# THE CREATION OF GENETIC CONSTRUCTS FOR MODIFYING THE SHIKIMIC PATHWAY IN THE BACTERIA *BACILLUS SUBTILIS*

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In the pharmaceutical industry, shikimic acid is used to create drugs, which is used in cancer chemotherapy, and is used for the treatment and prevention of diseases caused by the influenza virus, etc. [4].

In plants and microorganisms, shikimic acid serves as a precursor of a large number of functionally primary and secondary metabolites, such as: aromatic amino acids (phenylalanine, tyrosine and tryptophan), lignin, folic acid, tetracycline, ubiquinones, phenolic and carbolic compounds, alkaloids [3,5], therefore many of its derivatives are agricultural interest and are used as herbicides and antibacterial agents.Using of these compounds is based on their ability to block the shikimic metabolic pathway in organisms, because mammals and humans do not have the shikimic metabolic pathway, so without negative effect on mammals and humans [7]. For this reason, it is highly advisable to search for the alternative sources of this raw material and modify existing methods for its production.

The aim of the work was to obtain genetic constructs for modifying the shikimaic pathway in the bacterial strains B. subtilis, which are capable to increase tryptophan synthesis and are potentially suitable for producing shikimic acid.

These bacterial strains were used in the work: E. coli - DH5 and XL1 − Blue; B. subtilis 168 trpC2. Strains from the collection of the Department of Genetics of BSU, characterized by an increased level of tryptophan synthesis: B. subtilis KMBU 2003, B. subtilis VKPM5434, B. subtilis VNII Genetics-15, and its derivatives C10, D3 and D4, obtained by the mutagenesis of the B. subtilis Genetics-15 strain and then selected mutants, which resistant to tryptophan structural analogues; and plasmids: pMTL21C, pLAV1, pAL1 and pAL2, pMUTIN4.

Bacterial cultures grow in liquid and solid nutrient medium have different compositions: LB or Spizizen minimal medium. Plasmid DNA from E. coli and B. subtilis bacteria were isolated by alkaline lysis [1]; to isolate plasmids from B. subtilis bacteria, the culture of plasmid-containing bacteria grows at 30°C. The total DNA of the bacteria was isolated by the method of phenol-chloroform extraction and used as a matrix for PCR.

The transformation of E. coli and B. subtilis bacteria cells, previously transferred to the state of competence, was carried out according to the recommendations given in the work manual [6], [2]. The transformation of B. subtilis strains with an increased level of tryptophan synthesis was carried out according to a modified method.

The chromosome fragment of the bacterial B. subtilis, containing the tmrB and aroI – genes, which were amplified using two pairs primers. For the ShikF-NotI and ShikR-BamHI pair, the following amplification modes were used: 94ºC – 5 min (1 cycle); 94ºC – 30 s; 52ºC – 30 s; 68ºC – 2 min (10 cycles); 94ºC – 30 s; 54ºC –

30 s; 68ºC – 2 min (20 cycles); 72ºC – 10 min. This primer contains recognizable sites of the restriction enzymes NotI and BamHI at the 5'-ends, respectively.

For this pair primer ShikF1-EcoR1 and ShikR2-SacII, the following amplification modes were used: 94ºC - 5 minutes (one cycle); 94ºC – 30 s, 55ºC – 10 s, 54ºC – 25 s, 68ºC – 2 min (10 cycles); 94ºC – 30 s; 54ºC – 30 s; 68ºC – 1 min 30 sec (25 cycles); 72ºC – 5 min. The primer contains EcoR1 and SacII restriction sites at the 5'-ends, respectively.

Using the total DNA and the indicated primer pairs as the template for the PCR, the conditions for obtaining the target product were selected. To prove that the obtained amplicons have a similar structure to the expected, the obtained amplicons were verified using restriction enzymes HindIII and PstI, and then did the electrophoretic separation in an agarose gel.

By the Analysis of the electrophoregram shows that all the amplicons obtained are similar to the structure, but on the basis of the electrophoretic mobility can be divided into two groups. The first group can be attributed products obtained from total DNA of the following B. subtilis strains: VKPM5434, Genetics-15 D4 and KMBU 2003-2; the second group - VNII Genetics-15 D3, VNII Genetics-15 C10, KMBU 2003-1 and 168 trpC2. These differences may be a consequence of a series of mutagenesis, with the help, these strains used in the work were obtained.

Amplification products verified by restriction analysis were cloned into the pMTL21C and pMUTIN4 vectors. The selection of recombinant molecules was carried out using the transformation of E. coli bacterial cells with the ligation mixture, and with the key restriction sites, then selected on the mediam with X-Gal, IPTG, ampicillin 50-100 μg/ml. From the obtained transformants, plasmid DNA was isolated and tested by RFLP method and sequence analysis. According to the results of the RFLP, a number of molecules were selected, in which contain the key determinants---restriction sites as expected.

As a result of this work, initial amplification products were obtained for the creation of genetic constructs, intended for integration into the composition of the bacterial chromosome. Among these amplicons, genetic polymorphism was identified, which may be the result of mutagenesis used in the preparation tryptophan producing strains. Using the obtained amplicons, an integration system has been created, for the controlled directional inactivation of the shimate kinase gene in B. subtilis bacterial cells. These results obtained are an important basis for the further design of producers.