ANALYSIS OF THE INTERGENIC REGION FOR THE PRESENCE OF REGULATORY MOTIFS IN *PSEUDOMONAS CHLORORAPHIS* SUBSP. *AURANTIACA*

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As a result of a wild-type bacteria *Pseudomonas chlororaphis* subsp. *aurantiaca* B-162 (accession number CP050510.1) mutagenesis *P. chlororaphis* subsp. *aurantiaca* B-162/17 with an increased level of phenazine production was obtained. The quantity of phenazines in this mutant strain was almost three times higher than in the wild-type bacteria. Moreover, the B-162/17 strain was capable of producing phenazines on a minimal medium in contrast to the *P. chlororaphis* subsp. *aurantiaca* B-162. Phenazines are a group of heterocyclic nitrogen-containing compounds exhibiting a wide range of antibiotic properties, in particular, they have phytopathogenic and antitumor activity. Also, phenazines are widely used as pH indicators, components of microbial fuel cells and biofuel components in the biotechnology industry. Therefore, identification of mutations leading to phenazine overproduction on minimal nutrient medium becomes relevant.

Comparative analysis of wild-type and mutant genome sequences revealed potentially significant mutations in intergenic regions that can affect the level of phenazine synthesis. Using the SigmoID tool, the presence of motifs typical for the TetR and LuxR families of transcriptional regulator proteins was predicted in the intergenic region (coordinates 3443576 to 3443752 bp) located between genes expressing ABC transporter permease and LLM class flavin-dependent oxidoreductase.

To confirm the role of deletion which is located in described above intergenic region we decided to delete this region and analyze the resulting mutants. For this purpose, we have designed primers: CGGATCCTCCTTCCTCATCAAGCC (forward) and CGGATCCCAAAGGGCGTTGGTC (reverse), with BamHI restriction sites (highlighted in bold). The obtained PCR product was cloned into the pK18mob integrative suicide vector, which contains ampicillin and kanamycin resistance genes. The polylinker is located inside the ampicillin resistance gene. After entering the bacterial cell this genetic construction recombines with the chromosome and disrupts the integrity of the target sequence due to the presence of the homology region in it.

Using $CaCl_2$ transformation, the resulting vector was inserted into the *Escherichia coli* BW19851 bacteria, which are capable of conjugation. For this purpose, the donor bacteria *E. coli* BW19851 with the vector and the recipient *P. chlororaphis* subs. *aurantiaca* B-162 were mixed in a 70:30 ratio. The mixture of donor and recipient cells was incubated in a liquid LB medium for one hour and then, after precipitation, was transferred to the agar medium in the shape of medallions and incubated for 24 h at 28 °C. Cultured cells were transferred in a selective agar medium containing 50 μ/ml kanamycin and 50 μ/ml ampicillin (used to remove donor cells) and then incubated for 42 h at 28°C. The grown colonies were analyzed.

Analysis of *P. chlororaphis* subs. *aurantiaca* B-162 with deleted intergenic region showed that the phenazine level was higher than in wild-type *P. chlororaphis* subs. *aurantiaca* B-162 but lower than in *P. chlororaphis* subs. *aurantiaca* B-162/17. In this regard, the analyzed intergenic region contains regulatory motifs capable of affecting phenazine biosynthesis and excretion from the cell.