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FERMENTATIVE CHARACTERIZATION AND EVALUATION PROFILE OF A THERMOTOLERANT STRAIN OF *SACCHAROMYCES CEREVISIAE* FOR INDUSTRIAL APPLICATION

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1. INTRODUCTION

Currently, Brazil is the second ethanol producer worldwide using sugar cane as a fermentative substrate, since its high sucrose concentration is suitable for sugar and ethanol production (AMORIM HV, et al., 2011; WHEALS AE, et al., 1999). The fermentation occurs at approximately 6–8 h with high ethanol yield at the end of fermentation (90–92%) (LOPES ML, et al., 2016; WHEALS AE, et al., 1999). Fermentation occurs between 28 and 35 °C, and the maintenance of this temperature during the summer is only possible through water cooler use in fermentation tanks. Although this cooling process is crucial to maintain the temperature within an ideal range not exceeding 35°C, it is costly and requires large quantities of water (PAULINO DE SOUZA J, et al. 2018). Several strains that naturally adapted to the sugar cane fermentation process used in Brazil were isolated and broadly used in several mills. The producers have mainly employed two industrial yeast strains known as CAT-1 and PE-2 whose genomes were sequenced in 2009 (BASSO LC, et al., 2016; BASSO LC et al., 2011; BABRZADEH F et al., 2012). Although these strains have had a high performance for several years, the constitution regarding Brazilian fermentation substrates has been changing throughout the last years, especially after sugar cane burning was prohibited by law. As a result, there was a lower persistence of these strains in the fermentation tanks during the whole harvest that were then replaced by wild yeasts in several mills in Brazil (PAULINO DE SOUZA J, et al. 2018). Otherwise, the works of COSTA et al. (2019) show that CAT-1 and PE-2 were not able to ferment appropriately above 35 ºC, reducing their viability. Aiming to maintain productivity or even improve the Brazilian fermentation process, our research group has been isolating new strains from distilleries since 2009 to identify new *Saccharomyces cerevisiae* strains that can adapt to the new conditions or that have new characteristics as ethanol, sugar, and thermotolerance (PAULINO DE SOUZA J, et al. 2018). In this work, we demonstrate the identification and physiological and molecular characterization of a new thermotolerant S. cerevisiae strain that is able to grow at 40 °C with 12.5% more sugar conversion when compared to the CAT-1 strain. In addition, LBGA-01 shows more viability than CAT-1 when submitted in stress conditions such as lactic acid (4%) sucrose (30%) and ethanol (16%). Fermentation assays using Chemostats show that LBGA-01 has a superior profile of ethanol yields at 40°C in comparison at 30°C, the values of *q*Ethanol (rate of ethanol formation) were 11.50 and 8.79 respectively. When compared to

studies of PED-2 in Chemostat Fermentation, DELLA-BIANCA 2014 obtained lower values of ethanol yields (7.70) when compared with LBGA-01 at 40°C (11.50). Although the recycle experiments showed a drop in the viability of the LBGA-01 at 40°C, the ethanol production yields (86,03%) still remained high in this stress condition. In the control condition (34°C) the results for LBGA-01 were similar to studies using industrial strains such as PED-2 and Ethanol Red, 87.2 and 87.6% respectively (DELLA-BIANCA, et al. 2014). LBGA-01 strain has also shown important changes in the genes (GPD1, GPD2, ALD6, ALD4, ACS2, MAL31, AGT1, SUC, SNF1, AXT1) expression pattern in pathways involved in fermentation efficiency, which can provide information about its thermotolerant phenotype and associated fermentative performance. In addition, this strain is resistant to the stressors produced during the first and second-generation ethanol production, highlighting the attributes of this strain for employment in high-temperature fermentation, endeavoring improvements in the Brazilian ethanol production chain.

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