Description for the general public

Biosurfactants (or surface active compounds of biological origin) are a group of chemical compounds that have attracted a lot of attention among consumers and manufacturers. Biosurfactants have very similar properties to synthetic surface active compound (such as detergents), that we all know from everyday life: washing liquids or washing powders. At the same time, biosurfactants are biodegradable and non-toxic for human cells. What is even more surprising, biosurfactants can act as antibiotics, antifungals, and anticancer drugs or can be used even for the protection of medical devices against infection with human microbial pathogens (antiadhesive activity). Biosurfactants are therefore considered to become a "green" substitutes of synthetic detergents in the near future.

Biosurfactants are widely spread in the environment and are produced by different strains of *Pseudomonas*, *Bacillus* and *Candida*. Biosurfactants are also a very structurally diverse group of chemical compounds, for instance lipopeptides, glycolipids or phospholipids can be mentioned. There are a few theories trying to explain why microorganisms produce biosurfactants. Previous reports suggest that biosurfactants can help microbes with degradation of hydrocarbons (oil spills for instance). Other interesting theory propose a role of biosurfactants in attachment/detachment of cells from a biofilm structure, having therefore a role in the colonization of environments by microorganisms. Existing hypotheses do not cover all aspects of biosurfactant activities and are often contradictory. Hence, coming up with a cohesive theory explaining why microorganisms secrete biosurfactants can prove to be of great scientific merit.

Pseudofactin, a lipopeptide biosurfactant, is one of molecules discovered in the Department of Biotransformation at the University of Wrocław. Pseudofactin shows antibiotic, antifungal, anticancer and antiadhesive activity. Yet, importance of pseudofactin in the lifecycle of a pseudofactin-producing strain *P. fluorescens* BD5 is still unknown.

The aim of our project is to understand the significance of pseudofactin for its producer *P. fluorescens* BD5. We will be able to determine this by identification of genes in the genome *P. fluorescens* BD5, that are involved in biosynthesis of pseudofactin. Next, we will generate mutants deficient in pseudofactin production and analyze their phenotypes. **Results will allow us to explain the reason (or reasons) of pseudofactin production by the strain BD5.**

The proposed project will start with *in silico* analysis of the genomic sequence of the strain *P. fluorescens* BD5, that we have previously obtained. We will look for the operons (gene clusters) encoding NRPS (nonribosomal peptide synthetases) proteins. NRPS are large protein complexes involved in the production of lipopeptides, including pseudofactin. Our first goal will be the identification and characterization of NRPS operon potentially involved in the synthesis of pseudofactin. Next, we will use molecular biology techniques to silent pseudofactin NRPS operon and therefore generate mutants that do not produce pseudofactin. Comparative analysis of phenotypes (characteristics) of mentioned mutants with wild type (parental) strain will help us determine the natural role of pseudofactin. We will examine all aspects of microbial life that have been previously shown as potentially biosurfactant-dependent, e.g. growth rate, degradation of hydrocarbons, colony migration, biofilm formation and interactions with other microorganisms (e.g. *Candida albicans*) and other.

Our results will allow us to identify the NRPS operon responsible for pseudofactin production by the strain *P. fluorescens* BD5 and to determine the natural role (or roles) of pseudofactin. Moreover, we will compare our results with previous reports, which can shed new light on the theories concerning the role played by lipopeptides in the environment and improve our understanding of the mechanisms governing microbial populations. Finally, identification of the pseudofactin NRPS operon can lay the foundations for future research on pseudofactin. The obtained results will allow us to reduce cost of pseudofactin production and to redesign pseudofactin molecule to produce a molecule with even better physiochemical and biological properties. This may lead to a new class of 'universal' compounds that will act simultaneously as antibacterial and anticancer drugs and cleaning agents in the industry of medicine.