COMPARISON OF PHENYLALANINE PRODUCTION LEVELS IN *P. CHLORORAPHIS* SUBSP. *AURANTIACA* PHENAZINE PRODUCING STRAINS

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Phenazines designate a group of nitrogen-containing heterocyclic pigmented secondary metabolites with the pronounced capacity to inhibit plant pathogens. Phenazines are secreted by a diversity of bacteria, particularly *Pseudomonas* strains. Phenazines have received researchers' interest due to their wide-ranging antibiotic properties and have been widely used in the biological control of various fungal phytopathogens. Besides phenazines are considered potential anti-cancer agents.

Pseudomonas chlororaphis subsp. *aurantiaca* bacteria are producers of various biologically active substances, including phenazines. *P. chlororaphis* subsp. *aurantiaca* B-162 were obtained from the collection of microorganisms from the Genetics Department of the Belarusian State University. This strain demonstrated stable phenazine production. This wild-type strain was further used for the mutagenesis and selection of highly productive producer strains. Two mutant producer strains B-162/255 and B-162/17 was derived following sequential mutagenesis coupled with the selection on resistance to the toxic analog of aromatic amino acid.

Genomic sequencing of these strains revealed several mutations that could potentially cause an increase in phenazine production in *Pseudomonas* bacteria. In particular, a point mutation was found in strain B-162/255 in the gene encoding phenylalanine hydroxylase transcriptional activator PhhR involved in the degradation and homeostasis of phenylalanine, which in turn is a negative regulator of DAHP synthase implicated in the catalysis of biochemical reactions leading to the phenazines synthesis.

The purpose of the research was to evaluate the effect of a mutation in the *phhR* gene on phenylalanine production by mutant strains.

This experiment was conducted with *P. chlororaphis* subsp. *aurantiaca* strains B-162, B-162/255, and B-162/17 were cultivated on a minimal medium (M9) and a production media. After 12 h, 18 h, 1 day, 2 days, and 3 days of incubation, which correspond to the exponential stage of culture growth, the level of phenylalanine production was determined by a modified spectrophotofluorometric method of McCaman and Robins.

The wild type strain B-162 is characterized by minimal phenylalanine production level on a production medium among all three strains. By 12 hours of cultivation on a minimal medium, strain B-162 accumulates 3.8 times less phenylalanine than B-162/255 and 6 times less than B-162/17. The mutant strain B-162/255 is characterized by an increase in phenylalanine production by the third day of cultivation. The accumulation of phenylalanine by strain B-162/17 during cultivation on a production medium gradually decreases during the first day and increases again by the third.

In general, phenylalanine production for all three strains is higher on a minimal medium (M9). Based on the obtained results, it was concluded that the maximum productivity values for phenylalanine are characteristic of strain B-162/255. This correlates with the hypotheses that the mutation in the *phhR* gene affects the production of phenylalanine by *P. chlororaphis* subsp. *aurantiaca* B-162/255.