POPULAR SCIENCE SUMMARY

BacSp222 is a recently discovered in our group peptide bacteriocin produced by *Staphylococcus pseudintermedius* strain 222 - a commensal bacterium colonizing the skin and mucosal surfaces of household animals. The BacSp222 bacteriocin kills a wide range of Gram-positive bacteria, enabling the manufacturer cells to eliminate competing bacterial strains. Our previous research on BacSp222 has focused mainly on the molecular biology of peptide, its physicochemical mechanism of action against bacterial membranes, as well as on the determination of peptide structure by nuclear magnetic resonance technique (NMR). Nevertheless, our most intriguing results proved that the BacSp222 bacteriocin is not only capable of killing bacteria, but also shows significant activity towards the cells of the infected host organism. At higher doses the peptide acts cytotoxically, while at very small concentrations (nanomole/l) it acts as an immunomodulating pro-inflammatory factor, inducing in macrophage cell lines the activity of inducible nitric oxide (NO) synthase (iNOS), and stimulating tumour necrosis factor (TNF) secretion. The second intriguing discovery showed that the BacSp222 peptide is produced and secreted together with several isoforms identified as syccinylated (butanedioic) derivatives. In relation to these preliminary results, the presented project is devoted to extensive research on the mechanism of posttranslational modifications of bacteriocin BacSp222, on the influence of these modifications on the structure of the peptide molecule as well as on the pro-inflammatory activity of BacSp222 isoforms towards eukaryotic cells.

The research will be conducted using isoforms of BacSp222 bacteriocin isolated chromatographically from the post-culture medium. The native unmodified form of bacteriocin will be used as a control peptide. The mechanism of posttranslational modification of bacteriocin and the influence of environmental factors on this phenomenon will be studied by chromatographic techniques. The effect of succinylation on the structure of the BacSp222 molecule will be determined by NMR technique. Research on bactericidal and cytotoxic activity of isoforms will be carried out on different strains of bacteria and using different model animal and human cell lines as well as primary cells. Analysis of cytokines or growth factors produced by cells after exposure to bacteriocin isoforms will be performed using a flow cytometry and multiple cytokine immunoassays. Research on the effects of isoforms on iNOS expression will be carried out by direct measurement of the enzyme activity in cells and on the basis of RT-PCR measurements of the enzyme gene expression. The identification of cell receptors involved in bacteriocin isoforms recognition will be carried out using co-stimulation of cells by isoforms and receptor-specific antagonistic compounds, by measuring the secretion of specific cytokines, by measuring intracellular cAMP mobilisation and/or by using engineered cell lines expressing specific recombinant cell receptors. Determination of the effect of bacteriocin isoforms on particular transcription factors mobilization (e.g. AP-1, NFκB) will be performed using the electrophoretic EMSA technique. The effects of bacteriocin isoforms on cell morphology and verification of their internalisation into cells will be studied by confocal microscopy, fluorescently labeled peptide and/or immunochemical techniques.

From a biological point of view, bacteriocins are defined as peptides or proteins produced by bacteria on ribosomes, able to kill closely related strains living in the same physiological niche or ecological habitat. These molecules serve also as regulators of bacteria metabolism dependent on their population density or presence of other microorganisms. It is estimated that 99% of bacterial strains produce at least one bacteriocin so the biological and ecological importance of these factors is very substantial. Moreover, bacteriocins or bacteriocin-producing strains are widely used in food and forage industry, veterinary and medicine. Realization of the studies described in this proposal will shed new light on the mechanisms of posttranslational regulation of bacteriocins activity and will allow to determine whether these molecules can also play the role of virulence factors, able to modulate the activity of immune cells of the host.