POSTER - SYSTEMS BIOLOGY AND MODELING

PREDICTION OF TARGETS TO IMPROVE LIPID PRODUCTION IN LIPOMYCES STARKEYI BY GENOME-SCALE METABOLIC MODELING

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Oleaginous yeasts cultivation in low-cost substrates is an alternative for more sustainable production of lipids, oleochemicals, polysaccharides, and enzymes. The Ascomycota Lipomyces starkeyi can accumulate high amounts of lipids from different carbon sources, such as glycerol and lignocellulosic sugars. Recently, we reconstructed and validated the genome-scale metabolic model (GEM) of L. starkeyi (lista-GEM), which is a great framework for predicting metabolic engineering targets. Herein, we applied lista-GEM to determine the effects of both oxygen and carbon availabilities using phase plane analysis and overexpression targets by flux scanning based on enforced objective flux (FSEOF) to improve triacylglycerol (TAG) production with glucose, xylose, or glycerol as carbon sources. We performed modeling adjustments and simulations in the MATLAB environment (v. 2023a) using RAVEN (v. 2.7.9) and COBRA (v. 3.0) toolboxes. In the phase plane analysis, we varied the carbon uptake rate from 0 to 10 mmol/gDW h-1 and the oxygen uptake rate from 0 to 50 mmol/gDW h-1, setting the upper bound of biomass formation to 0.01 mmol/gDW h-1 while maximizing TAG (1-16:0, 2-18:1, 3-18:1) exchange. Meanwhile, in the FSEOF, we fixed the carbon uptake rate at 1 mmol/gDW h-1 and selected the most important reactions to redirect the flux from biomass

production toward TAG exchange. The higher TAG production rate was achieved using glucose, followed by xylose and glycerol (0.312, 0.248, and 0.170 mmol/gDW h-1). The phase plane analysis indicated that the carbon availability affected TAG production more than oxygen availability. Moreover, we found that the maximum TAG production using glucose and xylose as carbon sources required more oxygen than glycerol. These results indicated that enhancing the carbon assimilation capacity of L. starkeyi, as well as controlling the oxygen availability, might be suitable strategies for improving TAG production. Moreover, for the three carbon sources evaluated, the FSEOF indicated that following enzymes related to lipid synthesis in the endoplasmic reticulum were the main targets to improve TAG production: stearoyl-CoA desaturase, fatty-acyl-CoA synthase, diacylglycerol acyltransferase, and glycerol-3-phosphate acyltransferase. Enzymes associated with yeast growth such as pyruvate kinase, enolase, phosphoglycerate mutase, glyceraldehyde-3-phosphate dehydrogenase, and phosphoglycerate kinase were indicated as targets for superexpression. Besides, pyruvate decarboxylase, acetaldehyde dehydrogenase, acetyl-CoA synthetase, adenylate kinase, inorganic diphosphatase, and triose-phosphate isomerase were predicted as possible targets only when glycerol was the carbon source. In summary, we demonstrated that lista-GEM can provide multiple metabolic engineering targets to improve lipid production by L. starkeyi from carbon sources found in agricultural and industrial wastes.