DOI 10.18699/SBB-2022-275

Technological aspects of the search, cloning and production of enzymes for industrial use

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Key words: thermostable enzymes, extremotolerant bacteria

Motivation and Aim: The search for enzyme genes and the production of enzyme preparations based on them is an urgent topic of our time: enzymes are in demand both in industry and in scientific research. Our approach is based, firstly, on the large-scale collection of microorganisms from extreme environmental conditions – thermal springs, salt and soda lakes, industrial landfills and others. Secondly, on the screening of both the physiological characteristics of individual microorganisms and the search for homologues of known enzymes in genomes by bioinformatics methods [1, 2].
Methods and Algorithms: By screening methods on Petri dishes, we determine the presence of enzymatic activity in a particular microorganism, by biochemistry methods we test preferred carbon sources for biomass production, by bioinformatics methods we investigate the presence of the desired gene in the genome of the microorganism. The next stage is the production of a sufficient amount of culture fluid with sufficient content of the target enzyme for analysis, isolation and chromatographic purification of the target enzyme, its mass spectrometric identification. The data obtained in this way allow us to assess the prospects of an enzyme preparation before the laborious process of cloning a gene in the yeast producent Komagataella phaffii.
Results: Among the natural strains, we have identified producers of secreted enzymes of various classes: amylases, proteases, cellulases, effectively producing enzymes to the culture medium at pH 9–10 at 55 °C. 32 cultures of amylolytics were obtained, the best activity is demonstrated by representatives of the species Bacillus licheniformis. In the genomes of this species there are two amylases, intracellular maltogenic amylase and alpha-amylase. pH and temperature optimums of the chromatographic purified alpha-amylase 47018 were characterized. The adapted to expression in yeast K. phaffi enzyme gene sequence was chemically synthesized.
The search for thermotolerant destructors of protein (protease producer), cellulose (cellulase producers) and olive oil (lipase producers) was carried out at different pH values (7, 8, 9) and a temperature of 50 °C. 20 strains have been isolated that can use olive oil as the only source of carbon and energy. Three thermotolerant strains of Brevibacillus thermoruber and a strain of Thermomonas with secreted cellulases have been isolated. Isolated strain 6008 (Bacillus sp.) had high secreted proteolytic activity at 50 °C. 31 protease genes were found in the genome of strain 6008.

Conclusion: Among the natural strains, we identified producers of secreted enzymes of various classes (amylases, proteases, cellulases) and obtained chromatographically pure enzyme preparations. The developed preparations were studied according to biochemical parameters – operating temperature ranges and pH, resistance to metal ions. The genomes of the isolated producers were sequenced. Enzymes of the corresponding classes were found in the genomes of the strains.

Acknowledgements: This research was funded by the Ministry of Science and Higher Education of the Russian Federation, project No. 075-15-2019-1662 (Kurchatov Genomic Center, federal research center ICG SB RAS, Novosibirsk).

The study was funded by the Ministry of Science and Higher Education of the Russian Federation as a part of the comprehensive project on high-tech industry "Creation of high-tech production of high-quality plant food proteins" (The agreement on the provision of subsidies from the federal budget for the development of cooperation of a state scientific institution and organization the real sector of the economy in order to implement a comprehensive project for the creation of high-tech industry No. 075-11-2020-036 from 15.12.2020) in the framework of the Decree of the Government of the Russian Federation of April 9, 2010 No. 218 on the basis of the ICG SB RAS.

We thank the Collection of Microorganisms (supported by Ministry of Science and Higher Education grant No. FWNR-2022-0022) at the Federal Research Center ICG SB RAS.

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