

The present study focused on the analysis of the structure and function of enzymes produced by bacterial viruses (phage) active against pathogens from the ESKAPE group. ESKAPE is an abbreviation of bacterial names (*Enterococcus*, *Staphylococcus*, *Klebsiella*, *Acinetobacter*, *Pseudomonas*, *Enterobacter*), of which the multidrug resistant clinical strains are isolated.

The aim of this study is to prepare the recombinant proteins of novel phage depolymerases, degrading bacterial exopolysaccharides composing capsule or/and lipopolysaccharides (LPS). We are going to recognize its characteristic features and structure and to establish effects of their action. The first task to achieve this goal is to adjust conditions in every step of recombinant proteins production process using genetic engineering methods. The hydrolytic activity and specificity of obtained proteins will be determined on isolated saccharide polymers such as exopolysaccharide (EPS) and LPS using biochemical and biophysical methods.

We are going to study their structure on the basis of the crystal protein and determine the activity depending on different environment conditions in which they are placed. With structural analysis we will be able to identify the active sites of derived enzymes, which allow for the design of modified proteins with altered substrate specificity. Such recombinant enzymes will be tested for antibacterial and anti-virulent activities as potential therapeutic agents.

This project can build new, fundamental knowledge in order to improve our understanding how phage enzymes act, which hydrolyzing activity they exhibit, how broad is their activity spectrum against bacterial saccharides with different origin. The results will help to answer the question of whether exopolysaccharidases of viral origin can effectively degrade and remove bacterial biofilm structure, reduce the virulence of the harmful human pathogens, and thus provide an alternative or supporting agents for the standard antibiotic therapies.

The literature confirms the efficacy of depolymerases in combating infections in animal models, as well as permit the penetration of antibiotics into the biofilm due to the action of these enzymes. Although the proposed project is strictly cognitive, the answers to the questions may help to dispel at least some doubt about the hypothetical use of depolymerases in eradication of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* strains, among which both isolates of hospital and environmental origin are equally dangerous. Research on phage depolymerases specific for these two representatives of ESKAPE group, are not yet widespread, which is why we decided to explore the subject.