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DESCRIPTION FOR THE GENERAL PUBLIC

Bacteria of the genus *Staphylococcus* belong to the most important pathogens for both humans and animals. A crucial characteristic for this group of bacteria, is that they can easily acquire mechanisms of antibiotic resistance for a plethora of antibiotics currently in use for human and animal therapies. Therefore, there is a great need to find novel, non-antibiotic chemotherapeutics with marked antistaphylococcal activity. In our opinion, promising but still underestimated group of potential antistaphylococcal chemotherapeutics constitute bee products: honey, pollen, royal jelly, feremented pollen, and especially propolis. The antimicrobial activity of the fermented pollen is the consequence of presence in this product lactic acid bacteria (LAB) and the products of their metabolism – lactic acid as well as bacteriocins (proteinaceous ribosomally synthesized antimicrobial compounds). This project aims to: 1) investigate of antimicrobial activity of large collection of bee products obtained from polish apiaries; 2) screening for novel antimicrobial compounds from bee products, including honey and fermented pollen; towards isolation of bacteriocinogenic bacterial strains with specific antistaphylococcal activity; 3) develop a simple purification procedure for the most active peptides; 4) analysis of potential synergistic effect of bee products, new recognized bacteriocins and antibiotics; 5) confirmation of activity of the bee products and bacteriocins towards staphylococci growing in the form of biofilms; 6) establish a sequence of genes encoding the most promising bacteriocins; 7) chemical characteristic of selected bacteriocins including trials of determination of 3-D structure.

Determination of antibacterial activity of the products as well as new isolated bacteriocins will be conducted using a serial dilution method in microtiter plates under the conditions recommended by the National Committee for Clinical Laboratory Standards (NCCLS). Analysis of chemical composition of bee products and identification of constituents crucial for their activity will be conducted using gas and liquid chromatography, in both cases mass spectrometry detectors will be used. The research of an abroad partner, Prof Worobo and his coworkers revealed that honey and other bee products should be considered as a potential source of microorganisms producing promising antimicrobial compounds, especially bacteriocins. Identification and isolation of bacteriocinogenic microorganisms from Polish bee products will be carried out according classical method with using Top - agar. In this method the TOP - agar mixture inoculated with indicator strains will be poured onto the prepared bottom – TSA agar plates containing the replica of colonies of microorganisms isolated from honeys. After the overlay has solidified, the plates will be incubated at 37 ºC for additional 24 hours and growth inhibition zones of indicator strains will be observed. Species identification of strains producing interesting bacteriocins will be carried out by sequencing of the 16S rRNA gene. Preliminary attempts to obtain efficient level of production of bacteriocins in liquid media will be conducted at the laboratory scale (in flasks of volume of 250-1000 mL). In the case of the most promising peptides, optimization of their production will be performed in bioreactors using a volume of 10 L. Purification of selected peptides will be carried out with classical approach with using two basic techniques: ammonium sulfate precipitation and HPLC. Efficiency of bacteriocins production and effectiveness of its purification will be checked through polyacrylamide gel electrophoresis. The N-terminal sequencing of purified bacteriocins will be performed by Edman degradation method and electrospray ionization (ESI) – MS will be performed to determine the accurate molecular mass of the peptides. Other advanced spectrophotometric methods such as circular dichroism and NMR will be used in attempts to determine the chemical structure of the peptides. Synergistic activity of bee products, new identified bacteriocins and antibiotics will be checked with checkerboard method and bactericidal activity towards staphylococci growing in the form of biofilm will be determined calorimetrically with the use of tetrazolium salt XTT.

Results obtained from this project should allow verification that it is possible to use the aforementioned bee products or bacteriocins as an effective alternative treatment for anti staphylococcal antibiotics. If one or more compounds (bee product or bacteriocin) exhibit promising properties, then additional research could be conducted to determine application of those agents as treatments for human and animal *Staphylococcus*-based infections. From the scientific point of view, especially interesting, will be the advanced investigation of physicochemical properties of the bacteriocins, including trials of 3D structure determination.