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Regulatory network and interactome of inner membrane drug/metabolite transporters in bacteria – implications for physiology, drug resistance and virulence

Bacteria are unicellular organisms that adapt very well to environment through skillful management of resources and economical use of the repertoire of genes encoded in the genome. The transporter proteins encoded in each genome are responsible either for the uptake of essential nutrients such as carbohydrates, amino acids, and metals into the cell, as well as the efflux of toxins and antimicrobial agents out of the cell. Therefore they play key roles in keeping cellular homeostasis, providing substances needed for cellular processes as well as excreting toxic, unfavourable compounds. There are many different families of transporter proteins, with differing substrate specificities, structures, and mechanisms of transport. The prevalence of transporter genes in bacteria varies considerably, but usually there is the link between the environment, that the cell occupies and the number and the type of transporters a bacterium possesses, with the number of transporters in the genome being generally proportional to genome size.

Pseudomonas aeruginosa is a bacterium commonly found in various ecological niches and characterized by its ability to survive in highly unfavorable, variable environmental conditions. Being a dangerous opportunistic human pathogen, it is often the cause of nosocomial infections in immunocompromised patients, currently also struggling with COVID-19. The main goals of the project presume characterization of drug/metabolite transporters (DMTs) from *P. aeruginosa* as a model organism of Gram negative bacteria. The regulatory and interaction network of inner membrane DMTs with its possible implications for physiology, drug resistance and virulence of *P. aeruginosa* will be studied. The DMTs comprise poorly characterized group of proteins, classified to the secondary transporters. Only few members of the DMTs class was characterized so far in bacteria. In *P. aeruginosa* there are 33 drug/metabolite transporter genes. Three DMTs ArnE, ArnF, CntI have assigned function in *P. aeruginosa*, for others the role in biology of this bacterium awaits elucidation.

The main research hypotheses assume that: I/ the DMTs are involved in keeping homeostasis of the cell, by excreting toxic and/or unfavourable compounds, and possibly acting as messengers of nutrition to virulence, connecting metabolic status of the cell, adaptability potential with drug resistance and pathogenesis; II/ the DMTs are involved in regulation of membrane functions, e.g. those connected with LPS biosynthesis, maintenance of membrane integrity, drug resistance or secretion control; III/ the DMTs create intricate interaction network with their putative partners, involved in control of different cellular processes and survival strategies (transport; response to starvation, stress, antibiotics; motility; biofilm formation; virulence); IV/ the C terminus of some DMTs is putatively involved in interactions with cellular partners on the cytoplasm side of the membrane, allowing control of membrane functions, as those connected with secretion of toxins. It is unexplored and undiscovered interaction network which may serve as a promising research object and source of inspiration for designing new anti-bacterial therapies.

Research will include a combination of microbiological and genetic, molecular biology and high-throughput genomic techniques, e.g., construction of appropriate mutant strains of *P. aeruginosa* and their phenotypic characterization; cloning of genes and studying the effects of gene overexpression on bacterial cells; use of ChIP-seq and pull-down analysis to identify DMTs regulatory network; protein-protein interactions analyses by bacterial two-hybrid system and protein co-purification, proximity-mediated biotin protein labeling in living *P. aeruginosa* cells coupled with purification of labeled proteins and their identification using mass spectrometry.

Obtained results will provide insight into the DMTs family, their essentiality for bacteria and role in the interaction network and biology of *P. aeruginosa*. The knowledge obtained from the planned research will facilitate our understanding of the physiology and survival strategies of pathogenic bacteria such as *P. aeruginosa* and may help identify potential targets for antibacterial therapies. Furthermore, the gained results may help to design the valuable tools for the control of bacterial metabolism with potential applications in industry, environment, and for control of plant, animal, and human pathogens.