CRISPRi-based screening for *Pseudomonas aeruginosa* vulnerabilities to antibiotics

Genes, which cannot be removed or inactivated, have been identified in genomes of various bacterial species. These are so-called essential genes, encoding components of core biological processes, crucial for bacterial survival. As expected products of essential genes are the best targets of antibacterial drugs. For years gene "essentiality" has been regarded as a binary variable. The gene is either essential or not. Is it however possible that certain essential genes are more essential than others? The approach based on determining the relationship between the degree gene silencing and the inhibition of bacterial growth allows determining the degree of essentiality (sensitivity) of a given gene (and its product). This makes it possible to find weak points, i.e. genes, which even a slight silencing has a drastic impact on the fitness of bacterial cells. The products of such genes could be exploited as targets for new antimicrobial therapies.

In recent years, the CRISPRi technique has been developed, in which inactive dCas9 protein is directed to a selected region in the genome using a short 20-nucleotide sequence included in the so-called guide RNA (sgRNA). Binding of dCas9 to the targeted fragment results in selective gene silencing. By manipulating the guiding sequence, it is also possible to adjust the degree of silencing. The possibility to silence almost any gene to various degrees, the reversibility, and the possibility of the simultaneous analyses of many genes using pooled sgRNA libraries in recent years led to widespread use of CRISPRi in analyses of the consequences of gene silencing, including the silencing of essential genes, on the fitness of bacterial cells.

This project is multidisciplinary and includes research with the use of molecular biology and bacterial genetics along with high-throughput sequencing methods, bioinformatics, data analysis, and chemical syntheses. In the project, we will adapt the CRISPRi technique for use in the analysis of the genes of *Pseudomonas aeruginosa*. This bacterium is an opportunistic human pathogen, characterized by high adaptability and intrinsic resistance to antibiotics. It causes difficult-to-eradicate nosocomial infections, especially dangerous in immunocompromised patients and patients with cystic fibrosis. Using CRISPRi we will check, which essential genes in this bacterium are the most sensitive to silencing. Moreover, we will also check if this sensitivity changes when bacteria are exposed to antibiotics. Similarly, we also plan to assess the sensitivity of non-essential genes involved in *Pseudomonas aeruginosa* resistance to antibiotics. The expected goal of this research is the identification of genes, the expression modulation of which strongly sensitizes bacteria to the action of antibiotics. The products of such genes would be the most promising therapeutic targets, including their targeting in combined therapies.