Bacteria, in response to adverse environmental conditions such as nutrient deficiency or stress caused by the presence of antibiotics, develop series of defense mechanisms that enable them to survive. This has a consequences in increasing number of bacterial infections in humans and animals and the and often manifests in the occurrence of recurrent and chronic infections. Due to the therapeutic difficulties associated with an increase of antibiotic-resistance of bacteria and relatively recently discovered its ability to form in the fraction of population the cells particularly resistant to occurring conditions (referred to as *persister cells*) it is important to study the molecular mechanisms responsible for these phenomena. Recent studies show, that entities called toxin-antitoxin systems (TA systems) can play an important role in the production of *persister cells* and virulence of bacteria. Within the genomes of these microorganisms TA units are widespread and form operons consisting of the toxin gene, encoding a stable protein being toxic to the bacterial cells and antitoxin gene encoding an unstable inhibitor of toxin, having different biochemical nature (RNA or protein). Such systems are identified in the pool of plasmid and chromosomal DNA. The former have been implicated the role of the maintenance of plasmids in bacterial populations. There is also an evidence pointing at their involvement in regulation of the expression of genes responsible for the virulence of bacteria. The role of chromosomally encoded TA systems is currently the subject of wide discussion. On the one hand one indicate their potential contribution to virulence (by influencing the formation of *persister cells* or bacterial biofilm formation). On the other hand, they are considered as selfish genetic elements which after a change of their location from plasmid to chromosome can be degraded and become non-functional pseudogenes.

The object of the research presented in the project is *pemIK*$_{Sp}$, the toxin-antitoxin system, identified in the chromosomal DNA of *Staphylococcus pseudintermedius* strains. During bioinformatics analyzes, it was demonstrated high heterogeneity of the system manifested by the presence of two variants of the toxin and five variants of antitoxin. Importantly, it also shows the homologous plasmid-located TA system from *S. aureus*, which has a proven effect on the virulence of this bacteria. During preliminary studies it has shown that PemK$_{Sp}$ toxin possess the ability to degrade ribonucleic acid (RNA), which builds gene transcripts. In contrast, PemI$_{Sp}$ antitoxin is an inhibitor of toxin. Therefore, the main objective of the project is to determine the biological function of the *pemIK*$_{Sp}$ system in *S. pseudintermedius*, in the context of the change of the location of genes of this system from plasmid to chromosome. Secondly, the impact of structural changes in the different versions of the system on its activity will be assessed. The planned study will be divided into a few stages. The first will involve determination the sequence, recognized by both variants of the PemK$_{Sp}$ toxin, within which gene transcripts are degraded. This allows for identification of the potential role of TA system in the regulation of gene expression. In the second stage it is planned to investigate the properties of the PemI$_{Sp}$ antitoxins variants, which will answer the question whether a TA system is functional. Then, there will be performed the *in vitro* and *in vivo* tests of interactions of PemIK$_{Sp}$ system components, as well as their cross-interaction with proteins belonging to the homologous system from *S. aureus*. This will enable to obtain information about the evolution of these systems in bacteria. In the last stage we will attempt to crystallize the toxin PemK$_{Sa}$ to determine its crystal structure and compare it to the similar tests performed for PemK$_{Sp}$ toxins.

The subject of this project is a part of the current debate conducted on the role and evolution of chromosomally-located toxin-antitoxin systems. The realization of established research tasks will determine the functionality and action of *pemIK*$_{Sp}$ system in *Staphylococcus pseudintermedius*. In addition, we determine whether a possible different functionality is associated with the observed rearrangement of the system components. This will refer to reports in the literature about the possible degradation of toxin-antitoxin systems after changing the location from plasmid to chromosome, or on the contrary indicate this rearrangement to fulfilled a sophisticated regulatory role.