TITLE: EVOLUTIONARY ENGINEERING FOR IMPROVED HIGH-ADDED VALUE COMPOUND PRODUCTION IN INDUSTRIAL YEAST STRAINS

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ABSTRACT:

The increment in global energy demand, request the development of eco-friendly approaches. In this context, production of compounds with biofuels application naturally synthesized by yeasts, as ethanol and higher alcohols, shown as a viable alternative. However, higher concentrations of this compounds are growth inhibitory, avoiding achieve superior yields than actual. Using evolutionary engineering, through the increment of extracellular concentration of a specific alcohol for occurrence of natural mutations and selections, is possible to improve the yeast physiology such us resistance to alcohols. In view of the above, the objective of this work was to improvement industrial yeasts, for increase resistance to growth in inhibitory concentrations of alcohols. The tests were performed using natural sugar cane broth (120 g/L of sucrose, supplied with 5 g/L of ammonium sulfate). Industrials strains of *Dekkera bruxellensis (Db-CBS74) Sacharomyces carbelgensis (Sc-YK007) and Sacharomyces cerevisiae* (Sc-JP1, Sc-P25 and Sc-MI0), and alcohols as ethanol, propanol, isoamyl and isobutanol were tested. Yeasts were inoculated at 0.3 optical density (620mm), at 100 rpm and 32 °C. This initial condition with 0% v/v of alcohol, was used as 100% of growth. The alcohol concentration was gradually increased each 48h and the biomass was determined during 154 days. For define the adaptation grade, was determined the inhibitory concentration of 50% of cell population (IC 50%) of parental/original strains. The assays demonstrated different strains growth profiles in responds to the alcohols increment. In general, the IC 50% of the adapted strains augmented, in relation to the parental, indicating the increased resistance to the specific compound. For example, Sc-JP1 strain displayed an increment in the IC50% of 2.7-fold times (from 0.27 to 0.73 % v/v) using isoamyl alcohol as selection factor. Another strain Db-CBS74, using the same alcohol, displayed an IC 50% increment of 50 % (0.24 to 0.49 % v/v). The results showed that yeast strains respond differentially to alcohols, possible for the size or branched chain of the alcohol structure. Evenly, is confirmed the possibility of improve the resistance to toxicity of alcohols by industrial yeasts using adaptive evolution. At the same time, this approach could be use as alternative or concomitant with genetic modification, especially in the industrial context.

Keywords:: adaptation, higher alcohols, industrial yeasts

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