YEAST SELECTION FOR SECOND GENERATION ETHANOL INHIBITORS TOLERANCE BY HYBRIDIZATION AND ADAPTIVE EVOLUTION

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The sugarcane bagasse can be hydrolysed to obtain fermentable sugars to produce second-generation ethanol. However, due to inhibitors produced during pre-treatment and hydrolysis, novel yeasts strains with higher inhibitor tolerance profile are required. This study aimed at the selection of hybrids with increased multi-tolerance profile to cope with this fermentation. Industrial *Saccharomyces cerevisiae* strains (BG-1, CAT-1, PE-2 and SA-1) were sporulated (rafinose 1%, potassium acetate 2%, pH 6,8) and 604 haploids were isolated by tetrads dissection. The haploids tolerance was evaluated by growing in 96 wells microplate reader (OD 570nm at 30ºC during 24 hours in a Tecan GENions device) using selective medium (14% TRS, 2.5 g/L acetic acid, 1.5 g/L furfural, 0.5 g/L HMF, pH 4.7). The selected haploids (25) had their mating type (a and α) identified and used in 51 direct crossings allowing the rescue of 398 zygotes by micromanipulation. All the zygotes were evaluated in microplates using the same selective medium and the best 112 zygotes were saved for a cell recycling fermentation pre-screening based on growth and cell viability. At the same time pools of haploids from each parental strain were also used for "polycrossings" (random breeding among haploids from two parental and breeding involving haploids from all parental) resulting in 7 different populations which were subjected to an adaptive evolution for 50 generations, using the same selective medium. A total of 189 isolates (27 from each population) were rescued by micromanipulation and were also evaluated for growth on microplate reader as above mentioned. The 42 better isolates were also subjected to a cell recycling fermentation pre-screening in the same medium. Three better hybrids from each selecting approach (direct breeding and polycrossing followed by adaptive evolution) were assessed during cell recycling fermentation (5 cycles, with final ethanol titers ranging from 7 to 8,5%) using selective medium composed by bagasse hydrolysate and molasses (17% TRS, 1.74 g/L furfural, 0.89 g/L acetic acid, pH 4.8 at 30ºC). A hybrid between PE-2 and SA-1 (D10 lineage) stood out with greater biomass production, fermentation rate and cell viability during all cycles, when compared to the parental. We suggest that D10 strain could be an attractive candidate for genetic transformation aiming the xylose fermentation for the second generation ethanol production.

Keywords: *Saccharomyces cerevisiae*; 2G-ethanol; adaptive evolution; hybridization; bagasse hydrolyzate.

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