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The studies conducted in various organisms have revealed the existence of complex regulatory networks, cascade relationships, intertwined regulons on the level of gene expression control involving transcriptional regulators. Many transcriptional regulators remain classified as hypothetical regulators of unclear, unknown cellular function that has not been confirmed experimentally. Clarification of their involvement in the regulation of gene expression and organism adaptation processes may significantly contribute to our understanding of the complexity of living organisms. Particularly important is the accurate knowledge of the biology of pathogens and their adaptability.

The research object in the proposed project is bacterium *Pseudomonas aeruginosa*, an opportunistic human pathogen causing nosocomial infections in immunocompromised patients. This bacterium has the ability to survive in very adverse, variable environmental conditions. The high survival rate is related to a huge repertoire of the genes engaged in survival and adaptation to a specific niche, and an extensive regulatory network enabling rapid response to stimuli coming from the environment. Striking is the large number of transcriptional regulators (about 500) and two-component regulatory systems (more than 120) encoded by the *P. aeruginosa* PAO1 genome. Although intensive studies are conducted by many teams, over 30% of the *P. aeruginosa* genome remains annotated as hypothetical, unknown genes.

ParA and ParB proteins are encoded by the majority of bacterial genomes and take part in the proper distribution of chromosomes to daughter cells. Our studies of *P. aeruginosa* confirmed the participation of the ParA/ParB proteins in condensation of the newly replicated chromosomes, proper movement of *ori* domains of chromosomes to opposite poles of the cell during the cell cycle. It was also shown that the interactions of the ParA/ParB proteins with DNA throughout the cell cycle have a direct or indirect effect on regulation of gene expression. Of the 29 genes with altered expression in *P. aeruginosa parA* and *parB* mutants encoding putative transcriptional regulators, only nine were functionally characterized, and the others are described as putative and/or hypothetical, classified as transcriptional regulators based only on *in silico* analyses.

The aim of this project is functional characterisation of putative, unknown transcriptional regulators PA1196, PA2121, PA2577, PA3027, PA3458, PA3973 from the ParA/ParB regulon of *P. aeruginosa*. The research hypothesis assumes the existence of a cascade of regulatory networks in *P. aeruginosa*, in which the partitioning proteins play superior roles in controlling, among others, the production of the transcriptional regulators. The transcriptional regulators in turn, might be involved in the regulation of the expression of single genes, operons and/or in global regulation, also with the participation of other, well-known transcriptional regulators. The study will lead to the correlation of the processes regulated by individual transcriptional regulators with the *P. aeruginosa* cell cycle. None of the putative transcriptional regulators chosen for the study has been characterised so far and, what is interesting, each belongs to a different family of prokaryotic-type transcriptional regulators (NtrC/NifA, LysR, AsnC, AraC, MarR, TerR -type). Understanding how the transcriptional regulators work, what processes they control, how they are intertwined in the regulatory network of *P. aeruginosa*, will expand our knowledge of the biology of this important model organism. The defining of the regulatory potential and the role of the analysed transcriptional regulators in the bacteria life cycle will be valuable for the understanding of the biological system of the opportunistic pathogen, which is *P. aeruginosa*.

Detailed analysis using genetic; biochemical; modern, high-throughput genomic methods and structural studies in combination with systems biology and bioinformatics tools, will help to define the role of the examined transcriptional regulators in the functioning of the *P. aeruginosa* cells and allow to determine what processes depend on these regulators. The study will determine whether the characterized proteins belong to regulators responsible for the control of specific metabolic pathways (pathway-specific) so-called "local regulators" of strictly defined processes, or whether they are global regulators that control the expression of many genes. It is also important to determine whether the analysed transcriptional regulators depend on, and/or interact with other regulatory networks.

The knowledge acquired in the proposed project with the use of *P. aeruginosa* as a model organism, can help in the functional characterization of putative transcriptional regulators, not only in *P. aeruginosa* but also in other pathogenic bacteria, in which homologues of the tested proteins are present. The knowledge gained from the project, apart from the undeniable, cognitive advantage for such an important, from a medical point of view, research object as *P. aeruginosa*, will provide information on the protein-DNA interactions relevant to a broad scientific community.