„Characteristics of novel antibacterial peptides hidden in primary sequences of lytic proteins”

Monika Szadkowska, MSc

Among pathogenic bacteria, resistance to antibiotics is rapidly growing, becoming a global public health problem that threatens the effectiveness of therapy of many infectious diseases. In this respect, antimicrobial peptides seem to be an interesting alternative to combat bacterial pathogens. In this work, I presented a detailed characterization of the antibacterial peptide Intestinalin (P30), whose sequence corresponds to the N-terminal part of the LysC enzyme (aa 2-31) from the *Clostridium intestinale* URNW strain. This peptide exhibits bactericidal activity against clinical strains of Gram-positive and Gram-negative bacteria. Fluorescence microscopy and computer modelling revealed that the peptide oligomers form transmembrane channels that directly engage the negatively charged phospholipid heads of the bacterial cell membrane. This disrupts the electrochemical gradient of the cell membrane, which can negatively affect many vital processes, such as ATP synthesis, bacterial motility and nutrient transport, and these abnormalities may consequently lead to the loss of bacterial viability.

In order to search for proteins similar to the lytic protein LysC, I conducted *in silico* analyses that enabled the selection of three potential lytic proteins: PhiKo, GasC and CT4, derived from the bacteriophage phiKo of *Thermus thermophilus* HB27, *Clostridium gasigenes* and *Clostridium manihotivorum* CT4, respectively. Then, I performed bioinformatics analyses using the CAMP program to search for regions that may correspond to antibacterial peptides "hidden" in the lysins sequences. In order to test the antibacterial potential of selected proteins and peptides (named RAP-29, VVR-20 and IFR-20), I conducted antibacterial tests, e.g., against *A. baumannii* CRAB KPD 205 and *S. aureus* ATCC 25923.

Both the Intestinalin (P30) peptide, and the RAP-29 peptide hidden in the amino acid sequence of the PhiKo protein show high bactericidal activity. The conducted research may therefore be helpful in the search for new antimicrobial peptides, which in the future have a chance to replace the existing antibiotics.