Session 15: Biotechnology

Lectures

L15.1

Biogas from unusual substrates

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It is generally accepted as a fact in the biogas technology that protein-rich biomass substrates should be avoided due to inevitable process inhibition. Substrate compositions with a low C/N ratio are considered difficult to handle and may lead to process failure, though protein-rich industrial waste products have outstanding biogas generation potential.

This common belief has been challenged by using protein-rich substrates, i.e. casein and precipitated pig blood protein in laboratory scale continuously stirred mesophilic fed-batch biogas fermenters. Both substrates proved suitable for sustained biogas production (0.447 L CH_4/g protein oTS, i.e. organic total solids) in high yield without any additives, following a period of adaptation of the microbial community. The apparent key limiting factors in the anaerobic degradation of these proteinaceous materials were the accumulation of ammonia and hydrogen sulfide. Changes in time in the composition of the microbiological community were determined by next-generation sequencing-based metagenomic analyses. Characteristic rearrangements of the biogas-producing community upon protein feeding and specific differences due to the individual protein substrates were recognized. The results clearly demonstrate that sustained biogas production is readily achievable, provided the system is well-characterized, understood and controlled. Biogas yields (0.45 L CH_4/g oTS) significantly exceeding those of the commonly used agricultural substrates (0.25-0.28 L CH_4/g oTS) were routinely obtained.

The results amply reveal that these high-energy-content waste products can be converted to biogas, a renewable energy carrier with flexible uses that can replace fossil natural gas in its applications. Process control, with appropriate acclimation of the microbial community to the unusual substrate, is necessary. Metagenomic analysis of the microbial community by next-generation sequencing allows a precise determination of the alterations in the community composition in the course of the process.

Applications of the power-to-gas principle for the handling of surplus renewable electricity have been proposed. The feasibility of using hydrogenotrophic methanogens as CH_4 generating catalysts has been demonstrated. Laboratory and scale-up experiments have corroborated the benefits of the CO_2 mitigation *via* biotechnological conversion of H_2 and CO_2 to CH_4. A major bottleneck in the process is the gas-liquid mass transfer of H_2.

Fed-batch reactor configuration was tested at mesophilic temperature in laboratory experiments in order to improve the contact time and H_2 mass transfer between the gas and liquid phases. Effluent from an industrial biogas facility served as biocatalyst. The bicarbonate content of the effluent was depleted after some time, but the addition of stoichiometric CO_2 sustained H_2 conversion for an extended period of time and prevented a pH shift. The microbial community generated biogas from the added α-cellulose substrate with concomitant H_2 conversion, but the organic substrate did not facilitate H_2 consumption. Fed-batch operational mode allowed a 4-fold increase in volumetric H_2 load and a 6.5-fold augmentation of the CH4 formation rate relative to the CSTR reactor configuration. Acetate was the major by-product of the reaction.

Fed-batch reactors significantly improve the efficiency of the biological power-to-gas process. Besides their storage function, biogas fermentation effluent reservoirs can serve as large-scale bioCH_4 reactors. On the basis of this recognition, a novel concept is proposed, which merges biogas technology with other means of renewable electricity production for improved efficiency and sustainability.

L15.2

Application of metabolomics in biotechnology – future or past

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Biotechnology belongs to the field of science with very wide application in many branches of industry including of aquatic environment – blue biotechnology, agriculture – green biotechnology, health care – red biotechnology and white biotechnology related to use of cells, organisms or enzymes to obtain synthetic compounds or materials. In all these examples the metabolomics can be used as monitoring, discriminating or diagnostic tool. The metabolomics is the interdisciplinary science, which combines of analytical chemistry, mathematic and biology in one subject belonging to systems biology with holistic approach to investigated problem. Metabolomics is comparative science, which is based on qualitative and quantitative analysis of low molecular compounds MW<1500 Da, where many objects with many (thousands) of their features (variables) can be mutually compared. Metabolomics reinforced by metabolic flux analysis with use of isotopically ^{13}C enriched metabolites can yield of unique information about biochemical pathways and action of particular enzymes. This valuable information can be used in genetic studies, drug design and optimization of biotechnological processes. In this presentation the newest trends in application of metabolomics and fluxomics, in white and red biotechnology will be discussed, based on own research as well as worldwide scientific trends in microbiome monitoring and determination of cell processes for industrial application. Additionally, the use of metabolomics in cancer diagnostics will be also described.

L15.3

Novel approaches to understanding the behaviour of ovarian cancer cells and to overcome drug resistance

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Ovarian cancer is the most lethal gynaecological malignancy with very little achievement recorded on 5 year overall survival in the last couple decades. Despite the availability of several treatment options, the anticancer effectiveness of these options has been limited by lack of outstanding target specificity and/or the capacity to identify and fully understand the possible mechanisms of their actions and effects on cancerous cells.

We have taken a quantitative systems pharmacology approach to study and understand the proliferation behaviour of ovarian cancer cells and their hierarchical addiction to production, sequestration, tolerance and adaptation to reactive oxygen species (ROS). We used cytotoxicity, western blotting, immunocytochemistry, transcriptional reporter assays and knowledge-based approaches to quantify and to mathematically fit and model proliferation behaviour of cells with the dynamics of ROS and the regulation of the antioxidant response pathway by Nuclear erythroid related factor-2 (NRF2)-Kelch-like ECH-associated protein 1 (KEAP1) redox sensor system (NRF2-KEAP1) in these cells. In addition and using real time quantitative PCR assay, as well as data mining and bioinformatics analysis of *in vivo* xenograft data, we explored and unveiled the implication of cellular tolerance to ROS to promote proliferation, to induce dynamic changes and reprogramming of signalling pathways followingHER-targeted therapies, and to convey therapeutic resistance in ovarian cancers. Furthermore, this presentation will demonstrate a role for NRF2 function in regulating the HER receptor family and ROS and in mediating the response of ovarian cancer cells to receptor tyrosine kinase inhibitors (RTKi). We propose that NRF2 primes to set the limit and effectiveness of RTKi in anticancer therapy and as such a key anticancer target.

Key words: Ovarian cancer, ROS, proliferation, NRF2-KEAP1, HER-targeted therapy

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L15.4

Model-based concepts for advanced bioprocess control

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In the last years, computer-assisted methods for process design and control have become increasingly popular and were applied in many industrial processes. This resulted in the development and application of several novel sensors and powerful technologies. However, not all industries make use of this high potential for specifying model-assisted designs, process optimization and controlled reproducibility. This is especially true for biotechnological processes due to the comparably high complexity of biological systems.
Commonly, manual samples and off-line techniques are still the main methods used for process control and quality assurance. However, using these methods, only a handful of process variables can be monitored. Many important efficiency parameters such as the productivity of the biomass can neither be monitored nor controlled using this approach. Therefore models can serve as tools to bridge the gap between the information that is available and the efficiency parameters required for designing and optimizing a bioprocess.
Models may serve as powerful tools for advanced control applications. The techniques for this purpose are mainly classified according to three tasks: adaptive control, predictive control and other neuro-(fuzzy)-based approaches. The system of adaptive control uses information on changes in process dynamics to adjust control parameters accordingly to maintain correct operation. Model predictive control (MPC) can be regarded as a complex add-on for existing control systems. MPC relies on dynamic models with varying degrees of structure and complexity which are used to predict the future progression of process variables and compare this prediction to reference trajectories. The predicted data is then used to adjust the appropriate control variables thus eliminating future deviations from the reference trajectory.
As part of this contribution the application of models in bioprocess design, optimization and control are summarized. Furthermore, current challenges and future trends are highlighted by discussing emerging novel techniques for advanced applications in model predictive bioprocess-control.

Oral presentations

O15.1

Epigenetic modulation of the chalcone synthase gene activity in flax

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Recently, the growing number of reports indicating the epigenetics as a significant source of organisms' variability has been noted. The epigenetic modifications play an especially important role in plants, which are perpetually adjusting to the surrounding environmental conditions. Stress factors can leave a mark in the "molecular memory" of plant in the epigenome. This event enables a rapid response of the plant in the case of subsequent environmental changes. Moreover, it has been confirmed that the epigenetic modifications might be maintained during cell divisions and inherited through generations.
In plants the products of secondary metabolism constitute the first line of defense against some stress conditions, including pathogen infection. In flax (*Linum usitatissimum* L.), important group of the secondary metabolites are flavonoids. The key enzyme involved in the synthesis of flavonoids is chalcone synthase (CHS). The gene encoding CHS is extremely sensitive to stress factors, which may be reflected in the modified level of the transcript. Two isoforms of chalcone synthase gene were recognized in flax genome.
The purpose of this research was the modulation of activity of the chalcone synthase gene induced by the epigenetic changes. Observed modulation was compared to the genetically modified flax obtained by the introduction of the CHS cDNA sequence from Petunia hybrida. The epigenetic modifications were induced by: treatment with specific OLIGOs (short oligonucleotides homologous to the analyzed gene sequence) and influence of the stress factors (such as low temperature, darkness, attack of the non-pathogenic fungi).
In the obtained plants expression of the CHS gene and methylation level were examined. The CHS gene expression in epigenetically modified plants showed greater variability in the comparison to transgenic lines. A negative correlation between the total genomic methylation level and the CHS gene expression in the analyzed plants was confirmed. In the sequence of the CHS gene several CCGG sites were studied for cytosine methylation profile. The sites were either stably demethylated or showed great variability in methylation of the cytosines.
Obtained results prove that epigenetics is a promising tool for obtaining improved plants and may constitute a great alternative for genetic transformation.

O15.2

Inhibition kinetics of aryl thiosemicarbazone derivatives on mushroom tyrosinase

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Tyrosinase is a key enzyme in melanin biosynthesis. It is copper-containing oxidoreductase. Tyrosianse is responsible for pigmentation in animals and enzymatic browning in fruits, vegetables and fungi [1]. Excess production of melanin can be the reason of numerous dermatological disorders in humans and the cause of the loss of commercial value of plant and fungi-derived food. Tyrosinase inhibitors have application in a lot of fields including medicine, pharmaceutical industry, cosmetology and food industry [2]. Thiosemicarbazone derivatives seem to be promising tyrosinase inhibitors [3, 4].
Tyrosinase was isolated and purificated from mushroom using such methods as extraction with acetone, salting out and column chromatography (DEAE-Sepharose, Phenyl-Sepharose and Sephadex G-50). Activity, concentration and purity of obtained enzyme sample were checked using UV-VIS spectroscopy, Bradford method and SDS-PAGE, respectively. The activity of obtained enzyme was 26886 U/ml, the concentration was 0.49 mg/ml and the sample was pure enough to carry out kinetic investigations.
Inhibitory kinetics on the diphenolase activity of mushroom tyrosinase was investigated for a group of aryl thiosemicarbazone derivatives. The inhibitory effects of these compounds on mushroom tyrosinase were investigated using UV-VIS spectroscopy. For all investigated compounds K_i (or K_{is}), IC50 mechanism and type of action were determined using computer programs. Structure-activity relationship was also evaluated.
All investigated compounds belonged to reversible inhibitors of different type of action, competitive, non-competitive, uncompetitive and mixed-type. Their inhibition constants were determined and compared to each other and to kojic acid – literature reference compound [5].

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O15.3

A novel affibody-auristatin E conjugate that targets HER2-positive cancer cell lines

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Breast cancer is the most commonly diagnosed cancer in women worldwide. HER2 (Human Epidermal Growth Factor Receptor 2), whose overexpression occurs in about 25% of invasive breast cancers [1], is a molecular target of cancer therapies that relay on anti-HER2 monoclonal antibodies. Kadcyla (Genentech) consists of anti-HER2 antibody and a highly potent drug DM1. This FDA-approved antibody-drug conjugate (ADC) has turned out to eliminate cancer cells more effectively then the naked anti-HER2 antibody called Herceptin (Genentech) demonstrating the potential of the ADC strategy in clinics.
We developed and characterized a new cytotoxic conjugate based on a small affibody molecule, $Z_{HER2:2891}$, which specifically recognizes HER2 (Human Epidermal Growth Factor Receptor 2) [2]. The conjugate contains monomethyl auristatin E (MMAE), a highly potent drug that blocks tubulin polymerization, which results in mitotic arrest and subsequent apoptosis. To enable conjugation between the thiol group and the maleimide group present in the cytotoxic component, a Drug Conjugation Sequence (DCS) containing single cysteine was introduced at the C-terminus of the $Z_{HER2:2891}$ affibody. The resulting highly homogeneous conjugate efficiently killed the SK-BR-3 HER2-positive (IC_{50} value of 5.16 nM), comparable to the effect of free MMAE (1.76 nM). Consequently, the significant increase of the IC_{50} values was observed when the cell lines expressing lower HER2 levels were treated with the conjugate, which reflects the selectivity of our construct [3].
We demonstrated that proteins other than antibodies or antibody fragments can be employed as drug targeting molecules. Our affibody-based conjugate exhibits high efficacy and selectivity in *in vitro* settings. However, the small size of the affibody contributes to its short half-life in circulation. Therefore, we are going to increase the size of affibody molecule either by fusion with Fc fragment of IgG or by the use of PEGylated auristatins.

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Posters

P15.1

The use of *Callitriche cophocarpa* as biosorbent for As(III) and As(V) removal from aqueous solutions

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Callitriche cophocarpa (water starwort) is a perennial, aquatic higher plant. It grows in stagnant or slow-moving water in temperate climate all over the world, being the most common species of *Callitriche* genus in Europe. The ability of the representatives of Callitrichaceae family (*C. lustanica, C. brutia and C. stagnalis*) to phytoextract of arsenic was demonstrated by the group of Favas and coworkers investigating the polluted aquatic reservoirs in the central Portugal (Favas *et al*., 2012, *Sci Total Environ* 433, 390). Arsenic is one of the most toxic elements occurring in the environment. It is a well-known carcinogen for humans, causing tumours in the liver, lung, kidney, bladder, skin, and various human tissues. In natural waters it occurs mainly in the inorganic form as arsenite and arsenate oxyanions. The biosorption of arsenic from aqueous solutions using dried biomass as adsorbent has recently received much attention due to its availability and low cost. The good adsorption capacity of *Callitriche cophocarpa* toward chromium(VI) oxyanion was proved in our earlier work (Kyziol-Komosińska *et al*., 2016). Therefore, in this work we focused on the As(III) and As(V) oxyanions biosorption on dried *Callitriche cophocarpa*. The biosorbent was prepared from the shoots of *C. cophocarpa* collected in the plant's natural stand. The obtained material was washed with tap and distilled water, dried and ground. In order to determine the adsorptive properties of *C. cophocarpa* towards As(III) and As(V), the equilibrium studies were conducted. The experimental data were analyzed using theoretical isotherm models, which complemented with FTIR analyses, allowed to describe the process of arsenic phytoremediation. The obtained results revealed high sorption capacity of *C. cophocarpa* towards arsenic species – 30.0 mg As(III)/g and 8.0 mg As(V)/g. These values are comparable and sometimes exceeding adsorption capacity of many synthetic adsorbents used for arsenic removal from natural waters. We believe that the results of the work can be applicable to the biotechnological utilization of *C. cophocarpa* in the phytoremediation of As-polluted aquatic systems.

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P15.2

Comparative analysis of biological and physiochemical properties of two structural analogues of a lipopeptide biosurfactant pseudofactin

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Biosurfactants (BS) are surface active molecules produced by microorganisms. BS exhibit similar properties to synthetic detergents, are environmentally friendly and have compelling antimicrobial, antiadhesive, or anticancer activities. Wide structural variety of BS is the reason of their diverse properties. Activity of BS depends strongly on molecule structure, but the question on the structure-activity relationships is still open [1].

Pseudofactin (PF) is a lipopeptide biosurfactant produced by the Arctic strain *Pseudomonas fluorescens* BD5. Two structural analogues of PF: PF1 and PF2 were identified. Pseudofactins are composed of a palmitic "tail" and hydrophilic, cyclic "head" of eight amino acids. PF1 and PF2 peptide part differ only in one amino acid and compose of Gly-Ser-Thr-Leu-Leu-Ser-Leu-Val for PF1 and Gly-Ser-Thr-Leu-Leu-Ser-Leu-Leu for PF2 [2]. *P. fluorescens* BD5 produces approximately 10 times more PF2 than PF1 [2], therefore we previously revealed antimicrobial, antiadhesive, antibiofilm and anticancer activities of PF2 only [3-5]. Here we report successful purification of two structural analogues of PF: PF1 and PF2 followed by the comparative analysis of their biological and physiochemical properties. We revealed surprisingly significant differences in critical micelle concentration (CMC), emulsification index, micelle size, as well as microbial growth inhibition and antiadhesive activity.

We determined the CMC levels at approximately 30 mg/L and 19 mg/L for PF1 and PF2, respectively. Emulsification index of PF1 was greater than that of PF2 for the most of tested hydrophobic substrates. Micelle size, measured by dynamic light scattering, revealed significant concentration-dependent reduction in particle size of PF micelles. At the same time, PF1 micelles were 40–55% bigger then PF2 micelles. Also in terms of reducing microbial growth and adhesion, pseudofactins showed differences for some of testes strains.

Our observations prove that even small structural changes in lipopeptide molecules can cause a shift in macromolecular properties.

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P15.3

Highlighting the fullerene C_{60} impact on bacterial cell walls

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C_{60} fullerene (FC_{60}) is an example of carbon nanoparticle, which has a potential to directly interact with biomacromolecules, such as aromatic mutagens or anticancer drugs, and penetrate into cells. Its unique properties place FC_{60} in the centre of modern nanomedicine attention. It is believed that FC_{60} may serve as an efficient drug carrier in novel chemotherapy systems. However, there are some indications suggesting FC_{60} toxicity (eg. induced by interactions with cell walls), but these studies still remain inconclusive. Basing on such contradictory reports a question what exactly happens when FC_{60} reaches cell walls arises.

To shed a light on this problem we employed combined double Live/Dead staining on *Salmonella typhimurium*, which is commonly used in mutagenicity test, called Ames test. We have already demonstrated that FC_{60} can diminish biological activity of selected anticancer drugs towards *S. typhimurium* cells. Therefore, bacterial cells were incubated in the presence of FC_{60} in different concentrations and in diverse time. Chosen concentrations and times are similar to those recommended in the Ames test procedure. In employed approach first fluorescent dye selectively stains only living cells, in the contrary to the second one, which stains only dead cells or cells with destabilized membranes. Fluorescence signals were detected using confocal microscopy. Recorded pictures will provide data on FC_{60} uptake and its possible impact on the bacterial cell walls continuity and cells viability.

Moreover, obtained results will provide practical insight into FC_{60} impact on bacterial cells membranes, what is essential for further FC_{60} pharmaceutical application. What is more, such knowledge may be exploited in future cancer prevention or treatment.

P15.4

Factors affecting the synthesis of bioflocculants by *Rhodococcus* strains

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Flocculation is a physico-chemical process that involves special molecules known as flocculants. Depending on the source from which flocculants are obtained, they can be classified as [1] inorganic flocculants, [2] synthetic flocculants and [3] natural polymeric flocculants called bioflocculants. The most common sources of bioflocculants are bacteria, algae and fungi. Despite many investigated natural products about flocculating activity, still only a few of them are used in industry processes. However, new discovers are necessary to decrease the application of synthetic substances, harmful for environment and humans. Promising producers of substances with flocculating activity are bacteria from Actinobacteria class, known as a source of biologically active molecules applied in medicine and pharmacy. These microorganisms are able to excrete extracellular polymers, composed mainly of polysaccharides with chemically active groups such as amino, carboxyl and hydroxyl, participating in creation of bonds in flocculation process. Flocculating activity of these polymers depends on basic culture conditions like carbon and nitrogen sources, an initial pH value of medium, inoculum size and a presence of metal ions in culture broth.

In the present study, two strains of Actinobacteria were compared by their synthesis of exopolymers to culture broths during bacterial growth. Supernatants obtained from *Rhodococcus opacus* and *Rhodococcus rhodochrous* culture broths were analysed for flocculating activity using mixture of kaolin and calcium chloride as the standard suspension. The optimum culture conditions were determined for both strains of *Rhodococcus*, using different variants of culture, such as carbon and nitrogen sources, temperature, pH value of the medium and the speed of shaking. Additionally, two separate exopolymers were extracted from culture broths of *Rhodococcus opacus* and *Rhodococcus rhodochrous* strains using ethanol as a precipitate factor. Pellets formed after the precipitation were then lyophilised to obtain crude exopolymers for further analysis. Moreover, the morphological studies of extracted exopolymers were performed by scanning electron microscopy (SEM).

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P15.5

Efficacy of carriersmade of biotechnologically produced bioengineered silk for targeted drug delivery – *in vivo* study

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Silk-based drug delivery offers great biocompatibility and biocompatibility as well as outstanding mechanical strength. Therefore its application in the cancer treatment can help reduce harmful effects of cytotoxic drugs. Furthermore, such carriers can be easily modified by using genetic engineering to achieve greater specificity towards target cells. Results of our *in vivo* studies indicated that functionalized with tumor recognizing peptides the silk-based spheres can selectively transport cytotoxic drug (doxorubicin) into cancer cells.

The aim of this study was evaluation of efficiency of bioengineered spider silk spheres as drug delivery carriers in breast cancer treatment.

The recombinant proteins MS1 and its functionalized Her2+ specific variant H2.1MS1 were bioengineered based on repeating sequence of MaSp1 protein from *N. clavipes*. Spheres were obtained after mixing biotechnologically produced soluble proteins with kosmotropicagent (potassium phosphate). Efficiency of loading with doxorubicin (Dox) was calculated based on spectrophotometric methods. System was tested *in vivo* using Balb/c mice and Her2-positive breast cancer cells (D2F2E2/Luc). As control, the mice administrated with Her2-negative cancer cells (D2F2/Luc) were used. The distribution of carriers and therapeutic effect of delivered Dox were evaluated based on *in vivo* IVIS Spectrum imaging system. Histopathological examination allowed estimating possible toxicity towards organs after administration of Dox loaded spheres.

Fluorescent signal of functionalized silk spheres was observed at Her2-positive tumor sites indicating specificity towards Her2+ cells. Although initially signal was also observed in liver and lungs, after a week the signal in those organs was no longer present in contrast to Her2+ tumors. Furthermore, tumor growth was hampered compared to control group, indicating successful delivery of Dox. Histopathological examination showed no systemic toxicity after administration of spheres and drug loaded spheres.

Presented results confirm that bioengineered, functionalized silk carriers can be used as efficient drug delivery system in breast cancer therapy. Using biotechnology tools like genetic engineering and recombinant protein expression system gives the possibility to design carriers for drug delivery to other cancer types or beyond.

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P15.6

Two-stage anaerobic digestion of molasses: II. A tool for the production of gaseous biofuels

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Anaerobic digestion of biomass to methane and carbon dioxide is a common process in anoxic environments. It requires activity of many different groups of microorganisms responsible for hydrolysis, acidogenesis (hydrogen-yielding stages), acetogenesis and methanogenesis (methane-yielding stages). Acidogenesis is considered to be one of the most attractive biological methods of hydrogen production. However, only one-third of the biomass can be converted to hydrogen and large amounts of non-gaseous fermentation products are formed. Effective biomethane production from non-gaseous fermentation products could make biological production of hydrogen economically attractive. For the purpose of innovative technologies based on microbial processes, it is desirable to build modern biogas plants where the hydrogen- and methane-yielding stages of anaerobic digestion are separated to respectively favour the production of hydrogen and methane under controlled conditions.

Previously, we have developed and described a laboratory-scale two-stage anaerobic digestion system that produces hydrogen (stage 1) and methane (stage 2) from molasses as the primary energy substrate under mesophilic conditions. Initially, hydrogen is generated *via* processes of acidogenesis in a packed bed reactor by a hydrogen-yielding microbial community fermenting molasses. Subsequently, non-gaseous organic products from this first stage feed an UASB reactor in which methane is produced by a methane-yielding microbial community. Recently, the two-stage system for hydrogen and methane production has been successfully scaled up 10-fold and currently operates in one of the Polish sugar factories (Krajowa Spółka Cukrowa S.A.). We have obtained similar efficiencies of hydrogen and methane production in both scales that gave a positive answer to the question whether it is possible to gain a comparable performance of hydrogen- and methane-producing bioreactors in a small-laboratory scale and the enlarged-laboratory scale. A series of successful experiments of supplying a polymer electrolyte membrane fuel cell with air and a hydrogen-rich gas obtained by microbial fermentation of molasses were done. An energy efficiency of the two-stage anaerobic digestion of sugar beet molasses was calculated to be up to 50.0 %. Further research aimed at an innovative solution for the sugar factory as a producer of biofuels, biohydrogen and biomethane, from bio-waste and by-products of the sugar industry are warranted.

P15.7

CpG-siRNA delivery using functionalized bioengineered spider silk spheres

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Small interfering RNA (siRNA) promises high efficacy and excellent specificity to silence the target gene expression, which shows potential for treatment of various diseases. However, systemic delivery of siRNA into the cytosol of cells and construct stability in body fluids remain a major limitation. The CpG-siRNA molecules target TLR9-positive cellsand thus overcome a problem of cell-specific delivery. The bioengineered spider silk is biocompatible and biodegradable, and due to the self-assembly property can be processed into drug delivery vehicles.Moreover, its functionalization by adding peptide moieties such as nucleic acid binding domain enables development of nucleic acid delivery system. Silk spheres loaded with CpG-siRNA may protect construct from degradation and prolong its activity in target cells.

The aim of present study was the evaluation of CpG-siRNA cell uptake and its gene silencing potential when delivered in spider silk spheres.

The hybrid construct (MS2KN) was obtained by cloning a nucleic acid binding domain (KN) to spider silk (MS2). The AA sequence of MS2 was based on the MaSp2 from *N. clavipes*. Spheres loaded with CpG-siRNA were formed by mixing a soluble protein with potassium phosphate at silk: nucleic acid molar ratio of 1:1. Obtained spheres were characterized in terms of size, morphology (SEM), Zeta potential (ZP) and cytotoxicity (MTT assay). CpG-siRNA loading into particles was examined spectrophotometrically. The serum stability assay was performed by incubation of spheres loaded with CpG-siRNA in presence of mouse serum and then analyzed in acrylamide gel. Flow cytometry and confocal microscopy were used to evaluate the uptake of constructs by J774 macrophages. Real-time quantitative PCR was performed in order to measure the silencing effect of CpG-siRNA on target mRNA (*Stat3*).

Silk spheres protected siRNA from degradation in serum. MS2KN spheres loaded with CpG-siRNA were internalized by TLR9-positive cells. The intracellular localization of CpG-siRNA delivered in silk spheres was prolonged as compared to naked nucleic acid construct. Furthermore, extended silencing effect of *Stat3* mRNA was observed when CpG-siRNA was delivered in silk spheres comparing with naked construct.

Functionalized silk spheres can bind, prolong stability and function of CpG-*Stat3*siRNA in immune cells. Therefore, it might be a very promising strategy for development of therapeutic nucleic acid delivery system.

P15.8

Functional characterization of the *Helicobacter pylori* HP0377 – determination of the ability to reduce apocytochrome *in vivo*

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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 Dsb (disulfide bond) thiol 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*. HP0377 is essential for cell viability what prevents its functional characterization in the native host.

The aim of this study was to determine HP0377 ability to reduce apocytochrome *in vivo* in the heterologous host *Bacillus subtilis*.

PVK48 plasmid which allows the introduction of a gene of interest into the chromosome of *B. subtilis* at the *amyE* locus was used to generate *B. subtilis* strain carrying *hp0377* gene. Created plasmid was next transformed to *B. subtilis* LUL9 strain, a ResA (CcmG homolog) deficient derivative of the *B. subtilis* wild type strain. The production of HP0377 was verified by Western blot analysis. Preliminary test (TMPD staining) confirmed the proper HP0377 functioning in the heterologous host. In order to more precisely quantify the level of cytochrome *c* oxidase activity, a spectroscopic cytochrome *c* oxidase assay has been employed. In order to gain insight into the process the content of cytochrome *c* in the cell membrane has been also evaluated by a haem staining method.

We found that *B. subtilis* strain containing *hp0377* gene reveals high level of an active cytochrome *c* oxidase. The cytochrome *c* oxidase assay showed that the complementation of *B. subtilis* LUL9 strain with *hp0377* restores a wild-type phenotype. Thus it has been shown that HP0377 is involved in the cytochrome *c* maturation process and confirmed its ability to reduce apocytochrome *in vivo*.

P15.9

Effect of emodin on *Candida albicans* growth

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Candida albicans is a major human fungal pathogen. *Candida* pathogenicity is facilitated by a number of virulence factors, the most important of which are those for adherence to host tissues and medical devices, morphological transition between yeast and hyphal forms, biofilm formation and secretion of hydrolytic enzymes[1]. The intensive prophylactic use of anti-fungal drugs leads to emergence of resistant strains of *C. albicans*. This causes a great concern for finding suitable new therapeutics and antifungal compounds and drugs. One of the compounds is emodin with the proven antifungal activity.
Emodin (1,3,8-trihydroxy-6-methyl-anthraquinone) is a inhibitor of tyrosine and serine-threonine kinases. It is a natural compound isolated from roots and rhizomes of numerous plants. Emodin possesses a number of activities such as inhibitory activity of inflammation, immunosuppression, anti-virus, anti-cancer and anti-bacteria[2].
Our studies showed the activity of emodin on the growth inhibition of most common *Candida* species (*C. albicans, C. krusei, C. tropicalis, C. parapsilosis*). MIC's (Minimal Inhibitory Concentrations) were astimated by the microdilution method and ranged from 12.5 to 50 µl/ml.In addition, this compound showed the lethal effect with MFC (Minimal Fungicidal Concentration) in the concentrations 25–50 µl/ml against all tested strains. Emodin activity has been proven also against 50 clinical *C. albicans* strains obtained from gynecological patients (MIC ranged 12.5–50 µl/ml). Additionally, we found that emodin inhibits the hyphae formation. We tested, that this compound reduces the total protein phosphorylation in *C. albicans* cells and we confirmed its activity against protein kinase CK2 isolated from these yeast cells.

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P15.10

Physiological attributes of *Lactobacillus* spp. strains isolated from chicken intestinal tract

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Lactic acid bacteria (LAB) are a diverse group of Gram-positive, nonsporulating, low G+C content bacteria. Many of them have been given GRAS status (*generally regarded as safe*). Over the past two decades, intensive genetic and molecular research carried out on LAB, mainly *Lactococcus lactis* and *Lactobacillus* genus, has revealed new, potential biomedical LAB applications.
Chicken gastrointestinal tract is inhabited by various bacteria, fungi and archaea. The members of *Lactobacillus* genus are predominant in the chicken microbiota and some of them can be classified as probiotics. *Campylobacter* spp, microorganisms pathogenic for humans, are also a normal inhabitant of the chicken gut. Chickens harbor a high load of *Campylobacter jejuni* in their gastrointestinal tract (GIT). Contaminated poultry meat is considered to be the main source of human infections of *Campylobacter*. Disease caused by this bacteria is one of the most common foodborne human illnesses worldwide.
Lactobacillus strains can be used to decrease the number of *Campylobacter* colonizing chicken digestive tract. Additionally they can be employed as vectors to deliver *Campylobacter* immunodominant proteins to bird's immune system.
Our group has characterized 112 *Lactobacillus* strains of chicken origin. Affiliation to the *Lactobacillus* genus was evaluated by molecular analysis (PCR amplification and sequencing). We subsequently examined the *Lactobacillus* isolates for the inhibitory effect on the growth of *Campylobacter*. *L. salivarius* and *L. plantarum* strains exhibited consistent and strong anti-*Campylobacter* activity *in vitro*, whereas in case of *L. agilis*, *L. johnsonii* and *L. crispatus*, the antagonistic activity against *Campylobacter* depended on the strain used in the experiment. The only strain that had no effect on growth of *Campylobacter* was *L. reuteri*.
The objective of the presented study was to characterize the selected *Lactobacillus* spp. Strains isolated from chicken digestive tract. To better understand their potential probiotic properties, we evaluated their phenotypic characteristics such as: the ability of selected ten strains to grow on defined carbon source, their acid and bile tolerance and the ability to adhere to bare polystyrene (PS). The capability of the selected strains to inhibit the bird's intestine colonization by the pathogen was also examined.

P15.11

Cloning and expression platform for production of recombinant proteins

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The efficient production of soluble recombinant proteins has become increasingly important due to the increased demand for the target proteins in both basic research as well as in the drug discovery programs. The novel platform of *ligation* independent *cloning* for generation of a number of genetic constructs for the production of target proteins based on *E. coli* expression system has been applied. The presented platform relies on the series of linear expression vectors (LEV), which harbor various versions of essential components of expression vectors such as promoters, origins of replications, fusion tags, and regulatory elements. The variations of the vectors allow for relatively rapid selection of the optimal genetic constructs with the highest levels of production of given target protein. Both, LEVs and genes encoding the target proteins are generated by the standard PCR method, with specifically designed primers that contain the 15-bp complementary fragments that allow cloning using the specific PCR reaction, termed the circular polymerase extension cloning. The resulting clones are cultivated at the expression inducing conditions and the levels of production of target proteins are assessed in the multi-well plate format. The selected clones, with the highest levels of production of recombinant protein of interest are used for the scaled-up production, which are subsequently purified using the corresponding affinity chromatography based on common fusion tags. Each LEV contains the DNA fragment encoding for the specific protease recognition site, which together with the Clean-Cut, a novel, proprietary, unique and highly specific recombinant serine protease facilitates the efficient removal of fusion tag. The presented approach is cost and time effective and is ideal for the determination of the optimal conditions for the overexpression and production of the range of recombinant, heterologously produced proteins.

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P15.12

Novel fusion protein with cancer specific cytotoxic activity

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Safety and efficacy are the main goals of modern anticancer therapies. Although there is many molecules registered for clinical application, including several classes of small chemical compounds or monoclonal antibodies (mAbs) there is still need for development of new therapeutics that would be as safe and specific as antibody and effective as small chemotherapeutics.

Here we propose a new fusion protein AD-ON2.5 that displays efficacy as a potential cancer therapeutic. We have linked modified variant of human cytokine that already possess slight cytostatic activity on particular cancer cells, with strong cytotoxic, active domain of *Pseudomonas sp.* exotoxin. Two kind of receptors that are recognize by the cytokine component are overexpressed on many types of cancer cells, thus this strategy may be an alternative for direct targeting of unspecific cytotoxic agents directly into tumor.

ET expression system, and purified by affinity chromatography using histidine-tag on Ni-NTA resin. Obtained molecule was characterized by circular dichroism and SPR analysis for direct receptors binding. The molecule has been tested in MTT assay on the panel of 10 human cell lines displaying different sources of origin of cancer (organs). *In vivo* potential was examined on mice xenograft model of human lung carcinoma (A549).

AD-ON2.5 was analyzed regarding proper folding. The calculated secondary structure correlated with its component parts. Receptor binding kinetics parameters of fusion protein were similar for cytokine sole thus we confirmed that linking the cytokine to domain toxin doesn't disrupt its biologic properties.

In MTT cell cytotoxicity assays AD-ON2.5 displayed *in vitro* specific cytotoxic effect on various cancer cell lines (IC50 2-313 ng/ml) and very low activity on normal cells (IC50 >4000 ng/ml). Cytotoxic activity of single components was significantly lower on most cancer cell lines in comparison to fusion molecule.

AD-ON2.5 displayed *in vivo* tumor growth inhibition activity at the dose 0.1mg/kg. The inhibition was related to administration of the molecule.

Regarding this assumptions we had developed very promising molecule with high potential of cytotoxic activity against cancer cells and with potential therapeutic properties. Our strategy of creating fusion molecules of human cancer related cytokine acting as a specific carrier of effector domains may be considered as a novel promising anticancer solution.

P15.13

Effects of treatment of human keratinocytes with flax extracts

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Psoriasis is currently one of the most common skin disorders. This disease is affecting around 10 million people in European Union and in the USA only. Characteristic symptoms are red and slightly elevated skin lesions with successive layers of scales. The occurrence of this disease deteriorate skin condition, which interferes with proper body functioning. Through the stigmatization of sick people, psoriasis affects their mental condition too. Histologically, psoriasis include thickening of the epidermis accompanied by hyperproliferation of keratinocytes. The mechanism of psoriasis is not yet completely known.
Linum usitatissimum is an annual plant originating from Middle East and presently occurs in a Mediterranean and temperate climate zone. Due to its content of bioactive compounds like SDG (secoisolariciresinol diglucoside), α-linoleic acid, other polyunsaturated fatty acids or CBD (cannabidiol), flax can be used in pharmaceutical industry. Bioactive compounds present in flax may have, inter alia, antioxidant, anti-inflammatory, antibacterial or antitumor activity. As a result, substances isolated from flax may have potential use as medicines, in treatment of skin diseases, like psoriasis, atopic dermatitis, erysipelas or acne.
Impact of flax extracts on human keratinocytes induced with human IL-22 will be examined. Extracts will be obtained from different parts of plant (wild and GM types), using different solvents. Effects on keratinocytes will be studied using cell number and morphology analysis.

P15.14

Cyanidin as a potential scavenger of food-derived mutagens

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Cyanidin is an aglycone form of the most common anthocyanin present in raspberry (*Rubus ideaus*) fruits. As a nontoxic, small molecule with a high antioxidant properties and potential to form stacking (π-π) complexes it was analyzed in many research projects and found to possess lots of beneficial functions such as scavenging activity against free radicals, anticancerogenic properties, ability to prevent inflammation and antimutagenic activity. Cyanidin molecules can interact non-covalently with other anthocyanins in a process called copigmentation.
It is hypothesized that cyanidin can also act as an interceptor molecule, sequestering aromatic mutagens in stacking (π-π) complexes and in this way decreasing their bioavailability and, in consequence, mutagenic activity. To verify this hypothesis two representatives of heterocyclic aromatic amines (HCAs) – food-derived mutagens – was chosen. IsoIQ (2-amino-1-methylimidazo[4,5-*f*]quinoline) and MeIQx (2-amino-3,8-dimethylimidazo-[4,5-*f*]quinoxaline) are IQ-type HCAs and occur mostly in cooked meat but can be also found in polluted air, water or croplands. They are produced in high temperature (above 100°C), when free amino acids and monosaccharides react with creatine in Maillard reaction. HCAs interact with DNA (exactly with deoxyguanosine), forming adducts that induce mutations and in this way leading to cancer development.
In order to assess the ability of the cyanidin solution to interact with HCAs, a UV-Vis spectrophotometric measurements, followed by calculations using appropriate thermodynamical model of mixed aggregation, were performed. To analyze the influence of cyanidin on the mutagenic activity of HCAs, bacterial mutagenicity Ames tests based on *Salmonella typhimurium* TA98 strain were also carried out. Additionally, Autodock Vina docking procedure was used to receive sets of low-energy MeIQx/cyanidin and isoIQ/cyanidin complexes. Obtained results suggest that cyanidin molecules can form mixed stacking complexes with IQ-type HCAs. What is more, this phenomenon can decrease the mutagenic activity of HCAs.

P15.15

Two-stage anaerobic digestion of molasses: I. A characteristic of microbial communities

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Anaerobic digestion, a process that ultimately generates methane and carbon dioxide, is common in biogas plants and natural anoxic ecosystems where concentrations of nitrate, the oxidized forms of metals and sulphate are low. This complex process promoted by the interaction of many groups of microorganisms comprises four major steps: (i) hydrolysis of complex organic polymers to monomers; (ii) acidogenesis that results in the formation of hydrogen and carbon dioxide as well as non-gaseous fermentation products that are further oxidized to hydrogen, carbon dioxide and acetate in (iii) acetogenesis based on syntrophic metabolism, and (iv) methanogenesis. Current knowledge of microbial ecology and physiology, derived from culture-dependent techniques, is limited and incomplete because the majority of microorganisms have not been cultivated. Moreover, syntrophy is believed to be common in microbial communities, and syntrophic bacteria cannot be grown as a monoculture. We provided a detailed molecular characterization of a two-stage anaerobic digestion system producing hydrogen (in stage 1) and methane (in stage 2) from sugar beet molasses as the primary energy substrate under mesophilic conditions using optimized DNA extraction protocols and high-throughput pyrosequencing (454 Roche). The analysis of reactor performance data were also presented. The dominant bacteria the hydrogen-producing communities (stage 1) were representatives of the *Clostridiaceae, Enterobacteriaceae* and heterolactic fermentation bacteria, mainly *Leuconostocaeae*. Our results indicate that the phenomenon analogous to cross-feeding observed in the gastrointestinal tract involving lactate and acetate conversion to butyrate and hydrogen occurs in hydrogen-producing bioreactors. The overall biodiversity of the methane-producing microbial community (stage 2) was considerably high. The role of specific groups of bacteria in acetogenesis were discussed. In the domain *Archaea*, the order *Methanomicrobiales* was predominant, with *Methanoculleus* as the most abundant genus. The reasons for the dominance of the hydrogenotrophic pathway of methane synthesis in the methane-yielding bioreactor processing acidic effluent from molasses fermentation were discussed. Metabolic pathways of transformation of the main products of dark fermentation leading to formation of substrates for methanogenesis are poorly recognized.

P15.16

Transformed root extract of *Leonurus sibiricus* induces apoptosis through intrinsic and extrinsic pathways in various grades of glioma cancer cells

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Malignant glioma is the most aggressive primary brain tumors of the central nervous system, with a medium viability time of two years. This study determines the influence of transformed root (TR) extract of *Leonurus sibiricus* L. on various grades (I-III) of human glioma cancer cells derived from patients. This plant occurs in southern Asia and Siberia and is widely used as a medicinal plant with various biological activities. Chromatographic profile of TR extract have revealed the presence other polyphenolic compounds (4-hydroxybenzoic acid, gentisic acid, vanilic acid, 1,3-dicaffeoylquinic acid, *a*-resorcylic acid). We found TR root extract to have antiproliferative activity on glioma cancer cells after 24 hours of treatment. TR root extract induces apoptosis on various grades (I-III) of human glioma cancer cells by the generation of reactive oxygen species (ROS) along with concurrent loss of mitochondrial membrane potential, enhanced S and G2/M phases of the cell cycle, and altered mRNA and protein levels of Bax, Bcl-2, p53, Cas-3, Cas-8 and Cas-9 factors involved in apoptosis. This work for the first time demonstrate that TR extract from *L. sibiricus* root has the potential to activate apoptosis in grade I-III human glioma cells through the intrinsic and extrinsic pathways.

P15.17

Antioxidant and DNA repair stimulating effect of extracts from transformed and normal roots of *Rhaponticum carthamoides* against induced oxidative stress and DNA damage in CHO cells

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Rhaponticum carthamoides has a long tradition of use in Siberian folk medicine. The roots and rhizomes of this speciesare used in various dietary supplements or nutraceutical preparations to increase energy level or eliminate physical weakness.This is the first report to reveal the protective and DNA repair stimulating abilities of *R. carthamoides* root extracts in Chinese hamster ovary (CHO) cells exposed to an oxidative agent. Both transformed root extract (TR-extract) and extract of soil-grown plant roots (NR-extract) may be responsible for stimulating CHO cells to repair oxidatively-induced DNA damage, but CHO cells stimulated with extract from the transformed roots demonstrated significantly stronger properties than cells treated with the soil-grown plant root extract. These differences in biological activity may be attributed with differences in the content of phenolic compounds in these root extracts. Preincubation of the CHO cells with TR- and NR-extracts showed an increase gene expression and protein levels of catalase (*CAT)* and superoxide dismutase (*SOD2). R. carthamoides* may possess antioxidant properties that protect CHO cells against oxidative stress.

P15.18

Immobilization of α-1-antitrypsin on various biomaterials and their properties

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Proteolytic enzymes are a group of proteins that are responsible for hydrolysis of the peptide bonds in many bioparticles. Beyond basic function performed by proteases, which is the digestion of nutrients taken from food, they also control such mechanisms as activation of the complement system, the blood coagulation cascade or activation proenzymes and prohormones [1, 2]. The functioning of the proteases system is very complex, and its correctness determines systemic homeostasis [3]. A crucial role in the proper functioning of this system play inhibitors of proteolytic enzymes and one of them is α-1-antitrypsin. Currently protease inhibitors are very commonly used in the medical and pharmaceutical industry.
The aim of this study was to determine the optimal conditions for immobilization of α-1-antitrypsin inhibitor and to investigate the activity of the immobilized preparations in terms of stability during storage.

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P15.19

Aggregation of bacteriophage T4 by targeting ionic environment as a reversible process compatible with biological activity

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Bacteriophage is a complex biological object, with multiple interactions operating across several scales. Phages became powerful tools in biotechnology and they have great potential for medical treatment of infections (1, 2). The phenomenon of phage aggregation has sporadically been reported and suspected of major biological significance. In this work we used advanced imaging by SEM and AFM microscopic approaches, corroborated by DLS to demonstrate that the specific ionic strength of a bacteriophage T4-containing solution triggers aggregation/disaggregation transitions of phage particles. We compared the activity of T4 phage infection in *E. coli* B (expressed as pfu ml^{-1}: plaque forming units per ml) in standard culture media versus low-salt media. In low-salt media, T4 phage formed only half of the number of plaques formed under standard culture conditions. This result demonstrates that bacteriophage aggregation, which can be induced by changes in physico-chemical conditions, exerts inhibitory effect on phage infectivity therefore may affect bacteriophage biological life-cycle.

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P15.20

Stage specific apoptosis in mouse preimplantation embryo

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Apoptosis is a process of physiological cell suicide which is genetically programmed ("intracellular death program"). It involves an energy-dependent cascade of molecular events and is actively controlled by the induction of the corresponding genes and synthesis of specific proteins.
It is assumed that every cell has the potential ability to enter the path of apoptosis. This is also true for cells in the developing embryo, where whole groups of cells and structures have to be removed to shape the new organism. However, our observations supported by literature data, suggest that apoptosis does not occur until mid- blastocyst stage in the mouse embryo (64-cell stage), corresponding to first six cell cycles. To support this finding we present immunofluorescent analysis of apoptosis marker (cleaved effector enzyme – caspase 3) localization in carefully staged mouse preimplantation embryos. We also investigate regulation of apoptosis initiation timing in embryonic development – specifically, whether it is related to time of fertilization, cell number or it's stage specific (morula/blastocyst)? To answer this question we performed a series of micromanipulations, to uncouple these naturally related characteristics (such as creating 'older' embryos but with lower cell number). After manipulations and in vitro culture, embryos were analysed for the localization of phosporylated Histone H2AX (to detect dsDNA breaks) and cleaved caspase 3 by immunofluorescence. Although our manipulations resulted in developmental events such as compaction and cavitation (beginning of blastocyst stage) being initiated earlier in embryos with lower cell number compared to control, unmanipulated embryos, apoptosis still occured only at blastocyst stage in both manipulated and control group. Therefore our results indicate that caspase acivation and apoptosis in the preimplantation mouse embryo is independent of time since fertilization, but related to the stage of development defined by morphology and cell number.

P15.21

Immobilisation of extracellular laccase from *Pleurotus ostreatus* on novel porous carriers

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The immobilisation of enzyme, defined as the attachment to an insoluble supports, is very useful method for the improvement of enzyme applications because of the storage and operation stabilities enhancing. Among numerous of industrially useful enzymes, the extracellular fungal laccase is known as a very promising biocatalyst due to its ability to oxidise a broad range of substrates used in paper, food or textile industry. However, there are certain limitations in the application of this enzyme, such as still the high costs of its production, which may be overcome by laccase immobilisation, using physical or chemical enzyme-support interactions. Nevertheless, the efficiency of this process is different and depends on the applied technique and carrier. The parameters of immobilisation process should be adapted to the particular biocatalysis process, which should be sustainable and low-cost.
In the current study, the fungal laccase obtained from aerated cultures of *Pleurotus ostreatus*, was purified through ion exchange chromatography and then immobilised covalently or by adsorption on the three novel carriers from Purolite® about different porosity and structure. As a result of conducted experiments, we obtained three laccase-support preparations characterised by the high immobilisation efficiency. However, the wide discrepancies in the activity of immobilised laccase were observed, which probably were caused by the structure of carriers and related to this applied immobilisation technique. Based on obtained results, one carrier characterised by the highest immobilised laccase activity was selected for further optimisation studies.

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P15.22

Serum and urine ^{1}H NMR-based metabolomics in diagnostic of selected thyroids diseases

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Although there is many modern diagnostic methods for thyroid disease, setting final diagnosis is still very difficult and most often require biopsy of thyroid gland. Sometimes even examination of biopsy material is not sufficient to accurate and proper diagnosis. Therefore, developing of new diagnostic tools that could assist in routine examination is needed. Metabolomics methods could be a solution to this fundamental problem. Metabolomics tools allow to observe qualitative and quantitative changes of low molecular weight compounds (MW).
In this study we examine whether it is possible to differentiate patients with thyroid tumors from healthy individuals based on metabolomic studies of paired urine and blood serum. Second objective was to verify whether it is possible to discriminate different subtypes of thyroid tumors. Study was carried out on 50 samples of paired serum and urine fluids from patients and 17 samples of control group. As an analytical method ^{1}H NMR spectroscopy was used. The calculation were prepared based on each biofluids information but also with utilization of data fusion between both of them. With use of advanced chemometric and statistical methods potential diagnostic models were calculated. The studies showed that both urine and serum separately have the predictive potential in distinguishing patients with nodular changes from healthy individuals. Nevertheless, the stratification of tumor types and their differentiation, relative to each other has proved to be problematic. However, models based on data fusion allow to increase the possibility to distinguish between health and diseased and improve discrimination tumor subtypes. These studies although promising, require gathering and testing much larger cohort of patients and controls to be implemented in routine.

P15.23

In ovo immunization using GEM particles and liposomes as vectors for *Campylobacter* antigens

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Campylobacter strains are recognized as a major causal agent of bacterial diarrhoea both in developing and developed regions of the world. *Campylobacter* infection rates have been increasing steadily over the past decade. Mortality associated with *Campylobacter* infections is relatively low and no specific treatment is required for a vast majority of patients. However, *Campylobacter* infections constitute a serious problem due to the high number of cases, severity of neurological symptoms as well as high social and economic costs of the disease.

Consumption of infected poultry meat is a major source of *Campylobacter* infection. Current efforts to comply with hygiene and biosecurity EU regulations are insufficient to control or eliminate *Campylobacter* from the poultry food chain. Reduction the number of *C. jejuni* in the intestine chickens would significantly reduce the incidence of campylobacteriosis in humans and seems to be an alternative and more realistic approach for controlling *Campylobacter* contamination. However, anti-*Campylobacter* chicken vaccines are not commercially available yet.

We assessed the efficacy of *in ovo* immunization using various delivery systems: GEM particles and liposomes. The hybrid protein CjaAD, which is CjaA presenting CjaD epitopes on its surface, was employed as a model antigen. We found that CjaAD administered *in ovo* at embryonic development day 18 by both delivery systems resulted in significant levels of protection after challenge with a heterologous *Campylobacter jejuni* strain. In practice, *in ovo* chicken vaccination is used by the poultry industry to protect birds against several viral diseases. Our work showed that this means of delivery is also efficacious with respect to commensal bacteria such as *Campylobacter*. In this study, we evaluated the protection after one dose of vaccine given *in ovo*. We speculate that the level of protection may be increased by a post-hatch booster of orally delivered antigens.

P15.24

^{1}H NMR-based metabolomics in diagnostic of intrauterine infection in calves poor viability and stillborn

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Metabolomics is defined as "systematic study of the unique chemical fingerprints that specific cellular processes leave behind", while metabonomic studies are "the quantitative measurement of the dynamic multiparametric response of a living system to pathophysiological stimuli or genetic modification".Both these approaches are based on chemoanalysis of the low-molecular compounds profile, which are characteristic for pathological stage and healthy stage of the organism. The aim of the project was determination of metabolomic profile (metabolome) of calves (amniotic fluid, serum) basing on the NMR studies, which may be characteristic for healthy animals and these, which died due to pulmonary disorders in the first day after delivery or different pathological states developed during pregnancy period. Moreover the blood serum of their mothers (cow's serum) was also used for assessment of calves condition after delivery.

These studies were focused on two following issues:

Differentiating of compound profile for each group, correlation between elaborated metabolome and time of survival.

Attempts of delineation low-molecular markers of the pathological states.

All metabolomics studies were performed by use of nuclear magnetic resonance method (NMR) employing 1D and 2D techniques with applying of chemometric methods. The metabolom of amniotic fluids and serum samples from calves were determined. The chemometric analysis (PLS-DA and OPLS-DA model) of these samples showed simple differentiation between death and stillborn calves and healthy subjects. The analysis of serum samples obtained from calf mothers revealed lack of a suitable chemometric model for stratification. However, statistical analysis of individual metabolites showed that the control (mother healthy calves) is characterized by elevated levels of citrate and a reduced level of Unk_1. Very good classification was obtained for comparison groups of different infections of mothers: *N. caninum vs. N. caninum* antibody and also for comparison groups *L. hardjo vs. N.caninum*. These studies showed promising results for the future in diagnostic of intrauterine infection in calves poor viability and stillborn.