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"Construction of *Ogataea (Hansenula) polymorpha* strains capable to efficient conversion of glycerol to ethanol"

Summary:

The problem of waste glycerol utilization which is accumulated in huge amounts during production of biodiesel is of great interest. From year to year, the production of this biofuel is increasing, which carries a risk of excessive formation of contaminated by-product glycerol. Its purification, due to physic-chemical properties of glycerol, is too expensive. Therefore, the search for microorganisms capable of conversion of by-product glycerol to high-value products, including ethanol, is of great interest. For this, an important aspect is to elucidate the glycerol metabolism in the cells of microorganisms, especially in those capable of converting glycerol to ethanol at elevated temperature, and use methanol, which is a strong contaminant of the glycerol phase. Undoubtedly, the methylotrophic thermotolerant yeast *O. polymorpha* belongs to such very promising organisms. However, wild strains of this species produce too little amounts of ethanol from glycerol. Therefore, the aim of this work was to construct yeast capable of more effective conversion of glycerol to ethanol, as well as understanding the process of glycerol transformation in *O. polymorpha*, understanding the function of genes coding for enzymes responsible for catabolism of glycerol and analysis of ethanol as one of the products of this metabolism, as well as the construction of strains capable of more efficient conversion of glycerol to ethanol. To achieve this, overexpressing the *ADH1 and PDC1* genes coding for the enzymes, alcohol dehydrogenase and pyruvate decarboxylase, responsible for the synthesis of ethanol, were performed. In the second stage, it was planned to overexpress the *GCY1, DAK1* genes, as well as *GUT1* and *GPD1*, encoding glycerol dehydrogenase, dihydroxyacetone kinase, glycerol kinase and glycerol-3-phosphate dehydrogenase, respectively. These enzymes are involved in the initial stages of the two alternative catabolic routes of glycerol. Finally, the constructed strains were analyzed in terms of metabolic products, the level of activity and the level of expression of specific genes related to glycerol metabolism. As a result of these manipulations, the WT/ADH1/PDC1 # 98 strain was constructed in the first stage, which produced 4.3 g/l of ethanol, and after increasing the glycerol content in the medium from 100 g/l to 150 g/l and raising the temperature from 37 °C to 45 °C, this strain produced up to 5 g/l of ethanol. The overexpression of the *ADH1 and PDC1* genes has also slightly

improved the alcoholic fermentation on the substrate with xylose and glucose. In the next stage, the genes responsible for the initial stages of catabolism of glycerol were overexpressed. There are two such pathways in *O. polymorpha* cells. One based on glycerol kinase (Gut) and glycerol-3-phosphate dehydrogenase (Gpd), the other on glycerol dehydrogenase (Gcy) and dihydroxyacetone kinase (Dak). Overexpression of genes encoding mentioned enzymes of each pathway, resulted in an increase in ethanol production to 10,41 g/l and 10,71 g/l, respectively. This allows us to conclude that the genes involved in the primary stages of glycerol catabolism in *O. polymorpha* yeast cells are important in the conversion of glycerol to ethanol. Over-expression of *GCY1, DAK1* genes, as well as *GUT1 and GPD1*, contributes to the increase in ethanol production as well as significant increase in the rate of glycerol metabolism, and increase in the consumption of glycerol by cells of *O. polymorpha* modified yeast. It was found that the strains with overexpression of genes coding for initial steps of glycerol catabolism along with genes *PDC1 and ADH1* were characterized not only by improved production of ethanol from pure glycerol but also by elevated ethanol production from two analyzed samples of by-product glycerol. Thus, the yeast *O. polymorpha* is a promising organism for the conversion of waste glycerol to ethanol, with high yield.