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Industrial enzymes production based on genetically engineered microorganisms strains of super-producers

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Motivation and Aim: Enzyme technologies application greatly determines successful development of a diverse national economy sectors. Due to the biotechnological advances many chemical processes in various industry fields, such as textile, leather, food, pulp and paper, fruit and vegetable processing, feed production, and household chemicals, are now being replaced by enzymatic biocatalytic ones. In the Laboratory of molecular biotechnologies of the Federal Research Center Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences, we developed a scientific base of the so called «conveyor system» for industrial microorganism strains of enzyme producers.

Methods and Algorithms: The pipeline included creation of a wild type strains collection from previously unexplored unique extreme ecosystems, with further formation of a pure cultures strains sets with certain substrate and bio-catalytic properties. Next, 1860 complete genomes of the collection were fully sequenced and annotated. After, gene expression patterns of the most promising strains were determined, and metabolic activity of industrially significant substrates like lignin or oil sludge was studied. The ability of *Komagataella phaffii* yeast to secrete heterologous proteins in the culture medium was used to create strains producing target enzymatic activities.

Results: Using methods of genetic engineering, target enzymes gene transfer into the genome of *K. phaffii* was performed. The effective expression of target proteins in the culture medium was achieved by putting the target gene sequence under the control of the promoter-terminator system of the AOX1 gene, and the leader peptide. Using cross-diffusion filtration and high pressure chromatography preparations highly pure were obtained with subsequent identification by mass spectrometric analysis. To sum up, we developed the technology for preparations of target proteins or enzymes, including membrane methods.

Conclusion: Using the methods of modern microbiology, proteomics and bioinformatics, the process of identification, selection and scaled production of target proteins and enzymes for modern industrial purposes, including number of cellulases, alpha-amylases, and lipases, was developed.

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