doi 10.18699/bgrs2024-8.3-07

Genetic technologies: production of fermentative preparations for industrial needs based on the construction of producers using a collection of natural genes of bacteria and the genome of the yeast *Komagataella phaffii*

Peltek S.E.[1, 2]*, Zadorozhny A.V.[1, 2], Bogacheva N.V.[1, 2], Korzhuk A.V.[1, 2], Shlyakhtun V.N.[1, 2], Uvarova Y.E.[1, 2], Shipova A.A.[1, 2], Arbuzov G.D.[1, 2], Chesnokov D.O.[1, 2], Bannikova S.V.[1, 2], Pavlova E.Yu.[1, 2], Bochkov D.V.[1, 2], Bukatich E.Yu.[1, 2], Slyunko N.M.[1, 2], Bryanskaya A.V.[1, 2], Khlebodarova T.M.[1, 2], Vasilieva A.R.[1, 2]

[1] *Institute of Cytology and Genetics, SB RAS, Novosibirsk, Russia*

[2] *Kurchatov Genomic Center of the Institute of Cytology and Genetics, SB RAS, Novosibirsk, Russia*

* *peltek@bionet.nsc.ru*

Key words: strains-producers; enzymes; cultural liquids; proteomes

Motivation and Aim: The main objective of this work is to create a conveyor for constructing strains-producers of enzymes for industry, which will enable the import substitution of essential enzymes for industrial needs and the development of new enzyme (protein) preparations absent in global markets “on a turnkey basis”.

Methods and Algorithms: This task is partially implemented based on the Collection of microorganisms for biotechnological purposes of the Federal Research Center Institute of Cytology and Genetics SB RAS, more than 2,100 genomes of natural strains of which have been sequenced and annotated to date. A database of genes controlling the biosynthesis of enzymes used in various biotechnological productions has been formed. Experimental studies of the main properties of enzymes expressed by annotated genes have been conducted (substrate specificity, thermostability, optimum pH, activities, pI points, etc.). The nucleotide sequences of the studied enzymes were used for directed gene-specific obtaining of strains-producers of necessary enzymes.

Results: Using genetic engineering methods, their transfer into the genome of the yeast *Komagataella* phaffii has been performed, effective expression of target proteins in the cultural medium under the control of the AOX1 gene promoter and terminator and the leader peptide has been shown, and a technology for obtaining preparations of target proteins (enzymes) has been developed, including methods of cross-flow membrane filtration. During the cultivation of the strain T07 based on *K. phaffii* cells, a producer of alpha-amylase from *Bacillus licheniformis* 47018, studies were conducted to determine the target enzyme with confirmation of its structure and quantitative comparison of its content. of this enzyme in cultural liquids and proteomes. The created basic methods of qualitative and quantitative analysis of molecules of target enzymes make it possible to reliably determine their presence in cultural liquids and preparations.

Conclusion: The efficiency of the obtained conveyor has been tested during semi-industrial synthesis of alpha-amylase from *Bacillus licheniformis* 47018 under the conditions of a 100-liter bioreactor.

Funding: The work was carried out with the financial support of the “Kurchatov Genomic Center of the Federal Research Center Institute of Cytology and Genetics SB RAS” (No. 075-15-2019-1662) and the Ministry of Science and Higher Education of the Russian Federation (No. FWNR-2022-0022).