Bacterial toxin – antitoxin systems as novel target for the development of potential antibiotics

The emergence of bacterial strains of antibiotic resistance is a significant health problem. The World Health Organization has put infections caused by antibiotic-resistant bacteria at the top of the list of diseases which fighting and prevention should be given the highest priority. It seems that the scientific community ends up with effective antibiotics, while the pharmaceutical industry reduces the number of new antibiotics in research and development lines. This highlights the need for innovative antimicrobial solutions. However, developing de novo substances with antibacterial properties is an extremely complex and costly task. Therefore, a new look is required in the fight against bacterial infections. In this project, we want to use an "internal bomb," which is found in most bacteria - a toxin protein encoded in Toxin-Antitoxin (TA) systems.

TA systems are composed of two genes: a toxin whose product has a toxic effect on the cell and antitoxin, which interacts with the poison to inhibit its toxic effect. In this systems, the toxin is a stable protein while the unbound antitoxin is rapidly degraded by cellular proteases. Any disturbance in the ratio of both TA cell proteins in the cell leads to accumulation of the stable and active toxin in the cell, and consequently cell death. Prokaryotic TA systems are divided into six groups due to the type of antitoxin and mechanism of inhibition of toxins activity.

In this project, we want to investigate type II of the TA system as a potential target for the development of new antimicrobial agents. For this purpose we will use short, modified oligonucleotides that interfere with the expression of a bacterial gene at the translation level by complementary binding to bacterial mRNA, blocking the production of bacterial proteins, and inhibition of bacterial growth. Oligonucleotides will be designed to lead to artificial activation of the TA system by inhibiting the production of the corresponding proteins associated with TA systems.

The biggest advantage of the proposed strategy is that it can act as a selective inhibitor and precisely inhibit the growth of one type of bacteria. On the other hand, oligonucleotides can be designed to broaden the activity spectrum and inhibit the growth of different types of bacteria simultaneously. Also, the proposed strategy is universal and can be adapted to work with all bacteria with TAs. Besides, if mRNA mutations occur, the oligonucleotide sequence can be rapidly redesigned to overcome resistance.

All experiments will be conducted on pathogenic and multi-resistant strains of Escherichia coli. The project is interdisciplinary and combines bioinformatics and experimental research. The basic theoretical tasks of the project are 1) comparative analysis of selected mRNA sequences of the selected bacteria, 2) selection of oligonucleotide targeting sites and 3) designing of target oligomers sequences in the selected genes fragment. Particularly interesting cases are selected for experimental analysis and are characterized for new targets for bacterial growth inhibitors. Experimental investigations include, but are not limited to, the analysis of the antimicrobial properties of the designed oligonucleotides: determination of the minimum inhibitory concentration (MIC), selective inhibition of growth of one species of bacteria, and determination of synergistic action with conventional antibiotics (FIC). In addition, we will determine the effectiveness of the designed sequences to suppress the expression of selected genes (qRT-PCR) and also investigate the effect of antisense oligonucleotides on the formation of persister cells. In experimental work, we also use molecular cloning techniques and mutant constructions.

We propose an innovative solution that has not been investigated so far. The accomplishment of the project's objectives will provide basic knowledge about the use of bacterial toxins systems - antitoxin as new targets for bacterial growth inhibitors. The proposed strategy may be important in the future when designing new classes of antibiotics.