs are found in many, often extreme environments. To overcome the environmental stress, methanotrophs have developed diverse adaptive mechanisms including modification of the cell-wall, changes phospholipid composition and synthesis of various chemical compounds such as vitamins, antibiotics, biopolymers, single cell proteins and osmoprotectants, with the latter being of especially great value due to their broad application in medicine, cosmetology and science.

Synthesis of osmoprotectants is a cell response to growth at elevated salinity. In the current study we aimed to determine and compare osmotic tolerance of the methanotrophic consortia isolated from various environments such as deep subsurface sediments (coalbed and salt deposit) and plant endosphere (*Sphagnum* sp.). Osmotic pressures were made by addition of 1–5% (w/v) of salts including NaCl, potassium acetate and ammonium acetate. Reaction of the microbial communities was determined based on biomass growth and effectiveness in methane utilization.

Metabolic capabilities of the investigated methanotrophs revealed by high methanotrophic activity and intense growth show that they are of great biotechnological potential. Further research will be performed to elucidate what substances play predominant role in shaping osmotic tolerance of the bacteria isolated from various environments.

Key words: methanotroph, osmotic stress, osmoprotectant

IV.P.22

Association between susceptibility to photodynamic oxidation and the genetic background of *Staphylococcus aureus*

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The century of antibiotic therapy is getting to the end. Growing multiresistance of pathogenic microorganisms, especially *Staphylococcus aureus*, forces the alternative therapies development. Photodynamic inactivation (PDI) is a very promising therapeutic option that consist of three elements: photo sensitizing agent, appropriate wavelength light and oxygen. The excited state photosensitizer transfers its energy to ground state molecular oxygen producing reactive oxygen species (ROS) causing bacterial cell death.

The current study was aimed to investigate if the genetic backgrounds of *S. aureus* strains (SCC*mec* types and main clonal complexes) affect their susceptibility to protoporphyrin IX-mediated photodynamic inactivation. 97 MRSA and 20 MSSA isolates, with identified CCs and SCC*mec* type were used in the experiments. Additionally, 2 isogenic pairs and 9 reference strains of *S. aureus* for optimization of SCC*mec* type determination were used. PDI studies concerning planktonic cultures with PPIX at 25 μM concentration were performed. Cells were illuminated with red (620–780 nm) light (light dose of 50 J/cm²). After irradiation aliquots were taken to determine the colony forming units (CFU). Method used for Staphylococcal cassette chromosome *mec* (SCC*mec*) typing was SCC*mec* type-specific multiplex PCR.

The distribution of PDI responders among five different groups classified according to SCC*mec* types is similar- the type of SCC*mec* element does not have an impact on PDI response. Only clonal complex typing revealed significant differences in PDI response, for instance all CC1 strains were identified as PDI-resistant, all CC30 isolates were characterized as PDI-sensitive or intermediate-sensitive.

Enhanced investigation concerning the association of *S. aureus* genetic background with susceptibility to PDI could lead to precise identification of PDI-resistant phenotypes. Reaching the aim of these studies would benefit alternative therapeutic option development.

Key words: *Staphylococcus aureus,* photodynamic inactivation, SCC*mec* types

IV.P.23

Glucose dehydrogenase production and analysis of *Aspergillus* strains metabolic diversity, using carbon-source utilization profiles

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The organisms of *Aspergillus* genus due to their possibilities to the synthesis of various bioactive compounds and the capacity to produce specific proteins (e.g. enzymes such as α-amylase, glucose oxidase and glucose dehydrogenase) and other metabolites such as organic acids (e.g. citric, gluconic and itaconic acids) are considered as ones of the most economically important genera of microfungi. Glucose dehydrogenase produced by all studied *Aspergillus* strains is defined as oxidoreductases that are able to catalyze the oxidation of glucose in the presence of an electron acceptor is treated as one of the most often used biocatalyzer. Due to its electrochemical activity, glucose dehydrogenase (GDH) could be deployed as a part of a glucose sensor or a biofuel component. The hitherto reported till now GDHs may be divided into three distinct groups according to their electron acceptor including PQQ, FAD and NAD-dependent enzymes.

In this study twelve *Aspergillus* strains producing FAD-dependent glucose dehydrogenase were identified using internal transcribed spacer (ITS) region sequencing. All *Aspergillus* strains were also characterized using Biolog FF MicroPlates to obtain data on C-substrate utilization and mitochondrial activity. Biolog FF Microplate tests complement traditional genomic analysis by offering quantitative assessment of the organism's physiological response to different substrates. The Biolog systems are used for rapid identification of microorganisms using the principle of the "metabolic fingerprint". Due to assimilation of simple or complex carbon substrates, different species of fungi are characterized by a specific metabolic profile, which can be used in identification thereof.

We used the Biolog system to distinguish the 12 studied *Aspergillus* strains as well as to understand their metabolic differences. The ability to decompose various substrates differed among the analyzed strains up to three times. All the studied strains decomposed mainly carbohydrates.

Key words: *Aspergillus*, glucose dehydrogenase, metabolic diversity

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IV.P.24

Yeast and bacterial conversion of cellulose-containing substrates in protein feed products

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Bioconversion of cellulose-containing substrates for animal husbandry in enriched microbial protein and probiotics feed products is a way to increase the nutritive value of feeds. The instrument of bioconversion initial substrates is a joint solid phased fermentation by two groups of microorganisms. The first is a *Bacillus* (producers of cellulolytic enzymes and antimicrobial substances), carrying out the first phase of conversion – saccharification of raw materials. The second group is a special yeast strains – producers of protein.

Cellulose-containing raw materials – wheat bran, sunflower meal and rice husks were inoculated with daily bacterial culture. Cultivation was carried out at temperature 28–30°C for 10 days. The efficiency of fermentation or the ability of 12 strains of the *Bacillus* to grow on solid cellulose-containing substrates was evaluated by the changing of the content of cellulose.

During the study, was found that the loss of cellulose and hemicellulose in the bran was 2–6%,in rice husk and sunflower meal – 7–10% and 5–9% respectively. There were designed 11 mixed cultures, the use which increased the efficiency of destruction of cellulose in 2–3 times. The most active cultures: *B. licheniformis* G23+ *B. subtilis* NP-9, *B. cereus* G-7 + *B. subtilis* C-7, *B. licheniformis* G-7 + *B. cereus* P-5 hydrolyzed cellulose-containing solid substrates for 20–25%.

Then the substrate it was inoculated with cultures of yeast of the genus *Candida, Saccharomyces* and *Pichia*. The most productive strain turned of *Pichia guilliermondii IS-5*. These yeasts are capable of growing on the initial substrate up to 10^9 CFU/g for 3 days of cultivation. However, the use of bacterial pre-conversion increases yeast growth by 30% on average.

These obtained results demonstrate the possibility of gradual degradation of plant substrate by bacteria of the genus *Bacillus* and non-cellulolytic yeast *Pichia guilliermondii IS-5*, in which yeasts use reducing sugar produced by microbial conversion of cellulose.

Key words: *Bacillus*, cellulose-containing substrates, *Pichia guilliermondii,* bioconversion

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IV.P.25

Screening of bacterial cellulose-producing *Gluconoacetobacter xylinus* strains suitable for submerged culture

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Bacterial cellulose (BC) is a promising natural polymer with unique properties, such as high mechanical strength, good crystallinity and a very pure nanofibrillar network structure. This allows using BC film in many applications as nano-structured adsorbing bioactive material. Aim of investigation was to select optimal conditions for BC producer growth and biosynthesis of gel-film in submerged culture.
For isolation of *Gluconoacetobacter xylinus* pure culture – producing strain of BC, 5 samples of Kombucha and apple cider vinegar mixed culture were cultivated during 5 days on nutrient medium with different composition and pH for inhibiting the growth of unwanted microflora. High and low concentration levels of ethanol, acetic acid, various carbon and energy sources and temperature cultivation variants were used as selection factors. Strains productivity was evaluated by BC biomass measurement, which was preliminarily dried under 80°C, using gravimetric method. For its purification cellulose was washed first with 0.5–1% water solution NaOH by boiling, then with distilled water, 0.5% acetic acid solution and again with distilled water until neutral reaction. Obtained gel-fil was stored in distilled water under 5°C or after sterilization in hermetically sealed containers.
As a result of multistage screening new strain of *Gluconoacetobacter xylinus* C-3 capable to synthesis biopolymer on different carbon sources was isolated. Optimization of cultivation of *Gluconoacetobacter xylinus* in submerged conditions in which BC production higher in comparison with other strains was carried out. Maximum BC yield is achieved in submerged culture under 30°C on medium containing yeast extract, peptone, ethanol, wort and saccharose as carbon source, pH 5.9–6.0. BC film purification method was developed.

Keywords: bacterial cellulose,submerged culture, *Gluconoacetobacter xylinus*

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IV.P.26

Health-promoting properties of chosen *Lactobacillus helveticus* strains

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This research concerns the determination of some characteristics exhibited by *Lactobacillus helveticus* strains, what may lead to create a new approach towards a wider applications of those bacteria. *Lb. helveticus* is a thermophilic, homofermentative and acid-tolerant bacterium belonging to lactic acid bacteria (LAB). Those microorganisms possess the highest proteolytic activity among all LAB and generate bioactive peptides inducing a beneficial effects on health. Particularly relevant peptides reveal an antioxidative activities that can support a natural defenses of cells in human organism against the harmful oxidative stress effects.
The objective of this investigation was to evaluate an antioxidant activities of selected whey protein preparations fermented by *Lb. helveticus* strains. The aim of this study was also to identify the gene *slpA* encoding S-layer protein (SlpA).
Obtained study results indicate that the abilities to fermentation various type of selected whey protein preparations are very diverse among strains. *Lactobacillus helveticus* T80 allowed to obtain hydrolysates with the strongest antioxidant properties. Many of Lactobacillus strains are also characterised as probiotics and exert health benefits. In the probiotic activity of *Lactobacillus helveticus* (strain M92) the S-layer protein (SlpA) play an important functional role. This gene was identified in three of analyzed *Lactobacillus helveticus* strains (T80, B734, T105), what indicates their potential probiotic properties. In this study the abilities to production the isomers L(+) and D(-) of lactic acid by various strains were also determined and compared among all analyzed microorganisms.
A comparative studies on *Lactobacillus helveticus* strains in terms of their health-promoting properties might allow for a selection the most desired bacterium composition and application as a starter cultures in the production of functional food products.

Key words: *Lactobacillus helveticus*, antioxidant activity, S-layer protein

IV.P.27

Microbial preparation AgroMyc promoting productivity of triticale

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Formulation of complex microbial preparations made up by associations of microorganisms possessing a whole set of properties valuable for host plants is extremely significant for eco-oriented agriculture. Our studies were aimed at designing microbial product based on active nitrogen-fixing and phosphate-mobilizing cultures able to interact with popular in Belarus cereal crop triticale distinguished by high grain nutritive value.

Screening of efficient nitrogen-fixing and phosphate-mobilizing bacterial strains isolated from triticale rhizosphere was based on presence of *nifH*-genes and nitrogenase activity measured by acetylene method at gas chromatograph, on ability to synthesize indolyl-3-acetic acid (IAA) and to dissolve calcium phosphates, on growth-stimulating activity, competitive and plant root-colonizing capacity. Identification of bacterial isolates was carried out according to Bergey's Manual of Determinative Bacteriology (1994) and by analysis of 16S rRNA sequence.

Search, isolation and screening of efficient cultures of arbuscular mycorrhizal fungi (AMF) rapidly and intensively colonizing triticale roots was performed in compliance with recommendations of N. M. Labutova (2000). Identification of AMF forming symbiotic relationship with triticale was conducted by molecular-genetic methods engaging couples of primers: ITS1 and ITS4, NS31 and AM1, LSURK4f and LSURK7r.

Screening of 206 rhizobacterial isolates for the above-mentioned criteria yielded 2 most active variants identified later as *Rhizobium rhizogenes* 17 and *Pseudomonas lini* 10SK.

Analysis of PCR amplification products revealed presence of DNA fraction belonging to AMF of *Glomusmosseae-intraradices* group.

Highly active rhizobacterial strains *Rhizobium rhizogenes* 17, *Pseudomonas lini* 10SK and AMF of genus *Glomus* were selected as active principle of microbial preparation AgroMyc intended for presowing treatment of seeds and vegetating plants to raise productivity of triticale and to reduce doses of applied mineral fertilizers.

Presowing inoculation of winter triticale seeds, variety Prometheus with AgroMyc ensures early sprouting and 15.2% increase of average crop productivity as compared to the control.

Key words: triticale, microbial preparation, arbuscular mycorrhizal fungi, rhizobacteria

IV.P.28

Influence of some abiotic stresses on the biocatalytic activity of *Chrysosporium pannorum*

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The influence of pre-incubation of the fungus *Chrysosporium pannorum* in different stress conditions (organic solvents, medium pH and temperatures) on its activity in the oxidative bioconversion of α-pinene to verbenone and verbenol was examined. The total bioconversion activity increased over 2-fold after 15-min pre-treatment with 1.4-dioxane as an abiotic stress factor. Also, the change of the medium pH from the optimal 5.6 to 2.0 and 10.0 for 1 h before biotransformation enhanced product yield nearly 1.5-fold. Moreover, the use of dioxane, chloroform and ethanol and thermal stress at 50°C caused changes in the oxidation product profile (predominance of *trans*-pinocarveol over *trans*-verbenol). A maximum increase in the yield of verbenol was observed when the biocatalyst was subjected to 15-min dioxane stress (an increase from 13.7 to 33.1 mg×g^{-1} dry cell L^{-1} aqueous phase) before 48-h biotransformation of 1% (v/v) pinene.

A more active fungal biocatalyst for the biooxidation of a terpene may be obtained by subjecting the fungus used to some abiotic stresses prior to biotransformation. There also exists evidence that some abiotic stressors can lead to changes in the proportion of the main biotransformation products.

Key words: abiotic stresses, biotransformation, *trans*-verbenol

IV.P.29

Genome shuffling as a technique improving resistance to environmental stresses in *Saccharomyces cerevisiae*

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The aim of this study was to obtain a modified yeast strain of *Saccharomyces cerevisiae*, so it would have better of fermentation properties i.e. high resistance to environmental stresses formed during the industrial process of ethanol production (increased concentration of toxic by-products and high osmotic pressure).
The technique of genetic modification, known as genome shuffling was used to improve phenotypic traits. First there was conducted chemical mutagenesis of the yeast strain using ethyl methanesulfonate (EMS), then screening of the resulting mutants characterized by resistance to environmental stresses. As a result, after one round of mutagenesis there were obtained three mutants characterized by twice higher volumetric ethanol productivity and resistance to acetic acid than the starting strain. This allowed for the creation of parental library. The most outstanding mutants were subjected to the subsequent step which is called protoplasts fusion. In this process protoplasts were mixed, fused and regenerated. Cell fusion was carried out in the hope that the hybrids would have simultaneously a multiple traits present in single mutants e.g. resistance to toxins, and high osmotic pressure.
In the final stage, after a few rounds of genome shuffling there was selected a yeast strain showing not only distinctly higher volumetric ethanol productivity, but also twice higher resistance to several environmental stresses.

Key words: genome shuffling, environmental stresses, *Saccharomyces cerevisiae*, industrial strain

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Optimisation of *Bacillus subtilis* sporulation conditions

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Bacillus subtilis not only is an fundamental scientific model of endospore-forming bacteria, but also has significant influence on industry. Its spores have a number of applications, beginning with potential use in surface display system for example as a vaccine antigen carrier, following by probiotic features. Obtaining high yield and good quality of *B. subtilis* spores is a key issue of this study.
Endospore formation is promoted by the changes in cultivation conditions such as nutrient limitation or presence of specific chemical compounds. Final spore concentration can be improved by simple changes in media composition. Additional nutritions or cytotoxic factors have different impact on spore yield. Here we test the influence of various substances to optimise the efficiency of sporulation. Our goal is to find a factor that promotes sporulation and can be easy implemented to industial scale of spore production.

Key words: *Bacillus subtilis*, spores, sporulation