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## Abstract of the doctoral dissertation

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### **Kin discrimination and territorialism of *Proteus mirabilis* strains and their genetic and phenotypic diversity**

*Proteus mirabilis* is a Gram-negative bacterium associated with urinary tract infections. The bacterium is known from its swarming motility, the ability of cells to migrate over solid surfaces. The feature is considered to be the virulence factor of *P. mirabilis*. At the same time, it is related to the ability of strains to identify related (kin) cells, which is referred to as the Dienes phenomenon. The strains belonging to two different Dienes types compete with each other, and the interactions lead to the elimination of unrelated cells. Proteins transported between cells through the type VI secretion system are responsible for this effect. The Dienes phenomenon is an example of territorialism based on mechanisms classified as bacterial kin discrimination. Kin discrimination allows for the elimination of genetically distinct cells.

There are no comprehensive data on genetic diversity of *P. mirabilis* in the aspect of kin discrimination of this species. Additionally, although the presence of seven sets of genes potentially involved in the identification of kin between strains was indicated, their genetic conservatism was not determined. Moreover, despite the observation of the dominance of one of the competing strains, it is noticeable that the studies so far have been carried out using only a few strains or the mutant/wild-type system. It has not been determined whether in a larger group of strains, the presence of strains predisposed to a dominance over the others can be indicated. Also, the relationship between the territoriality of the strains and the phenotypic diversity (including the virulence factors) has not been studied.

For this reason, the first goal of the presented studies, carried out on two groups of genomic sequences (obtained as part of the sequencing of the genetic material of the strains owned and obtained from public databases) was the phylogenetic analysis of the genome sequences of *P. mirabilis* and investigation of the pan-genome, as well as the comparison of the results of this analysis with: 1) diversity of genes clusters responsible for kin discrimination,

2) pattern of kinship of strains determined in laboratory conditions. Another goal was to assess the potential of *P. mirabilis* strains to competition and to determine the relationship between their territorialism and the intensity of selected virulence factors.

As part of the presented research, a collection of 21 strains of *P. mirabilis* was characterized in terms of the relationship between them. A group of five strains showing Dienes relatedness was distinguished, while the other strains competed with each other. Strains were also assigned a level of competitiveness based on how often they dominated the rest. Strains predisposed to dominate the others and those that were losing the competition were indicated.

Then, the genomic DNA of the strains was sequenced using Next Generation Sequencing (NGS) technology. Along with 197 sequences available in public NCBI databases, they have been subjected to extensive comparative analysis using a range of bioinformatics tools. The degree of genomic relatedness between the strains was determined using the ANI coefficient, and the phylogenetic analysis based on SNPs present in the genomes was performed. Next, the *P. mirabilis* pan-genome was characterized and described as open. In addition, the phylogenesis of the strains was presented on the basis of the sequence of core genes (common to all studied genomes). The core genes were annotated in order to assign them potential functions. The diversity of the strains in terms of the total pool of genes encoded by their genomes (pan-genome based phylogeny) was also presented.

To characterize the *P. mirabilis* pan-genome, an analysis of the interactions of the identified genes (gene association and dissociation) was performed and the basic panGenome Wide Association Study (panGWAS) analysis was performed. The above research was completed with the analysis of non-coding regions, intergenic regions - IGR. The conducted analyzes allowed for a wide characterization of the genetic relationship of strains, and compared to the results of the Dienes test.

In the next step, the distribution of seven clusters of genes related to the identification of kinship among the studied sequences was determined and their genetic diversity was presented. Diversity of kin discrimination related genes was compared to the kinship observed in the comparative analysis of genomes and to results of the Dienes test.

In the last stage of the work, the intensity of selected virulence factors was assessed in the collection of 21 *P. mirabilis* strains. The focus was on assessing the ability for swarming motility and biofilm formation, and the relative expression level of genes responsible for ureolytic, proteolytic and hemolytic activity. Statistical tests allowed to determine the correlation between virulence factors and territorial abilities of strains. The results of the

presented research extend our understanding of the phenomenon of kin discrimination of *P. mirabilis* strains, both in terms of genetic variability, as well as phenotypic diversity.

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