Session 28: Marine Biotechnology

Lectures

L28.1

Biotechnological potential of Baltic cyanobacteria

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Pioneering studies into the biotechnological potential of marine natural resources began in 1970s. It was soon discovered that tropical marine cyanobacteria constitute one of the most promising sources of anti-disease activities. Less attention was paid to organisms from temperate ecosystems. Our studies on the Baltic filamentous cyanobacteria resulted in the discovery of tens of new bioactive metabolites of potential biotechnological application. Majority of the identified compounds were classified to nonribosomal peptides: spumigins, aeruginosins, anabaenopeptins, cyanopeptolins and nostocyclopeptides. Of the ribosomal peptides, four new aeruginosamide variants were found. The compounds were isolated from bloom-forming cyanobacteria and from species rarely or never reported from the Baltic Sea. In our recent work we focused on structure-activity relationship of cyanopeptolins and nostocyclopeptides produced by *Nostoc edaphicum* CCNP1411 and the unknown class of peptides produced by *Pseudanabaena galeata* CCNP1313. Structures of the peptides were determined based on LC-MS/MS and NMR analyses. Biological activity of the compounds against serine proteases, proteasome 20S and proteome was assessed. Due to strong and selective activity, the peptides are considered to be promising candidates for drug discovery process.

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L28.2

Fish models for biomedical research.

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Zebrafish (*Danio rerio*) and medaka (*Oryzias latipes*) probably are the most famous small (3-5 cm) and easy to maintain in a laboratory condition fish with a known genome. Both of them have a short life cycle (2-3 months), transparent bodies (at least at early stages of development), and produce many eggs which are externally fertilized. For both species large collection of mutants and transgenic lines are available. This, in combination with well-established methods and protocols, allow easy manipulations (up to a point of sending both species to space and having their development time-lapsed). Zebrafish and medaka show high genotype/phenotype similarity to humans and this has been confirmed in numerous studies on e.g. melanoma, leukemia, tuberculosis, epilepsy, depression, deathless, blindness, addiction, ageing, neurodegenerative diseases such as Parkinson and Alzheimer, various storage or developmental diseases as well as arthritis, multiple osteochondroma or other skeletal disorders. Moreover, zebrafish is particularly suited for high throughput compound screens as shown by a few groups whose discoveries entered clinical trials. The two species mentioned so far are not the only one. Diverse fish already have been or are being established for modelling of human disease. For example, rainbow trout (*Oncorhynchus mykiss*) that for decades has been used as a toxin indicator serves now also as a model for carcinogen-induced cancer. Recently, turquoise killifish (*Nothobranchius furzeri*) and blind cavefish (*Astyanax mexicanus*) meet more and more attention. They natural properties make them particularly suited for studies on ageing / ageing-related diseases (short living *N. furzeri*), retinal degeneration / sleep disorders (*A. mexicanus* with its partial or total sight loss). For illustration, a highlight of some fish projects conducted at the IIMCB in Warsaw will be presented.

Oral presentations

O28.1

Characterization of biological activities of marine cyanobacteria-derived peptides

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Cyanobacterial metabolites represent a wide variety of unique structures and show strong activity in an array of biochemical and cell– based assays. Among them, the cyclic and linear oligopeptides constitute the largest group and comprise approximately 65% of all bioactive structures reported from cyanobacteria. Other compounds produced by cyanobacteria are explored as potential antiviral, antimalarial, antifeedant, herbicidal or immunosuppressive agents. In this work, we have characterized biological activities of several cyanopeptides produced by *Anabaena* CCNP1406, *Pseudanabaena galeata* CCNP1313, *Spirulina subsalsa* CCNP1310, *Lyngbya aestuarii* CCNP1324, and *Nostoc edaphicum* CCNP1411. Cytotoxicity against T47D human breast cancer, HeLa cervical cancer, and HDFa normal human fibroblasts cell lines, as well as viability and proliferation of cells treated with tested compounds have been assessed. Induction of apoptosis was also tested as a potential mechanism of activities of the cyanopeptides. Differential effects of various tested compounds on particular cell lines have been observed. The whole genome sequence of *Nostoc edaphicum* CCNP1411 has been determined in order to clone gene clusters containing genes coding for enzymes responsible for synthesis of certain cyanopeptides (non–ribosomal). Such a strategy may allow for production of large amounts of these compounds in recombinant *Escherichia coli* cells.

O28.2

Marine bacteria as potential producers of polyhydroxyalkanoates

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Polyhydroxyalkanoates (PHAs) are bacterial polyesters accumulated as carbon and energy reserve materials under nutrient limited conditions. Because they have properties similar to petrochemical plastics and could be biodegradable, they have gained much attention in recent years. However, the high cost of production has prevented their wide use. Therefore, bacterial strains that can synthesize PHAs efficiently and are able to grow in low-cost substrates are needed. Promising bacteria are isolated from many environments, however only recently the marine environment is considered as a source of bacteria having ability to synthesize high amounts of PHAs. The potential of marine bacteria remains largely unexplored. The use of marine bacteria has a number of advantages in terms of commercial applications therefore more and more often bacteria isolated from seawater environments are tested as the polyester's producers. In the presentation, the genetic and metabolic background of microbial PHAs synthesis will be evaluated. The advantages and disadvantages of using marine bacteria as PHAs producers will be revealed and discussed.

Posters

P28.1

Profiles of miRNAs and their isomiRs in the rainbow trout (*Oncorhynchus mykiss*) eggs exposed to X-rays for the androgenetic purpose

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X-irradiation of fish eggs is applied to damage chromosomal DNA during induction of androgenetic development. Irradiated and inseminated eggs develop as androgenetic haploids. Radiation-induced DNA damages initiate a DNA damage response that is regulated by i.a. microRNAs. Thus, analysis of miRNAs in the irradiated fish eggs may shed a new light on the process of cellular responses to the X-rays. In the present research, rainbow trout eggs were irradiated with 350 Gy, a dose applied for the androgenetic purpose. Total RNA from irradiated and non-irradiated eggs was extracted and purified. 500 ng of RNA was used to construct microRNA libraries that were subjected to next generation sequencing. MicroRNA length and sequence variants (isomiRs) were identified. On the genome-wide level, irradiation of rainbow trout eggs did not result in significant alteration of the microRNAs expression. On the pointwise level, irradiated eggs exhibited differential expression of 9 known and 14 potentially new miRNAs. The most statistically significant changes concerned down-regulation of ssa-miR-206-3p, ssa-miR-148a-3p, ssa-let-7b-5p, ssa-miR-199a-3p, ssa-miR-146d-5p and upregulation of ssa-miR-146a-5p, ssa-miR-202-3p, ssa-let-7b-5p, ssa-miR-22a-3p. Multiple variations in the miRNA sequences known as *isomiRs* were observed in the irradiated eggs. For the majority of differentially expressed miRNAs, we observed over- or underexpression while for ssa-let-7b-5p we observed both, up and downregulation depending on its isomiR variants. Comparison of miRNA expression profiles in the irradiated human cells and rainbow trout eggs exhibited a similar pattern of response to the radiation.

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P28.2

Microbial fuel cells as a new method of electrochemical evaluation of biochemistry of unicellular aquatic organisms and bacteria – preliminary studies of the new concept

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Microbial fuel cells (MFC) are bioelectrical systems that are classically used to obtain electricity, but under certain conditions, they can be applied to the more sophisticated applications. It is possible thanks to a close relationship between the obtained current (or potential) and biological processes occurring in the organisms within the system. Using such tools as, for example, cyclic voltammetry (CV) we can in some cases distinguish the signals associated with independent oxidation or reduction processes that occur in the biological component of the system. This would allow, from a biochemistry point of view, to perform many measurements especially made in situ.

In this case, the use of MFCs is unfortunately limited mostly to single-cell organisms that are capable of producing biofilms. Only then, the system works correctly and the signal represents an average of the interactions of the entire population with the electrode. An additional practical limitation is the fact that only a small number of organisms within this group are able to grow on the surfaces of metal electrodes or interact with them ensuring electron transfer. In the presented work, we are proposing a novel solution to this problem, a third component, besides the electrode and microorganism, i.e., a polymer nanolayer that promotes adhesion, but at the same time does not isolate the surface electrically. The use of such tactic made it possible to obtain bioelectrode using organisms that have not been previously used in such systems. The system described in this work consists of gold (gold, despite good electrical parameters, is rarely used in the MFC due to its antibacterial properties against most bacteria) electrodes covered with a layer by layer nanolayer of cationically modified dextran. Bacteria *Lactobacillus rhamnosus GG* (a noninfectious and widespread bacterium) and *Rhodospirillum rubrum* (photosynthesizing bacterium with complex biochemical activity) were used as biological models.

Using the bioelectrical device proposed here, by performing CV measurements we observed organisms' response to such basic biochemical parameters as the change in the concentration of glucose (source of energy). The presented preliminary studies allow assuming that such a system can be used to study the biochemistry of aquatic/marine organisms in the future with the use of electrochemical methods, which will allow for an in situ signal registration.

P28.3

Characterisation of a novel bioflocculant produced by *Rhodococcus opacus* strain

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Actinobacteria commonly occur in seas and oceans, the soil, the roots of plants, anthills, glaciers and are known as a source of compounds with valuable biological properties, such as antifungal, antibacterial, antiviral and even antitumor activities. The high biotechnological potential has the genus *Rhodococcus*, the representative of Actinobacteria. This strain is able to produce bioflocculants that can interact with different ions and particles in flocculation process, which is considered as a dynamic and efficient method to remove suspended solids, colloids or cell debris of organisms from water solutions.In the present study, the bioflocculant from *Rhodococcus opacus* FCL1069 was characterised. The basic colorimetric reactions were performed to establish the main bioflocculant components such as protein, sugars, uronic acids and amino sugars. The acidic hydrolysis was carried out with the addition of 8 M trifluoroacetic acid. The hydrolysed samples of bioflocculant were dried and dissolved in Milli-Q water. Thin-layer chromatography analysis was performed using propanol – acetate – distilled water (4/0.5/0.5, v/v) as the mobile phase. The monosaccharides were determined based on Rf value of bioflocculant samples and standards (e.g. glucose, lactose, fructose). Additionally, the morphological structure of extracted product was observed by Scanning Electron Microscopy.

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