IV.O.1

Alcoholic fermentation: problems and perspectives

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Alcoholic fermentation is the oldest biotechnology known for more than 6000 years. Currently, production of fuel ethanol, even without alcoholic beverages, firmly occupies the first place regarding the sales among biotechnological products. Last year, there was produced near 100 billion liters of fuel ethanol which cost 50 billion US dollars. Currently, most of fuel ethanol on the market is that of the 1st generation produced from sugar and starch. It is difficult to increase significantly production of 1st generation ethanol due to limitation of feedstocks. However, it is possible to increase ethanol yield from glucose due to decrease in production of byproducts, glycerol and yeast biomass and thus to produce more ethanol from the same amounts of feedstock. We developed method to drop biomass accumulation during cultivation of laboratory or industrial strains of *Saccharomyces cerevisiae* by manipulation with intracellular ATP level which was achieved due to overexpression of vacuolar alkaline phosphatase encoded by gene *PHO8*. Consequently the strains were constructed which accumulated up to 10% more ethanol from corn meal hydrolyzates. The method of positive selection for more efficient ethanol producers based on the use of toxic inhibitors of glycolysis and some other pathways has been developed. This permitted to isolate mutants which accumulated 5–10% more ethanol relative to parental wild-type strains.
Substantial increase in fuel ethanol production will be possible to achieve only after development of the feasible technology which uses lignocellulose as feedstock. One of bottlenecks in such technology is the absence of yeast strains capable of efficient fermentation of the second abundant lignocellulosic sugar, xylose. We have found that xylose could be fermented under elevated temperature by thermotolerant yeast *Hansenula polymorpha* though ethanol yield and productivity in the wild-type strains is quite low. Using combination of the methods of metabolic engineering and classical selection, we have constructed the strains of *H. polymorpha* which accumulate 20–25 times more ethanol from xylose at elevated temperature 45°C up to 12.5 g/L. For this, protein engineering of xylose reductase along with overexpression of genes of the first three initial reaction of xylose metabolism (*XYL1, XYL2, XYL3*), deletion of the global transcriptional activator *CAT8* and isolation of the mutants defective in using ethanol as carbon source and resistant to anticancer drug 3-brompyruvate have been employed. Further increase in ethanol yield and productivity from xylose is planned to achieve due to activation of xylose transport and expression of heterologous genes involved in xylan degradation. Current state and perspectives for further increase in ethanol production of the 1st and 2nd generation is discussed.

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IV.O.2

New methods for ethanol, methanol and formaldehyde determination in environment and food products

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Environmental monitoring, including monitoring of food and drinking water is becoming increasingly important in programs to improve the living conditions and human nutrition. There is a need for the development and use of methods of analysis of ethanol, methanol and formaldehyde in foods. Ethanol is a major component of a variety of alcoholic beverages and is an ingredient of a number of foodstuffs. Formaldehyde and methanol are produced on the large scale and are very toxic and have mutagenic and carcinogenic action on living organisms. The presence of toxic methanol and formaldehyde in foods can lead to severe poisoning and even death.

Most of the known methods for determination of ethanol, methanol and formaldehyde are not very selective and sensitive enough or too expensive. Are more promising enzymatic methods using alcohol oxidase (AOX) isolated from strains superproducer *Hansenula polymorpha*. AOX capable of oxidizing methanol in vitro, aliphatic alcohols, including ethanol, has important biotechnological value. Another *H. polymorpha* enzyme having bioanalytical value is formaldehyde dehydrogenase (FdDH). Described two enzymatic methods for assay of formaldehyde in fish food products using AOX and FdDH isolated from *H. polymorpha*. AOX-based method exploits an ability of the enzyme to oxidise a hydrated form of formaldehyde to formic acid and hydrogen peroxide monitored in peroxidase-catalysed colorimetric reaction. In FdDH-based method, a monitored coloured formazane is formed from nitrotetrazolium salt during reduction by NADH, produced in formaldehyde-dependent reaction. The both methods were demonstrated that some fish products (hake and cod) contain high formaldehyde concentrations (up to 100 mg/kg wet weight).

The determination of formaldehyde using biosensors have been developed such as biosensors based on cells or enzymes, either AOX or FdDH. The measurement of formaldehyde is based on enzymatic oxidation of formaldehyde by means of formaldehyde dehydrogenase to formic acid with ensuing reduction of NAD^+ as electron transfer to NADH.

Key words: ethanol, methanol, formaldehyde, biosensor, enzymatic methods of determination

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IV.O.3

Enhanced production of polygalacturonase by solid state fermentation — process conditions, the enzyme isolation and partial characterization

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The pectinolytic enzyme from *Penicillium chrysogenum* was obtained by solid state fermentation. The microorganism cultivation was conducted for 5 days on orange peels supplemented with solution of inorganic salts ($(NH_4)_2SO_4$, $MgSO_4$). Such prepared medium served as a good nutrient source and rich pectin substrate for polygalacturonase (PG) production as well as a carrier for biomass attachment. The enzyme was isolated by extraction with buffer pH 5.0 or water at various periods of time (4–120 min) and intensities of agitation (50–230 rpm). The extract was purified only by dialysis that allowed to obtain preparation with detectable PG activity and avoid expensive steps of purification. The highest polygalacturonase activity was found after 48 h of peels inoculation. During next 3 days activity has lowered to 60% of initial value which was affected by the decrease of pH medium. The effect of buffer (pH 6.5–8.0) addition to cultivated biomass has significantly influenced the protein productivity and enhanced the PG activity. The best results were obtained by using buffer at pH 8.0 for which the protein concentration (mg per gram of orange peels) and PG activity (U/mL) have increased from 3.5 to 7.7 and from 190 to 1270, respectively. For preparation with the highest protein content, the enzymatic characteristic were performed at the pH and temperature range of 2.5–8.0 and 4–57°C, respectively. The results of measurements showed that optimum for PG activity is at pH 5.0 and 43ºC.

Key words: polygalacturonase, solid state fermentation, constant pH maintaining, enzyme kinetic characterization

Acknowledgements: Project supported by Wroclaw Centre of Biotechnology, programme The Leading National Research Centre (KNOW) for years 2014-2018.

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Molecular identification of *Paramecium bursaria* syngens and studies on geographic distribution using mitochondrial cytochrome *c* oxidase subunit I (*COI*) as marker

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The *Paramecium bursaria* (EHRENBERG 1831, FOCKE 1836) is composed of five syngens, which are morphologically indistinguishable but sexually isolated. The aim of the present study was to confirm by molecular methods (analyses of *COI* mtDNA) the identification of *P. bursaria* syngens originating from different geographical locations. The phylograms constructed using both the neighbor-joining and maximum-likelihood methods based on a comparison of 34 sequences of *P. bursaria* strains and *P. multimicronucleatum*, *P. caudatum* and *P. calkinsi* strains used as outgroup have revealed strains grouping into five clusters, which refers to results obtained previously by mating reaction. Our analysis show the existence of 24 haplotypes for the *COI* gene sequence in the studied strains. The interspecies haplotype diversity value was Hd=0.967. We confirmed the genetic polymorphism between strains of *P. bursaria* and the occurrence of correlation between geographical distribution and the correspondent syngen.

Key words: *Paramecium bursaria*, *COI*, syngens, geographical distribution, phylogenetic methods

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IV.O.5

Exposition of influenza A matrix protein 1 on the surface of recombinant *Bacillus subtilis* endospores

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The influenza virus due to its high infectivity and adaptability is one of the most dangerous pathogens infecting warm-blood edvertebrates worldwide. To reduce the spread of influenza virus in environment, there is a need to develop an effective vaccine. Most existing vaccines owes its effectiveness to the ability to induce the production of neutralizing antibodies directed against hemagglutinin (HA) and neuraminidase (NA). However antibodies to HA and NA provide potent but strain-specific virus protection. Due to the frequent antigenic drifts and antigenic shifts of the circulating virus there is a need for annual vaccine re-formulation and vaccination. Although mechanism of action of commercially available vaccines is based on induction of neutralizing antibodies production, a cellular response is also crucial for eradication of virus infection. Influenza virus-specific cytotoxic T lymphocytes (CTLs) have been shown in animal studies to limit influenza A virus replication and to protect against lethal influenza A virus challenge. CTLs can recognize relatively conserved viral proteins such as the nucleoprotein and the matrix 1 protein, they are able to contribute to heterosubtypic immunity between different subtypes. It has been recognized that CTLs play a key role in eliminating influenza virus infected cells by inducing apoptosis through contact-dependent interactions. Bacterium *Bacillus subtilis* is a gram-positive bacilli which produce endospores. Being metabolically dormant, spores are resistant to many environmental stressors such as UV radiation, desiccation, heat or freezing. There are evidence that oral or intranasal administration of spores presenting antigens induces a specific, both cellular and humoral immune response which can protect animals from infection. In our study, using a genetic approach we constructed *Bacillus subtilis* strains producing spores presenting influenza virus M1 antigen on their surface. M1 protein was fused to spore coat CotZ protein and was stably exposed on the spore surface as demonstrated by the immunostaining of intact, recombinant spores.

Key words: *Bacillus subtilis*, spore surface display, influenza virus vaccine

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IV.O.6

Influence of the silver nanoparticles on fungal community in different environments

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Despite the continuous increase in the production and use of products containing silver nanoparticle in industry and health protection, there is still no sufficient information on their impact on the fungal community in the different environment. The aim of the study was to assess the influence of AgNPs on fungi in environments. The silver nanoparticles (AgNPs) preparation (10–80 nm, 90 ppm) was obtained by chemical method of $AgNO_3$ reduction by sodium citrate, with PVP as an anti-aggregation factor. Tested environments were soil extract, river water and sewage from grit chamber. Model studies were performed using pure culture collection strains: *A. niger, P. chrysogenum,* environments and AgNPs. The fungi numbers equalled to 5.3×10^3 cfu/ml in soil extract, 4.0×10^3 cfu/ml in water and 1.8×10^3 cfu/ml in sewage. The addition of silver nanoparticles (45 ppm) to tested environments reduced the number of fungi by 33–85%. The fungi present in the environments showed different sensitivity toward AgNPs, depending on the inhabited environment. Two mould strains were eliminated from the environments: *Alternaria alternata, Penicillium glabrum.* The sensitive fungi were yeasts: *Candida* sp., *Cryptococcus laurentii.* The fungi with low sensitivity were: *Mucor hiemalis, Rhodotorula mucilaginosa.* In model studies, the addition of silver nanoparticles at MIC concentration to the culture medium with particular environment, reduced the mould biomass of *A. niger* and *P. chrysogenum* by 35.3–63.6% in soil extract, 28.6–41.7% in water and 35.7–66.7% in sewage after 14 days of incubation. In any of the tested environments, we have not noted the protective properties of the environment for moulds, in contrast, the addition of AgNPs medium containing environment intensified the effect of AgNPs inhibition of mould growth.

Key words: silver nanoparticles, moulds, environment

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