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Isolation, identification and characterization of biomedical properties of biosurfactants

Nowadays, surfactants are produced on a large-scale by chemical synthesis. These compounds are often toxic and unbiodegradable and therefore can be dangerous for environment. Intensive development of biotechnology, genetic engineering and increase of human responsibility for environment protection contribute to promising application of natural surfactants.

Biosurfactants are compounds produced by bacteria and yeasts, with different structural composition. Due to its properties, these compounds have an extensive applications in recovery of oil, bioremediation of soil, production of lubricants, paints, coatings and pharmaceutical, as well as cosmetics, paper and textile industries.

The aim of this study was to determined, whether arctic bacteria are able to synthesize biosurfactants and how the culture conditions influence on the production of these compounds. Another objective was to purified biosurfactants, determined the chemical structure and investigated the properties of the isolated compounds. In the studies three Arctic strains of *Pseudomonas fluorescens* BD5, *Pseudomonas putida* BD2 and *Rhodococcus fascians* BD8 were used.

It was shown that tested Arctic strains have various capacity to produce tensides, which depends on the culture conditions. The yield of biosurfactants synthesed by Arctic bacteria was mostly depended on the availability of carbon sources in the medium, temperature and the degree of aeration of the medium.

Surfactants produced by *P. fluorescens* BD5, *P. putida* BD2 and *R. fascians* BD8 were successfully purified using preparative chromatographic techniques such as RP-HPLC and PLC. Using MALDI-TOF/MS, GC-MS, ESI-MS and NMR spectroscopic techniques chemical structure of the analyzed biosurfactants was determined.

It was found that biosurfactants produced by *P. fluorescens* BD5 are two new cyclic lipopeptides, which were named pseudofactin I and II. Biosurfactants produced

by *P. putida* BD2 were diramnolipid and phosphatidylethanolamines, while biosurfactant synthesized by *R. fascians* BD8 was trehalose lipid.

Different chemical structure of biosurfactants was responsible for their different surface activity properties. In terms of these studies, reduction in surface tension value of 71 mN/m (water) to 31 mN/m was observed for rhamnolipid Rha_2-C_{10}-C_{10}. Similar properties were shown for pseudofactin II and trehalose lipid, which reduced surface tension to 31.5 mN/m and 34 mN/m respectively.

An important factor in surface activity of biosurfactants is their ability to disperse hydrophobic compounds and stability of the formed emulsions. It was found that the ability to disperse hydrophobic substances and the stability of the emulsion was depended on the type of compounds. In the case of non-polar aromatic compounds and aliphatic, biosurfactants solutions were better than synthetic surfactants Tween-80 and Tritonu-100.

One of the most advantageous property of biosurfactants synthesized by *P. fluorescens* BD5, *P. putid*a BD2 and *R. fascians* BD8 was their ability to inhibit adhesion (the first stage of biofilm formation) of bacterial: *Escherichia coli*, *Enterococcus faecalis*, *Enterococcus hirae*, *Staphylococcus epidermidis*, *Proteus mirabilis*, and *Candida albicans* to the plate surface. Moreover, this property of biosurfactants was not related to the mortality of pathogenic bacteria or yeasts. With the use of confocal microscopy, it was shown that pseudofactin II and trehalose lipid inhibit biofilm of bacteria and yeast formation on the surface of polystyrene, glass and silicone.

It was shown by *in vitro* studies that melanoma A375 cells are sensitive to the cytotoxic effects of pseudofactin II and trehalose lipid. In contrast to cancer cells, normal human dermal fibroblast cells (NHDF) showed lower sensitivity to the antiproliferative effects of the tested compounds. Induction of apoptosis by biosurfactants in A375 cells was examined on the basis of appearance of internucleosomal DNA fragmentation, as well as changes in the structure of the plasma membrane (translocation of phosphatidylserine to the outer layer of membrane).

Characteristic morphological changes observed after stimulation of the cells by increasing concentrations of pseudofactin II and trehalose lipid were related to the changes in the shape of cells and their nuclei, as well as to the fragmentation of its nuclei. Another known characteristic of apoptosis is the loss of asymmetry of the cell membrane. The results obtained by staining cells with annexinV-fluorescein

isothiocyanate (FITC) indicated that pseudofactin II and trehalose lipid caused the loss of membrane asymmetry in melanoma A375 cells.

Immunocytochemical studies performed using monoclonal antibody showed that expression of the protein caspase-3, involved in the mitochondrial pathway of melanoma A375 cells apoptosis induced by pseudofactin II and trehalose lipid. The ability of pseudofactin II and trehalose lipid to induce apoptosis in tumor cells allows to consider them as a potent and promising antitumor agents.

Results presented in this studies showed, that structurally diverse biosurfactants synthesized by Arctic bacteria exhibit properties typical for other chemical surfactants and can be useful in medicine and industry.