Metabolomics studies of *Pseudomonas aeruginosa*

ABSTRACT

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Pseudomonas aeruginosa is a gram-negative bacterium that is widely distributed in the environment. At the same time, the same species strains are dangerous opportunistic pathogens of humans, resistant to antibiotics. *P. aeruginosa* was classified, along with other bacterial strains, into the so-called "ESKAPE" group. Searching for the sources of virulence of these microorganisms is necessary for the effective treatment of infections. Microorganisms quickly acquire resistance to antibiotics, which is related to the widespread use of therapeutics in medicine and industry. Strains growing in hospital environments are particularly dangerous to human health and life. Investigating antibiotic resistance mechanisms at different molecular levels allows for finding previously unknown metabolic changes and antibiotic resistance biomarkers. One of the sciences that can contribute to understanding the differences between the above-mentioned strains is metabolomics, which deals with the analysis of small-molecule compounds reflecting the "current state" of the bacterial cell.

The main objective of the research conducted as part of the doctoral dissertation was to learn and compare the metabolomic profiles of various *P. aeruginosa* strains, enabling the understanding of the processes of the microorganism's adaptation to changed environmental conditions or the acquisition of antibiotic resistance. The presented doctoral dissertation is a collection of thematically coherent articles - it consists of one review article and three papers containing original experimental results. The experimental studies focused on analyzing the metabolomic profiles of microorganisms, with particular emphasis on *P. aeruginosa*.

In the first work, a literature review, the concept of metabolomics was defined and the possibilities of its use in experiments on microorganisms were discussed. These studies concern the identification of microorganisms as well as the influence of external factors on *P. aeruginosa*.

The next article presents the results of a methodological experiment in which, in addition to the *P. aeruginosa*, the metabolomic profiles of five other bacteria were also analyzed. A comparative analysis of three methods of bacterial cell disintegration was carried out - sonication, sand mill, and tissue disintegrator. The results showed that using each of the above-mentioned methods, we obtain the same qualitative results. The changes are quantitative and concern differences in the concentrations of individual metabolites. Additionally, the study proved that ^{1}H NMR spectroscopy is a tool that discriminates against metabolites of the studied microorganisms. The results confirm that in

the case of metabolomics studies, each step of the experiment must be carried out in the same way, and any changes during the preparation of the samples lead to the incorrectness of the analysis.

The second experimental work concerns the comparisons of intracellular metabolites of strains from different sources. For analysis, strains of *P. aeruginosa* from the natural environment and isolated from the sputum of patients suffering from cystic fibrosis were used. A comparison of the metabolomic profiles using the NMR technique was performed. The results of one- and multivariate data analyzes showed differences in the relative concentrations of the identified compounds. These changes mainly concerned compounds involved in the pathways of amino acid metabolism.

The last of the works is a study comparing another feature of *P. aeruginosa* - resistance to antibiotics. Strains isolated from patients with different sensitivity to antibiotics were selected for the study. In the experiment, in addition to the metabolomic fingerprint - the pool of intracellular metabolites, also the metabolomic footprint - a pool of extracellular analytes derived from the medium. The performed statistical analyzes allowed to identify the metabolites differentiating both strains. Additionally, the results of comparisons of the post-culture medium allowed us to determine the compounds used at the beginning in the metabolism of the bacterial cell. The altered metabolism, also, in this case, is mainly related to the degradation and synthesis pathways of amino acids.

The results of the conducted experiments showed that the strains from different environments have different metabolomic profiles. The presented results confirm the presence of differences in small-molecule compounds of *P. aeruginosa* strains having different resistance to antibiotics. These differences were mainly due to altered amino acid metabolism. In addition, it was confirmed that in metabolomics experiments, each stage of sample preparation should be identical, and the selection of an appropriate disintegration method is of particular importance in the case of targeted analyzes. Moreover, the potential of NMR as a tool to distinguish and identify different types of bacteria has been demonstrated. Future research should focus on the analysis of altered metabolic pathways using additional analytical techniques such as LC- and GC-MS.