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## Session 12. Agrobiotechnology and Industrial Biotechnology

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### Lectures

#### L12.1

##### Are we ready for GMO? Biotechnology and legislation A.D. 2013

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Knowledge Based BioEconomy [KBBE] is based mainly on taking advantages of modern, innovative technologies, including genetic engineering and biotechnology. Genetically modified products and technologies used for their production [particularly in the case of agro-food industry] raise a lot of emotions among the public. Majority of people is not oriented that:

- the competitiveness of the agri-food industry is the result of low-cost feed,
- Polish (as well as UE) nutritional self-sufficiency is not higher than 50%.

In this situation public opinion is a political and economical factor. Legislation friendly for innovation is a major enhancer for scientific and technical progress. The answer for the question “Are we ready for GMO?” is critically important and will be discussed in this lecture.

#### L12.2

##### Significance of biotechnological processes in food production

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The scope of the lecture covers the following problems:

1. Characteristics of biotechnology as a scientific discipline,
2. Directions of development in food biotechnology,
3. Advances in production and use of biocatalysis and its applicatory significance,
4. Enzymatic modification of food components, including
  - saccharides
  - proteins
  - lipids,
5. Biotechnological methods in food quality control,
6. Biotechnological processes in management of by-products and wastes from the agri-food industry,
7. Perspectives for applying biotechnological processes in food production.

## L12.3

### The utilization of crude glycerol in biotechnology processes with *Yarrowia lipolytica* yeast

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Biodiesel (fatty acid methyl esters e FAME) is an alternative diesel fuel made of vegetable oil, animal fat, cooking oil, waste grease, or other suitable lipid feedstock. The crude glycerol is the major by-product of this production. According to the European Biodiesel Board, in 2011 the total European biodiesel production capacity reached 22 million tones. The applicability of crude glycerol is highly limited due to its composition, mainly impurities. Furthermore, glycerol now produced in large quantities as a by-product from the biodiesel manufacturing affords new opportunities for biotechnological transformation reactions. Glycerol and crude glycerol were successfully used for the production of various metabolites by *Y. lipolytica* yeast strains. Unconventional and nonpathogenic *Y. lipolytica* yeast has been addressed in various studies conducted in many research centers, and in recent years has been perceived as an especially attractive microorganism for many applications of glycerol. This yeast was used for the production of many value-added products from glycerol such as (i) biomass rich in proteins and oils, as food and feed additives, (ii) organic acids (citric, pyruvic and  $\alpha$ -ketoglutaric acid), (iii) erythritol, mannitol and (iv) invertase. Especially for the Food Industry, an interesting perspective of glycerol valorization may be the production of sugar alcohols by *Y. lipolytica* yeast used in food as sugar replacers [1]. At the optimal pH conditions and application of crude glycerol-containing media, the Wratislavia K1 strain produced 170 g/L of erythritol, in the fed-batch culture with the total glycerol concentration of 300 g/L (2).

#### Acknowledgments:

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## L12.4

### Copy number polymorphism — plant genome breeding

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Intraspecies copy number variation (CNV — also called copy number polymorphism) is a DNA polymorphism manifested by the existence of differences in the number of DNA segments in the genomes of the individuals. Typically the polymorphic segments encompass relatively large DNA segments, from 1 kb to several Mb. The postulated mechanisms leading to CNV are duplications and/or deletions resulting from nonallelic homologous recombination or from template switching by the replication complex. CNV is prevalent in plants, animals and humans. Although it mainly affects non-coding genome regions of little functional importance, it may also alter the gene structure or copy number, thus influencing its expression. Over the last decade, a growing number of studies have highlighted the importance of CNV as a factor affecting human phenotype, and individual gene copy number variants (CNVs) have been linked to risks of severe diseases. In plants, the exploration of the extent and role of CNV is still just beginning. Initial genomic analyses indicate that CNV has greatly affected plant genome evolution. As in humans, also in plants multiple CNVs encompass protein coding genes, mainly the members of large families of functionally redundant genes. Thus, the effects of individual CNVs on phenotype are usually modest. Nevertheless, there are many cases in which CNVs for specific genes have been linked to important traits such as flowering time, plant height and resistance to biotic and abiotic stress. Recent reports suggest that rapid copy number expansion of particular genes involved in resistance to persistent stress like presence of herbicides, may take place. In many such cases, CNV affected the same key genes independently in different populations or species, which allows to hypothesize that this natural phenomenon may be recruited for directional plant improvement.

## L12.5

### Molecular basis of maize differential resistance to herbicides

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Herbicides, chemicals commonly known as weed killers, are compounds used to destroy or inhibit the growth of plants, especially weeds. The most popular weed killers, widely used in the maize fields, are nonselective, which means that they affect all green plants that are growing in the sprayed area. Biological organisms constantly exposed to environmental stimuli (stresses) are capable of establishing mechanisms of protection and adaptation. Because of their sedentary life style, plants are restricted to tolerance, resistance, and avoidance mechanisms only and thus require efficient short-term strategies. As a result, plants have developed exquisite adjustments to stresses at all levels (anatomical, morphological, cellular, biochemical and molecular) to guarantee their survival under adverse conditions.

Maize has strikingly dynamic genome. Analyses demonstrate that landraces and worldwide varieties are highly diverse since two maize varieties show as much DNA sequence variation as that observed between two different species. The 10 chromosomes of the maize genome are structurally diverse as a result of dynamic changes in chromatin composition (pieces of chromosomes inverted, exchanged, transposed, further duplicated, or lost).

Phenotypic variation in plants under stress is classically attributed to DNA sequence variants. It is known that mutations can alter the allelic DNA variation that will influence the genetic architecture and how these alleles will be expressed and passed from parents to offspring. Years ago it has been observed that some maize lines show higher sensitivity to herbicide spraying than others but molecular players in such heightened resistance remain unknown to this day. Therefore our goal is to identify molecular basics of plant's increased/decreased resistance to herbicides and identify a molecular marker that can be used as an indicator of plant's resistance to herbicides. We started our experiments on the transcriptome level analyzing changes in gene expression and alternative splicing events. The Next Generation Sequencing method was used in order to identify differences in small RNA populations and the degradome. Since it was recently shown that abiotic stresses cause long-term regulation of gene expression, mostly conferred by epigenetic gene regulatory mechanisms, we're also tracking changes in epigenome analyzing various histone modifications or DNA methylation.

## Oral presentations

### O12.1

#### The comparison of laccase-producing fungal biomass in textile dyes synthesis

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Basidiomycota fungi like *Cerrena unicolor*, *Pleurotus ostreatus*, *Pleurotus pulmonarius*, *Nematoloma frowardii* and *Rhizoctonia praticola* are able to synthesize ligninolytic enzyme called laccase. Fungal laccase, the main oxidase produced by many of wood-rotting strains, catalyzes oxidation of a broad range of organic and non-organic, producing new structures among them coloured products. Therefore, the use of laccase as biocatalyst and fungal biomass naturally secreted laccase, makes an alternative to chemical synthesis of existing or new dyes.

In this study the capability of laccase for transformation of different organic precursors to textile dyes was tested. This process was conducted in two ways: as a homotransformation where single phenolic precursor was used and as a heterotransformation where a mixture of two different precursors was transformed. During this transformation products about different colors were obtained, according to the structure of precursor, the mode of transformation and the type of fungal strain. Moreover, the intensity and time of dyes biosynthesis were specific to each fungal strain. What is interesting, an optimal value of pH characterized for the activity of laccase secreted by biomass is also very important factor during colourless precursors biotransformation to products about very interesting colours. Some of the tested species were not able to transform certain precursors into dyes what was confirmed in two different kinds of cultures.

Our experiments show the potential of fungal biomass as biocatalysts for biosynthesis of dyes about commercial importance, replacing the uses of enzyme, which are still too expensive for the industrial purposes.

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## O12.2

### Extraction method for trace analysis of antibiotics and other biologically active compounds in edible products

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Pest control is an important aspect considering production yield in agricultural farming. Strict norms and public pressure forces researchers to work with natural compounds rather than synthetic. The eco-trend mandates the use of natural pesticides and herbicides. In products of natural origin the concentration of main/active compounds fluctuate depending on various environmental factors. This led to a demand for a fast, reproducible sample preparation and analysis method that will meet today's standards. One of the main problems of the preparation process is the structural and chemical variety of biological compounds. High water solubility and ballast substances like sugars present in natural extracts demanded more targeted approach especially considering only small amounts of the active compound can be present in the sample. The technique we implemented fulfills those requirements. Our method is based on a salting-out liquid-liquid extraction process with a subsequent concentration of the sample. Low need for organic solvents and the absence of toxic additives to the process makes it more eco-friendly than most used extraction methods. This method base can be applied and optimized to various applications including but not limited to sulfonamides, alkaloids, naphthoquinones and other biologically active compounds determination and concentration analysis. Our method was successfully employed in sulfonamide analysis in beekeeping industry. The extraction process can be applied not only to trace analysis in the foodstuff production process but also extraction of small molecule organic compounds like plant secondary metabolites.

## O12.3

### Influence of fermentation media supplementation with mineral compounds on fermentation activity of *Saccharomyces cerevisiae*, and technological indicators of alcoholic fermentation process

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The need of optimization of the alcoholic fermentation process stems from the rise in demand on the ethyl alcohol used by the fuel and chemical industry. Application of high gravity mashes (HG) in alcoholic fermentation is one of the possible directions to improve the economy of the process [1]. The aim of the study was to determine the influence of the HG mashes supplementation with phosphate, Zn<sup>2+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, and inositol on the activity of *S. cerevisiae* D<sub>2</sub> strain. The selection of the medium enriching compounds was made by the analysis of the compounds released from the phytinian complexes during the hydrolysis process [2]. The raw material used in the preparation of HG mashes (22% w/w) was milled maize grain with characterized technological parameters. The process of the enzymatic starch hydrolysis was conducted with application of the Novozymes<sup>®</sup> company preparations. During the alcoholic fermentation the basic technological indicators (alcohol concentration, productivity, yield, and fermentation energy), indicators of the physiological condition of yeast (number, viability, phosphorus concentration in yeast cells) and changes in the composition of fermentation volatile by-products (GC method) were determined. The analysis of variance and determination of standard deviation was carried out by using STATISTICA software, ver. 10. The variant with the addition of Ca<sup>2+</sup> was characterized by the highest ethanol concentration on the level 10.8±0.1% v/v after 72h. This concentration was about 1.2% v/v higher than the control variant. This variant was also characterized by the highest productivity and yield of the fermentation. Independently of the supplementation dose, inositol and phosphate ions increased the number of yeast cells during the first 24 hours of the fermentation. Analysis of the volatile by-products concentrations demonstrated the lack of statistically significant differences between control variant and the variant with the addition of Ca<sup>2+</sup>. These distillates were characterized by the lowest concentrations of volatile by-products. In relation to control variant, the variant supplemented with Mg<sup>2+</sup> showed more than 2-fold increase of aldehydes concentrations (290.5±27.6 mg/L). Addition of phosphate ions increased the concentrations of higher alcohols from 1461.9±20.0, observed in control variant, to 3071.0±290.0mg/L.

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## 012.4

### Construction of hybrid spider silk for cell transfection

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**Background:** Bioengineered spider silks as well as their natural analogues — are biomaterials with unusual properties like mechanical strength, toughness, biocompatibility and biodegradability. Moreover, the recombinant spider silks can be tailored via genetic engineering in terms of sequence, molecular weight and other important features which allow functionalization of the silk biomaterial. Functionalization of the spider silk with domain responsible for binding nucleic acid enables development of the gene delivery system.

**Aim of the study:** Construction of hybrid bioengineered spider silk for cell transfection.

**Materials and methods:** The spider silk repeat unit was based on the consensus motive derived from the native sequence of MaSp2 from the spider *Nephila clavipes*. The bioengineered spider silk-15mer (MS2) consisting of fifteen repeat units and its hybrid variants (KNMS2 and MS2KN) containing additional nucleic acid binding domain (poly-lysine) were constructed. Spider silk proteins were produced in *E.coli* and then purified by the organic acid extraction and thermal denaturation. The complexes of silk proteins with plasmid DNA encoding GFP were prepared by mixing at various molecular ratios. To prepare the spheres, the silk protein and plasmid were mixed with 2M K<sub>3</sub>PO<sub>4</sub> pH 8.0. The complexes and spheres were characterized by agarose gel electrophoresis and fluorescence analysis, respectively.

**Results:** The constructs of MS2, KNMS2 and MS2KN were obtained. The control and hybrid variants of silk were successfully expressed and then purified by acid extraction method. The functionalized spider silks KNMS2 and MS2KN but not the control protein MS2 bound plasmid DNA. Spheres made of functionalized spider silk were obtained.

**Conclusions:** Biodegradable and biocompatible silk based nucleic acid delivery system may be potentially applied not only for cell transfection, but also for *in vivo* delivery of therapeutic nucleic acids. Further modifications of silk with domains that bind to a receptor on the cell surface is another step in the functionalization of the sphere.

## 012.5

### Antimicrobial polysiloxane polymers show affinity to bacterial membranes

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The advantage of antimicrobial polymers in comparison to many antibiotics and disinfectants is that they do not release low molecular weight toxic compounds into the environment, possess a strong biocidal potency and do not lead to the development of antimicrobial resistance. Although they have many potential uses in medicine and industry their mechanism of action remains unknown.

Investigation into the mechanism and site of action of polysiloxane polymers in bacterial cells was carried out using a polysiloxane copolymer grafted with t-butylaminoethyl methacrylate and attached fluorescent group (fluorescein). Fluorescence microscopy and fluorometric measurements were performed in order to examine the site and strength of attachment of the studied polymer to bacterial cells. It was established that the polymer forms a stable link with bacteria, most likely with their surface structures. Galactosidase enzyme assay with the use of ONPG as a substrate in the presence of polymer was performed and the results show that the polymer has a damaging effect on bacterial membranes. In order to confirm that membranes are the primary target for polysiloxane polymers, the bacterial cells were incubated with the polymer and subsequently their membranes were isolated and fractioned in sucrose gradients. It was proved that the polymer attaches to and damages the outer bacterial cell membrane, whereas it has less destructive effect and smaller affinity to inner membrane.

## O12.6

### Bacterially synthesized nanocellulose as a potential scaffold for tissues regeneration

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Bacterial nanocellulose (BNC), synthesized by *Gluconacetobacter* strain, is the natural biopolymer of the highest purity and outstanding biocompatibility. Bionanocellulose is the material of known properties being already investigated in a wide range of industrial and medical applications, including clinical trials for wet wound dressing and as a potential implant e.g. in vascular surgery (small vascular grafts), bone, trachea and cartilage replacement, peripheral nerves regeneration and many others. Many publications confirm that as a nondegradable biomaterial BNC implants fulfil their function by appropriate high hydrophilicity, inertness, lack of cytotoxicity, outstanding biocompatibility and stability in a wide range of temperatures and pH. It was recently investigated that the neurotubes made of BNC are of very good biocompatibility and allow the accumulation of neurotrophic factors inside, thus, facilitating the process of nerve regeneration. The successful application of BNC tubes in trachea reconstruction was also described. Going further, tissue regeneration applying specifically modified BNC scaffolds might be enhanced and stimulated by the application of various factors (e.g. drugs, cytokines, growth factors), which could improve the regenerative processes. The chemical or physical introduction of stimulating agents into BNC membranes is of interest especially for hardly regenerating tissues, such as nerves and cartilage. The preliminary *in vivo* trials were performed for nerve growth factor enriched BNC neurotubes, giving very promising results of significantly faster motor recovery. Following the obtained results the direction of the current research is set to the improvement of BNC scaffolds *in vivo* functionality. Since the properly adjusted structure of BNC membrane determines the success of tissue-engineered constructs adaptation, it is, therefore, necessary to improve the manufacturing process to produce a number of both suitably dense and porous BNC scaffolds, in order to create the efficient drug delivery system, as well as the proper conditions for the proliferation of host and *in vitro* introduced tissue-engineered cells.

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## Posters

### P12.1

#### 3D *in vitro* epithelial tissue model — how to use to study of antifungal agents?

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Three-dimensional (3D) organotypic tissue models have been recently engineered as a new approach for investigations of processes associated with various type of diseases. These models closely resemble the situation occurring in the *in vivo* environment and therefore may successfully replace the animal models, whose use is limited by ethical and cost reasons. Moreover, *in vitro* tissue models provide more controllable and reproducible conditions and can be utilized for testing a large number of experimental combinations. Several different 3D *in vitro* models have been developed including skin substitutes, liver, cardiac and bone tissues [1]. Among commercially available models, reconstituted human oral epithelium (RHE) is one the more frequently used to study bacterial or fungal infections. The epithelial tissue is formed by *in vitro* cultivation of the buccal squamous cell carcinoma cell line (TR 146) on a polycarbonate filter at the air-liquid interface in a chemically defined medium [2]. Importantly, multilayered tissue expresses major naturally present markers of the epithelial differentiation and basement membrane and demonstrates the mechanisms of tissue repairing, making it similar to the human *in vivo* epithelium [3].

In our studies, we have focused on the opportunistic oral infections caused by pathogenic yeast, *Candida albicans*. These infections are becoming a dominant problem in population of people with immune system dysfunctions, e.g. HIV and cancer patients. The aim of the project was to use the 3D epithelial tissue model to create an *in vitro* fungal infection in order to evaluate the effectiveness of the model for testing of antifungal compounds. The subject of our investigations was a Nystatin, which is the most popular agent for the treatment of oral fungal infections. In the first instance, an antifungal activity against *Candida albicans* and cytotoxicity on human keratinocyte cell line were examined separately. Subsequently, reconstituted human oral epithelium was infected with *Candida albicans* and the colonization and invasion of the tissue in the presence of antifungal drug was analyzed using confocal laser scanning microscopy (CLSM). We have observed that epithelial tissue model can be used both for assessing the antimicrobial and cytotoxic effect of the drug at the same time. Nystatin efficiently inhibited the progress of the infection in comparison to a drug-free control. Based on CLSM images we defined a percent of live and dead *Candida albicans* and epithelial cells upon the Nystatin treatment. Following this path, several new antifungal agents were also tested.

#### Acknowledgements:

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## P12.2

### Studies of antifungal activity of tetrahalogenated benzimidazoles

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The biological significance of benzimidazoles have long been established. Derivatives of these compounds are known for their antibacterial, antifungal, antioxidant, antiparasitic, antiviral, anti-inflammatory and antitumor activities. These compounds also inhibit photosynthesis and, therefore, exhibit appreciable herbicidal activity.

In addition, benzimidazoles can be classified as one of the most important groups of fungicides with systemic activity and are well-known for their pronounced ability to control a large number of fungal diseases. Benomyl, carbendazim, thiabendazole, tiophanate metyl and fuberidazole are examples of this fungicide class. The success with these compounds stimulated the search for new biologically active derivatives.

Present communication deals antifungal evaluation of 4,5,6,7-tetrabromobenzimidazole, 4,5,6,7-tetraiodobenzimidazole and its new derivatives against three cosmopolitan species of fungi: *Colletotrichum gloeosporioides*, *Fusarium culmorum* and *Sclerotinia sclerotiorum*.

This report examines 16 benzimidazole analogs and shows that some of them showed in vitro potent antifungal activity, especially 4,5,6,7-tetrabromo-1-karboxypropyl-2-dimethylamino-benzimidazole which displayed the highest activity among the test compounds against *Sclerotinia sclerotiorum* (MIC= 28 µg/ml). The 4,5,6,7-tetraiodobenzimidazole have shown good activity against *Colletotrichum gloeosporioides* (MIC=155 µg/ml). Both compounds also have shown maximum inhibition against *Fusarium culmorum* (MIC=280 µg/ml and 311 µg/ml).

## P12.3

### Identification of *Ga. xylinus* genes affecting the biosynthesis of bacterial cellulose

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*Gluconacetobacter xylinus* is a well-known producer of bacterial cellulose. This biopolymer, due to its unique properties, is widely used in the food, paper, textile and chemical industry, as well as in medicine [1]. Increased use of cellulose and its derivatives in various industries causes that work on improving efficiency and reducing the cost of the polymer synthesis and modification of its properties are the subjects of intensive research. Since the knowledge in this field is still insufficient, it is of high importance both for industrial and scientific purposes to do the research in the understanding of mechanisms of biosynthesis of cellulose. The research conducted in the Institute of Technical Biochemistry by using SSH-cDNA technique led to receive the seven fragments of transcripts differentiating the cells able to produce cellulose (Cel<sup>+</sup>) from those non-producing ones (Cel<sup>-</sup>). Among the identified transcripts, overexpressed in Cel<sup>+</sup> cells, the genes such as *clpP* (encoding ATP-dependent Clp protease proteolytic subunit) and *tpi* (encoding triosephosphate isomerase) were found. Analysis of the nucleotide sequences indicate that they can be associated with production of polysaccharide biofilm and thus the changes in their expression might affect the biosynthesis of cellulose. ClpP protease plays an important role in the stress response and the degradation of misfolded proteins and regulation of biofilm formation in many pathogenic bacteria [2]. Triosephosphate isomerase plays a key role in carbon metabolism as well as in bacterial morphology and in the *in vivo* fitness of a bacterial pathogen [3].

The aim of current investigation is to analyze the relation between the previously selected genes (*tpi*, *clpP*) and the biosynthesis of cellulose. The research aimed at obtaining overexpression and deletion of selected genes in cells *Ga. xylinus* is being carried out. Further investigations will focus on the use of the identified genes so as to obtain mutants of *Ga. xylinus* producing cellulose with greater efficiency and productivity than the wild-type strain. The results may also serve to synthesize cellulose membranes displaying specific structure and properties.

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## P12.4

### Potential application of yeast *Yarrowia lipolytica* in dietary supplements

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Yeast *Yarrowia lipolytica* is a strictly aerobic microorganism capable of producing metabolites important for food and dietary supplements (e.g. amino acids, vitamins and microelements). Furthermore it assimilates alkanes, alkenes and oils, degrades pesticides and has intense secretory activity that attracts the interest of researchers especially in the detergent, food, pharmaceutical and environmental industries. This strain is nonpathogenic and several processes based on this organism were classified as generally recognized as safe (GRAS) by the Food and Drug Administration. *Yarrowia lipolytica* has been accepted in EU as biomass for feedstock.

In this work we investigate *Y. lipolytica* in respect of production of valuable components such as proteins, vitamins, microelements, and amino acids during growth on different media and to test the possibility of using biomass obtained as a dietary supplement for humans. The results of the preliminary analysis suggest that the *Y. lipolytica* A-101 strain is a good candidate for a source of high-quality protein ( $\approx 480$  g/kg) and exogenous amino acids (e.g. aspartic acid  $\approx 31.9$  g/kg, lysine  $\approx 26.6$  g/kg, serine  $\approx 20.8$  g/kg). Moreover it is a rich source of microelements and vitamins (e.g. B<sub>1</sub>  $\approx 95$  mg/kg, B<sub>2</sub>  $\approx 15.9$  mg/kg, folate  $\approx 18$  mg/ml) for athletes and a dietary supplement for people after recovery. *Yarrowia lipolytica* also produces vitamin B<sub>12</sub> ( $\approx 60$   $\mu$ g/kg), which is the only vitamin not occurring in *Saccharomyces cerevisiae*.

#### Acknowledgements:

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## P12.5

### Evaluation of the endoglucanases activity secreted by filamentous fungi isolated from cellulolytic industry materials

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Cellulose is linear  $\beta$ -D-glucopyranose polymer, in which individual units (8–12 thousand) are connected with  $\beta$ -1-4-glycosidic bonds. No other chemical compound of the polysaccharides group is produced in the natural environment, in such large amounts. At present obtaining pure fibres of cellulose does not provide any technological problem. However, due to the presence of the  $\beta$ -1-4-glycosidic bond, and the supermolecular complex structure, efficient enzymatic degradation of cellulose to simple sugars is not a simple task. The hydrolysis is assisted by a complex action of three different enzymes. The activity of endoglucanase is essential for this process [1, 2]. The aim of the study was to evaluate the enzymatic activity of endoglucanase (CMC-ase) produced by selected strains of filamentous fungi isolated from cellulolytic industry materials. The study was conducted with the use of: birch sawdust, recycled paper waste, industrial cellulose, sewage sludge, and 90 strains of filamentous fungi isolated from the above-mentioned materials. The analysis covered: the total number of fungi and specific cellulolytic fungi [CFU / g ds], the degree of cellulose hydrolysis in agar medium containing 1% of carboxymethylcellulose (CMC), the concentrations of released reducing sugars, pH changes, the quantity of biomass grown in liquid Vogels medium supplemented with 1% of CMC [3]. In selected isolates the activity of CMC-ase and total protein concentration (Bradford method) was determined. All analyses of the enzymatic activity and concentrations of reducing sugars were conducted according to the IUPAC procedure (International Union of Pure and Applied Chemistry). Conducted analyses allowed to select three strains showing enhanced ability to release reducing sugars from cellulose (0.073 - 0.063 g/100 ml of culture medium). The CMC-ase's activity reached 0.8773, 0.279, 0.2864 mg of glucose/ml/min, respectively for *Aspergillus nidulans* (I6), *Trichoderma* sp. (II47), and *Trichoderma* sp. (II50). The highest activity per 1 mg of protein was determined for *Trichoderma* sp. (II50). Its total CMC-ase activity was 13.638 mg of glucose/min/mg of protein; even though the CMC hydrolysis grade reached only 47%. The isolated native fungi will be used in further studies leading to acquisition of high activity enzyme preparations.

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## P12.6

### Aptamer-modified superparamagnetic nanoparticles in targeted hyperthermia

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During the last two decades nanotechnology spread out into new areas of potential applications, including biotechnology and pharmacology. Many nanomaterials possess unique physical, optical and chemical features which make them useful both for cancer diagnosis and treatment.

Magnetic nanoparticles gather strong interest due to hyperthermic effect and ease of conjugation with biological component. A physical phenomenon named superparamagnetism causes increase in their temperature when subjected to alternating magnetic field. The rise of temperature causes cancer cells damage by apoptosis or necrosis and sensitizes the tumor to standard radio- or chemotherapies.

In the present work, we designed multifunctional magnetic nanoparticles conjugated with anti-HER2 aptamer and used them for targeted hyperthermia. Our results show much stronger effect of targeted hyperthermia to kill cancer cells, compared to the one induced with non-targeted magnetic nanoparticles.

In the first step we synthesized dextran coated magnetic nanoparticles (NP@Dex) and conjugated them with aptamer anti-Her2 (NP@Dex\_Apt).

We used SK-BR3 cell line which is characterized by over-expression of Her2 receptor and U-87 MG cell line as a control. The cells were incubated in full medium with either magnetic nanoparticles (NP@Dex) and targeted nanoparticles (NP@Dex\_Apt) followed by application of the alternating magnetic field (2 times for 30 min. with 30 min. interval). After overnight incubation the viability of the cells was evaluated.

The results showed, that high concentration of particles (1 mg/ml) is required for efficient diminution of the cells viability. We also found that to efficiently destroy HER2 cells there is no need to increase the time and frequency. With optimized these parameters of the magnetic field, we were able to destroy up to 50% of cells.

In case of application of targeted nanoparticles, we applied 91 times less nanoparticles to gain equivalent or better results. The magnetic field parameters were kept constant in both experiments.

To summarize, the targeted nanoparticles combined with hyperthermia efficiently cause cell death in *in vitro* experiments, which may decrease the side effects of anticancer therapies.

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## P12.7

### Effect of multigenerational cadmium exposure of *Spodoptera exigua* on detoxification response after bioinsecticide (spinosad) application

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*Spodoptera exigua*, beet armyworm is an agricultural insect pest in tropical and subtropical areas of the world. In the present study, we established differences in biochemical defence between two strains of *Spodoptera exigua*: exposed over 100 generations to sub-lethal concentration of cadmium (44 mg Cd/kg dry weight of larval diet) and kept in controlled conditions. This differences were found among various developmental stages of this species: larvae, pupae and imagoes. The aim of our study was to assess the consequences of cadmium exposure also on eggs exposed to spinosad *in vivo* as well as *in vitro*. We measured biochemical defence indices: total antioxidant capacity, esterases (carboxylesterase toward p-nitrophenyl acetate and cholinesterase toward acetylthiocholine iodide) and glutathione transferase (toward 4-chloro-1,3-dinitrobenzene) activity. We assessed the latter enzyme activity also in *in vitro* assay using as inhibitor biopesticide — spinosad. We detected a variation in detoxification response between eggs originated from both strains. We also found the differences in changes of the glutathione transferase and esterases activity, between eggs derived from experimental strains in concentration specific mode.

## P12.8

### Application of chromatographic methods for genetically modified food identification

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Nowadays, the area of genetically modified crops covers about 170.3 mln ha [1] and genetically modified plants are present in food production for nearly 20 years, when genetically modified tomato (Flavr Savr) was introduced into the market. The aim of genetic modifications is to improve crop characters, a.o their technological features during storage and processing as well as sensorial and functional features of food products. However, genetic modifications provided much controversy. From a practical point of view the most significant problem is the development of credible and commonly acceptable methods of GMO identification. Determination methods of GMO quantity in plant material are mostly based on the identification of nucleic acid sequences (PCR) and protein analyses (ELISA). Besides, routine techniques employed for this purpose in recent years have paid increasing attention to the application of chromatographic methods. For instance, the chromatographic analyses of oil allow to detect changes in fatty acids contents and profiles in transgenic plants [2]. An example represents the detection of trierucin in seed oil from transgenic rapeseed containing the lysophosphatidic acid acyltransferase gene from *Limnanthes douglasii* L. In this case gas chromatography is a very sensitive and selective analytical method for this particular transgenic trait. A large group of compiled methods, where liquid chromatography or gas chromatography is coupled with mass spectrometry is well-founded for profiling proteins in extracts. LC-MS has been evaluated for the profiling of relatively low-molecular weight protein species in genetically modified and non-transgenic maize [3] and was shown to allow a rapid separation of maize proteins with high resolution. A further interesting approach is the application of GC-MS for quick evaluation of amino acids profiles of genetically modified maize and soybean [4]. Chromatographic methods were also successfully implemented for profiling of volatile organic compounds, carbohydrates, organic acids, carotenoids, and alkaloids used to distinguish between transgenic and non-transgenic papaya [5]. Over the last years classical PCR procedures gained the status of a basic diagnostic tool in analyses of GMO. However, chromatographic methods allow accurate, efficient and fast identification of GM food. Nevertheless, there is a need for further specification of analytical methods that would allow reliable identification and quantification of several different GM food products.

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## P12.9

### Endopolyploidy and genome size in the selected Fabaceae species

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Endoreduplication is an alternative form of the cell cycle in somatic tissues, in which the repeated rounds of nuclear DNA replication occur without subsequent cell division. Increases in DNA content in the nuclei through endoreduplication lead to endopolyploidy; organs and tissues where endopolyploid nuclei occur are polysomatic. Although endoreduplication is widespread in plants, its biological significance is still unclear. It is suggested that it promotes cell enlargement and high gene expression to facilitate the rapid growth and maturation of a tissue/organ. Endoreduplication may represent also an evolutionary strategy, which substitutes for a lack of phylogenetic increases in nuclear DNA. The Fabaceae family is a large and economically important family of flowering plants. The high diversity of species in this family with respect to genome size, life cycle type and life habit makes it a good model to study endopolyploidy. The present study aimed at comparing the endoreduplication intensity in the cotyledons and mature leaf blades in 18 species of the Fabaceae family. Analyses of endoreduplication intensity and genome size were performed using flow cytometers: Partec CCA for endoreduplication analysis and Partec CyFlow Green for genome size estimation. The proportion of nuclei with different DNA contents, the mean ploidy (C-value) and genome size (2C/pg) were established. Flow cytometric analysis revealed the occurrence of endopolyploidy in cotyledons of all investigated species, except in *Medicago sativa*. The highest endopolyploidy (up to 128C; 5 endocycles) in this organ occurred in *Phaseolus vulgaris*, and the lowest (1 endocycle) in *Medicago x varia* and *Trifolium* species. In the mature leaf blade, however, no endoreduplication or only one endocycle was observed. Genome size (2C/pg) of the investigated species ranged from 0.88 pg (*Trifolium pretense*) to 26.87 pg (*Vicia faba* var. *minor*). Endoreduplication intensity was organ specific and characteristic for a particular species. There was a negative correlation between endoreduplication intensity and genome size. It also appears that there is less endoreduplication in perennial than in annual species.

## P12.10

### Synthesis, biotransformations and biological activity of selected chalcones

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In recent years many researchers have turned their attention to numerous advantages of microorganisms and the possibilities of their biotechnological applications [1]. Flavonoids are a group of natural polyphenolic compounds which are widespread in the plant kingdom [2]. Due to their health-promoting properties there is growing interest in methods of obtaining flavonoids and their applications as food additives. The use of microorganisms may be a highly efficient method of producing these compounds. This process is considered to be more effective than extraction from plants and classical chemical synthesis. The major advantage of the microbial biosynthesis of flavonoids is the possibility of production cost reduction by using modified microbial strains or low-cost cultivation medium components.

The primary scientific aim of the project is to obtain flavonoid compounds: chalcones and their methoxy-, hydroxy- and amino derivatives and to select bacterial strains which are capable of enzymatic transformation of this group of compounds.

The aim of research was to define a metabolic pathway of flavonoids in bacteria and yeasts and to determine the specificity generic individual strains which are able to carry out planned reactions.

The present studies report a development of highly efficient protocol for chemoselective reduction of  $\alpha,\beta$ -unsaturated carbonyl compounds in aqueous media using the bacterial and yeasts cultures [3-4].

Total antioxidant capacity using the method of evaluation of free radical scavenging activity against DPPH and antifeedant activity of chalcones and its derivatives against *Myzus persicae* also were examined.

#### Acknowledgements:

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## P12.11

### *Debaryomyces hansenii* extracellular exo- $\beta$ -1,3-glucanase is involved in killer action against *Botrytis cinerea* cells

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Killer phenomenon in yeast, first described for *S. cerevisiae*, was further observed in many yeast strains from different genera. Structurally diverse secreted proteinaceous killer toxins cause growth inhibition of sensitive cells by the use of various mechanisms, such as changing cytoplasmic membrane permeability, arresting cell cycle by the inhibition of DNA replication or through their enzymatic activity.

We have described earlier a killer activity of a few strains of *D. hansenii* directed against *Yarrowia lipolytica* cells, both isolated from Rokpol cheese. The crude killer toxins of *D. hansenii* strains KI2a, AII4b and MI1a are also active against another yeast (*Candida*, *Schwanniomyces*) and filamentous fungi (genera: *Trichoderma*, *Aspergillus* and species *Botrytis cinerea*), but not against gram-positive and gram-negative bacteria.

Searching for a possible involvement of enzymes in *D. hansenii* killer activity, we have found an extracellular  $\beta$ -glucanase, distinct however from the killer toxins active against *Y. lipolytica* cells. As the inhibitory effect of exo- $\beta$ -glucanases on *B. cinerea* growth was described so far only for a few killer yeast species, i.e. *Pichia anomala*, *P. guilliermondii*, *Tetrapisispora phaffii*, we have decided to characterize the *D. hansenii* enzyme and examine its potential activity against *B. cinerea* cells.

The secretion of  $\beta$ -glucanase starts at late exponential phase and extends into a stationary phase of *D. hansenii* growth. This is a 20 kDa enzyme, active at pH range 3.5–8.0, with a broad pH optimum at pH 4.0–5.0, hydrolysing laminarin but not barley glucan. It has exo- $\beta$ -1,3-glucanase activity, releasing glucose as the only final reaction product of laminarin hydrolysis, with  $K_m = 7,52$  mg/ml.

The isolated exo- $\beta$ -1,3-glucanase preparation significantly inhibits growth of *B. cinerea* cells; also the addition of exo- $\beta$ -1,3-glucanase to the crude toxin increases the level of killer activity of this preparation in an additive manner, which suggests direct involvement of this enzyme in *Botrytis* killing. A growth inhibition was not observed in parallel experiments where *Y. lipolytica* was used as a sensitive strain. The potential involvement of *D. hansenii* exo- $\beta$ -1,3-glucanase in killing of *B. cinerea* cells is also suggested by a found correlation in a production rate of the enzyme and killing activities.

These findings open a perspective for further studies on application of *D. hansenii* exo- $\beta$ -1,3-glucanase as a biological control agent against *B. cinerea*.

## P12.12

### Covalent immobilization of pepsin onto various porous carriers

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Pepsin (EC 3.4.23.1) is one of the key enzymes of the digestive system and it belongs to the aspartate protease family. Pepsin cleaves the peptide bond between aromatic and hydrophobic amino acids (for example tryptophan, phenylalanine or tyrosine). High probability of peptide bond hydrolysis by this endopeptidase occurs if there is a sulphur-containing amino acid near to the bond, which has an aromatic amino acid. Pepsin is active in acidic environment with optimal pH between values 1.5 and 2.0 and inactive at pH 6.5 and above. The optimal value of temperature is from 37°C to 42°C. Pepsin has many applications in food and leather industries and also in the synthesis of functional fragments from antibodies.

In the present study the properties of pepsin from hog stomach immobilized on various porous carriers were investigated. Pepsin was immobilized by covalent bonds on silica-gel carriers, acrylic beads and cellulose-based carriers, which were functionalized by the presence of -OH, -COOH, -NH<sub>2</sub> and -glycidyl groups on their surfaces. Carriers were activated with three different cross-linkers: glutaraldehyde (GLA), carbodiimide (CDI) and divinyl sulphone (DVS). After the immobilization process the storage stability and the stability under different conditions of pH and temperature values for each carrier were tested in comparison to native enzyme. Obtained results showed that the immobilized enzyme is much more stable than native enzyme and may be widely used in many industries.

## P12.13

### Chemoenzymatic synthesis of xylosides and oligoxylosides from lignocellulosic biomass for biological applications

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The valorization of non-food plant biomass is currently a major issue in the development of the bio-refinery concept. The processing of co-products from agricultural origin is the subject of extensive studies. Straw and bran, abundant co-products of the wheat industry, are rich in hemicelluloses. These polysaccharides mainly consist of two pentoses: D-xylose as the major one and L-arabinose as the minor one.

In recent years, our research work has been devoted to the use of various hemicellulases to perform transglycosylation reactions leading to the preparation of pentosides and oligopentosides [1]. Recently, the use of xylanases has enabled us to develop, from xylans or directly from agricultural co-products such as wheat bran, a synthesis of alkyl oligoxylosides with interesting surfactant properties [2]. Some beta-D-xylosides with a hydrophobic aglycon moiety can initiate biosynthesis of glycosaminoglycans (GAGs) by an exogenous pathway [3]. To target new applications for xylose-based molecules, we were interested in the production of GAGs, macromolecules that give human tissues its main physico-chemical properties (elasticity, hydration...).

The objective of the present work is :

- To develop enzymatic or chemoenzymatic routes towards the preparation of original xylosides and xylobiosides featuring aromatic or functionalized aglycons from xylans;
- To evaluate their biological activities for applications in the cosmetic or pharmacologic purpose.

The 'click' chemistry applying xylanase permitted to synthesize the xylosides or oligoxylosides with covalently attached aglycon moiety. We synthesized five different xylosides. Some of these xylosides are soluble in aqueous media and present different cytotoxicity level depending on aglycon structure. Their potential for the synthesis of GAGs was evaluated. The soft 'ecologic' chemistry which applies the reaction in aqueous media is a valuable tool to produce different bioactive compounds.

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