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New Yarrowia lipolytica Strains Engineered for Erythritol and Citric Acid Biosynthesis from Glycerol

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Abstract

Due to the ease of genetic manipulation, undemanding cultivation conditions and short generation time, yeasts are increasingly perceived as attractive microbial cell factories. *Yarrowia lipolytica* is a particularly intensively researched microorganism presenting great potential in the production bulk. Moreover, this non-conventional yeast has the remarkable ability to assimilate carbon sources from various agro-industrial wastes or by-products, including sugars from lignocellulosic biomass hydrolysates, fatty acids derived from animal fat and waste cooking oils or crude glycerol which is the main by-product of the biodiesel industry. Moreover, biodiesel production is projected to reach 53.2 billion liters in 2026, therefore large amounts of waste glycerol will be available as well. *Y. lipolytica* in the presence of hydrophilic substrates produce e.g. organic acids, polyols, and carotenoids, used as food additives (Bilal et al., 2021). The aim of the study was to evaluate *Y. lipolytica* yeast transformants obtained in the Wratislavia 1.31 strain in terms of growth and ability to metabolize glycerol towards overproduction of citric acid and erythritol. The strains were constructed by overexpressing the *GUT1* gene, encoding glycerol kinase, *CIT1*, encoding citrate synthase and *YALI0E34672g*, encoding the mitochondrial succinate fumarate transporter (Tomaszewska-Hetman et al., 2021). As a result eight strains were obtained, which contained multiple copy of one, two or three genes.
Growth parameters, such as ODmax and the maximum specific growth rate (μmax) were determined in the cultures performed in the Bioscreen apparatus. Next, strains were compared in terms of glycerol utilization rate and citrate production in shake-flasks experiment. Production cultures were carried out in 5-L bioreactor BIOSTAT B Plus with a working volume of 2 L. The growth curves obtained in the Bioscreen cultures indicated that transformants reached the stationary phase later than Wratislavia 1.31 strain but at a higher ODmax value. The value of the μmax parameter ranged from 0.065 h^{-1} (1.31.GUT1/6) to 0.097 h^{-1} (1.31. CIT1/3), while for the parental strain it amounted to 0.081 h^{-1}. In shaken cultures, under the conditions of growth limitation by low concentration of the macronutrient – nitrogen, the strains with an additional copy of the

GUT1 gene were distinguished due to the rate of glycerol utilization and presented a 1.4-times higher citrate production compared to the Wratislavia 1.31 strain. In the bioreactor culture, the strain 1.31.GUT1/5 produced 98.7 and 77.4 g/L of citrates from 150 g/L of pure and waste glycerol, respectively. The rate of citrates production from waste glycerol was higher for the transformant 1.31.GUT1/5 (0.97 g/Lh) than for Wratislavia 1.31 (0.79 g/Lh). Under conditions of increased osmotic pressure (for erythritol biosynthesis), strains with the *GUT1* gene utilized glycerol the fastest. However, the high dynamics of glycerol consumption was not related to the production of erythritol. In the case of strains 1.31.GUT1/5 and 1.31.GUT1/6 mannitol production was the highest. Three strains, 1.31.GUT1/5.CIT1/3.E34672g, 1.31.GUT1/6.CIT1/3.E34672g and 1.31.CIT1/3, produced more erythritol than the parental strain, while the productivity was higher for all transformants, ranging from 0.6 to 0.8 g/Lh. As a result of the transformations, strains with improved dynamics of glycerol utilization and good biosynthesis parameters of both citrates and erythritol were obtained.

Keywords: Yarrowia lipolytica, transformants, crude glycerol, citric acid, erythritol

References

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