Introduction

Brazilian bioethanol is exclusively produced by means of the “first generation ethanol” industrial process. Although considered a mature process with narrow margins for improvements, additional gains could be achieved in the fermentation step. One of such approaches is the very-high-gravity (VHG) fermentation, attaining 15 to 18% (v/v) ethanol titers, as is performed with cereal based substrates, near the double of the ethanol content found in Brazilian distilleries. Great economical and environmental advantages arose from the VHG fermentation, due to the lesser volume of stillage and reduced water usage and energy consumption during distillation, suggesting an increased energy balance of the produced ethanol.Several factors are known to act upon industrial yeasts, compromising yield, as high ethanol content, high osmotic stress (due to sugar and salts), high acidity and high temperature. All such stressing conditions are intensified by cell recycling, a unique trait of the Brazilian process. In a proposed VHG fermentation, not only ethanol stress will be higher, but also osmotic and acidic stresses will be augmented. One of the limiting factors for the VHG fermentation technology is the absence of proper strains to face the very harsh fermentation condition, where several stresses are simultaneously imposed to the fermenting yeast. In the present work, *Saccharomyces* strains were searched for multitolerance to cope with cell recycling VHG fermentation.

Methodology

Yeast isolates (280) from several Brazilian distilleries (20), assigned as *Saccharomyces sp* by means of electrophoretic karyotyping, were evaluated for their growth rate (umax) and 24 h biomass formation (OD at 600 nm) at 30^{o}C in 24% (w/v) total sugar industrial substrate (cane juice and molasses), using 96 well plate reader. Simultaneously, *S. cerevisiae* PE-2, CAT-1 and SA-1 strains were sporulated, complete tetrads were dissected, and segregant haploids were screened as above mentioned. Directed crosses between the fast growing haploids (from the same and different asci of all strains) were performed, and again screened for vigorous growth under the above conditions. Additionally, a pool of hybrids, from crosses involving all strains, were subjected to an adaptive evolution during 70 generations in cane molasses substrates with increasing sugar concentration (from 12 to 16% total sugar). Prevalent evolved variants were identified by electrophoretic karyotyping, and also screened for higher growth rates. Cultures presenting higher umax values and/or biomass formation were evaluated for their fermentation performance at 30^{o}C under recycling conditions, simulating the industrial process with cell acid washing and sugarcane based substrate. The following fermentation parameters were estimated: ethanol yield, glycerol formation, residual sugar, biomass gain, cell viability and intracellular trehalose content.

Results and discussion

During the screening procedure in 24% sugar concentration, umax value and final biomass presented great variation amongst the isolated strains from distilleries, but only 5% of them presented higher values for both parameters when compared with the reference strains (PE-2 and CAT-1). Direct crosses of previously selected haploids (for vigorous growth) showed to be more efficient

than randomly crosses in generating better variants when compared with parentals. After the adaptive evolution, at least 25% of the isolated colonies showed to be superior when compared to the parentals, regarding umax value and biomass formation. The selected cultures for higher umax and biomass formation also showed better fermentation parameters when compared to the parental (PE-2 and CAT-1), during very-high-gravity fermentation trails, simulating the industrial process with cell reuse. Not only higher viability was displayed, but also high ethanol yield could be achieved with the evolved strains. The polygenic nature of industrially desirable phenotypes, as ethanol tolerance, makes yeast improvement a difficult task by a rational approach. In this work a more “blind” procedure was devised and multitolerant strains were selected. Their fermentative performance to cope with a final ethanol concentration of 15% (v/v) using molasses based substrate, enable such strains for a very-high-gravity fermentation.

Author publications

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