ADAPTIVE LABORATORY EVOLUTION OF *YARROWIA LIPOLYTICA* TO ENHANCE GROWTH AND LIPID PRODUCTION UNDER HIGH CONCENTRATIONS OF CRUDE GLYCEROL: VALIDATION OF NOVEL EVOLVED POPULATIONS

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ABSTRACT

Yarrowia lipolytica has drawn the attention of the scientific and technological community as an ideal microorganism for single-cell oil production, as well as for catabolizing crude glycerol as preferred carbon source. The combined features of enhanced lipogenesis and favorable utilization of the low-cost crude glycerol (as a byproduct of the biodiesel industry) render this yeast a workhorse for sustainable lipid production. Improvements of lipid biosynthesis are achieved in various cases mainly through metabolic pathway engineering.[1,2] In strain development for biomanufacturing, adaptive laboratory evolution (ALE) is employed as an alternative tool. In this process, the microorganism is cultured over multiple generations under a specific selection pressure, and its fitness improves as beneficial mutations in the population are fixed over time while metabolism rewires to support the enhanced phenotype.[3]

In this study, *Yarrowia lipolytica* was cultivated in a synthetic medium with increasing glycerol concentration (2 - 18% v/v) to investigate the biomass formed after 24h by monitoring the OD_{600nm} and the total cell number. After 104 generations, the growth of the evolved population (EP) was studied under different glycerol concentrations (2.5 - 17.5% v/v) using a microplate reader. Due to the heterogeneity of the EP, single colony isolation was carried out using the highest glycerol concentration (18% v/v) and the growth profiles of the isolated evolved strains were reproduced in flasks.

The increased glycerol concentration extended the lag phase of the WT *Yarrowia lipolytica*, whereas the biomass formation of the EP was accelerated under all glycerol concentrations compared to the WT. When reducing the initial carbon source concentration, the growth rate and final OD_{600nm} in both cases (EP, WT) slightly increased. The final biomass concentration of the EP showed a two-fold increase after a 48-hour fermentation with 2.5% v/v glycerol into the culture medium. Several evolved strains attained a four-fold higher dry biomass concentration (g/L) on flask experiments without a negative effect on lipid content. Selected evolved strains are currently under evaluation as lipid and biomass producers in lab-scale bioreactor fermentations, as well as under transcriptomic analysis, to assess the metabolic changes enabling the adaptation to high glycerol content in synthetic media.

KEYWORDS: *Yarrowia lipolytica*, Adaptive Laboratory Evolution, Lipid Production, Crude Glycerol Valorization

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