MOLECULAR ANALYSIS OF THERMOSTABILITY OF ENDOLYSINS FROM EXTREMOPHILIC BACTERIOPHAGES ON A WAY TO COMBAT GRAM-NEGATIVE BACTERIA.

Bacterial resistance to antimicrobials is a natural process that has been observed since the first antibiotic, penicillin was discovered by Sir Alexander Fleming in 1928. The overuse of antimicrobials has increased the rate of resistance development and new actions are urgently needed to develop alternatives to conventional antibiotics. The rise of multidrug-resistant bacteria is particularly alarming in case of Gram-negative bacteria, including *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, which are etiological agents of a broad spectrum of nosocomial infections. Bacteriophage-derived enzymes, called endolysins, that rapidly degrade peptidoglycan (PG) layer of bacterial cell wall to release the phage progeny at the end of the lytic cycle, represent one possible solution.

Addition of recombinant endolysin to Gram-positive bacteria induces osmotic lysis and a consequent cell death. In case of Gram-negative bacteria the exogenous application of endolysins is hindered by the outer membrane (OM) that shields the peptidoglycan layer. Projects concerning this group of enzymes were often described as risky and defined as so-called no go zone. However, the successful usage of phage lysins against Gram-positive bacteria and the pressing need for novel bactericidal agents targeting Gram-negative pathogens pushed the scientists to find a solution to overcome the OM barrier. Nowadays, many different techniques are used to enhance the antibacterial efficacy of engineered endolysins against Gram-negative bacteria. Within the project, one of such technique, a VersaTile-driven platform (novel DNA assembly method) will be used to design engineered lysins against Gram-negative pathogens.

Although, the solubility and temperature stability are also of great importance in the study of endolysins, the projects investigating this phenomenon in the context of lytic enzymes are almost not existing. We have extensive experience in working with endolysins from extremophilic environments discovering two (Ph2119 and Ts2631 endolysins of *Thermus scotoductus* bacteriophages), out of the few known thermophilic lytic enzymes and thermostable proteins are superior in medical and technical processes due to their resistance to denaturation and proteolysis.

Therefore, the main objective of this proposal is to determine the molecular basis of the thermostability of globular endolysins from bacteriophages infecting bacteria of genus *Thermus*. State-of-the-art nano Differential Scanning Fluorimetry (nanoDSF) will be used to study thermal stability of globular endolysins and their variants.

In the aspect of development of novel technologies allowing effective permeabilization of bacterial outer membrane we aim to select the thermostable endolysin variant with enhanced activity against mesophilic Gram-negative pathogens.

We expect that our results will provide insight into, and lay groundwork for further studies of thermostable lytic enzymes directed towards Gram-negative bacterial pathogens.