Biotechnological and pharmacological potential of bee products microbiota.

For centuries honey bee products have been important component of the human diet. In addition, honey and propolis belong to the most common and most effective drugs of folk medicine. The issue is that very little is known about the microbiota of these products. Several recent studies have confirmed that bee products are rich sources of diverse bacteria that produce a broad spectrum of metabolites, e.g. enzymes, peptides, biopolymers (extracellular polysaccharides/proteins), and variety of secondary metabolites with high potential for clinical and agricultural application. We now aim to investigate the pharmacological and industrial potential of bacteria (and their metabolites) isolated from honey, pollen, and bee bread produced in Polish apiaries. The pharmacological potential include ability to production of metabolites (bacteriocins, peptides, secondary metabolites) of antibacterial and antifungal activity and probiotic properties of isolates – lactic acid bacteria (LAB) would be especially desirable. Ability to production of enzymes of particular industrial importance (cellulases, amylases, lipases, esterases, and proteinases) as well as secretion of biopolymers will be the main criteria for assessment of biotechnological potential of the honey isolates. A variety of screening assays will be performed in the first step of the proposed research for the identification of bacterial strains producing these compounds and exhibiting probiotic properties. Subsequently, growth conditions of the producing strain will be optimized for maximal efficiency of production of metabolites of interest. Antimicrobial agents will be purified using HPLC and elucidation of their chemical structures will be performed with mass spectrometry techniques. Bactericidal/bacteriostatic as well as fungicidal/fungistatic efficacy of the produced agents will be verified against a broad spectrum of pathogenic bacteria and fungi. Purification of the enzymes also will be performed with chromatographic methods and genes coding for the most promising proteins will be cloned and produce in *Escherichia coli* and *Pichia pastoris*. Whole genome sequencing will be performed for identification of genes/clusters of genes responsible for production of metabolites exhibiting the highest antimicrobial activity and for identification of genes coding for the enzymes that exhibit the highest application potential. Elucidation of the preliminary structures of the produced biopolymers, and investigation of their physicochemical properties will be performed with advanced analytical techniques, including: Fourier transform infrared (FT-IR) spectroscopy and differential scanning calorimetry (DSC) and scanning electron microscopy (SEM) will be used to investigate their surface morphology. The project will be implemented in cooperation between researchers from two Polish Universities, namely Gdańsk University of Technology (GUT) and University of Wrocław (UWr) and research group of Professor Randy Worobo from the Department of Food Science, Cornell University (USA). The researchers from GUT have large, confirmed with publications experience in: cloning, purification, and characterization of enzymes of particular industrial importance – Dr. hab. inż. Marta Wanarska from the Department of Molecular Biotechnology and Microbiology; production, purification, and characterization of microbial biopolymers – Dr. hab. inż. Hanna Staroszczyk from the Department of Chemistry, Technology and Biotechnology of Food and investigation of antimicrobial agents – Dr. hab. inż. Piotr Szweda (PI of the project). Prof. Dr. hab. Zbigniew Szewczuk and Prof. Dr. hab. Piotr Stefanowicz from Wroclaw University are specialists in the area of using mass spectrometry techniques for determination of chemical structures of peptides. Professor Randy Worobo is a world class specialist in the area of food microbiology. Production and investigation of bacteriocins is one of his leading research subjects. The expected result of the realization of the project is selection of bacterial strains producing compounds of high antimicrobial activity – candidates for new antimicrobial drugs as well as enzymes and biopolymers with high potential for pharmaceutical and industrial applications.