

Quantitative physiology and stress tolerance of yeast strains from the industrial production of fuel ethanol in Brazil

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Global interest in the production of fuels from renewable feedstocks has raised dramatically in the past years, due to environmental, economical and strategical issues. Ethanol occupies a central position in this scenario and Brazil has been the leading country in the production of ethanol from sucrose-containing feedstocks. The fermentation step is carried out by the yeast *Saccharomyces cerevisiae*, without complete asepsis and therefore the industrial fermentation vessels represent an environment for the competition among different microorganisms. The main industrial strains, as we currently know, which have been selected along decades of industrial practice, have recently been isolated and characterized in a preliminary manner. However, to our knowledge, no rigorous quantitative physiological characterization of these strains has been made and published in the scientific literature to date. In the present work, the *S. cerevisiae* strains PE-2, CAT-1, BG-1 (isolated from mills located in the south-eastern region of Brazil) and strain JP-1 (isolated from mills located in the north-eastern region of Brazil) were cultivated in a completely defined medium, in parallel with the popular laboratory strains CEN.PK113-7D and S288c, as well as with a strain employed in the industrial production of baker's yeast (Fleischmann strain). The main parameters of microbial physiology, such as specific rates of growth, metabolite formation and substrate consumption, as well as different yields, were determined during exponential growth on glucose as the sole carbon source, at pH 5.0 and temperatures of 30 °C and 37 °C. Furthermore, serial dilution spot assays in the presence of different stressors (oxidative, saline, heat, high ethanol, and low pH), at different levels, were carried out. The results show that JP-1 is the most tolerant strain to heat stress, among all strains investigated here. The industrial Fleischmann strain (baker's yeast) is the most tolerant towards oxidative stress, which was investigated here by the use of hydrogen peroxide. In general, the industrial strains present an improved performance, in terms of growth and ethanol formation, under ethanolic or acid stress, when compared to the laboratorial strains. Acid or low pH stress was the stress factor that mostly distinguished the strains isolated from the industrial production of fuel ethanol from their laboratorial counterparts and also from the industrial baker's yeast strain (commonly employed as starter strain), indicating that this might be the most relevant selective pressure under industrial conditions of fuel ethanol production, as the process is carried out in Brazil. During submerged cultivations, the industrial strains show higher specific growth rates at 37 °C than at 30 °C, a phenomenon that was not observed with the laboratorial strains. The glycerol yield on glucose was higher at 37 °C than at 30 °C for all strains. The ethanol yield on glucose at 37 °C were higher for the industrial strains, when compared to the corresponding parameter calculated for the laboratorial strains. These data indicate that the high temperatures achieved in the industrial fermentation vessels, far above the desired values around 30 °C, has been one of the main variables exerting selective pressure on the yeast strains in this process.

1. Rocha, Saul Nitsche ; Abrahão-Neto, José ; Cerdán, María Esperanza ; Gombert, Andreas Karoly ; González-Siso, María Isabel . Heterologous expression of a thermophilic esterase in *Kluyveromyces* yeasts. *Applied Microbiology and Biotechnology*, v. 89, p. 375-385, 2011.
2. Koetsier, Martijn J. ; Gombert, Andreas K. ; Fekken, Susan ; Bovenberg, Roel A.L. ; van den Berg, Marco A. ; Kiel, Jan A.K.W. ; Jekel, Peter A. ; Janssen, Dick B. ; Pronk, Jack T. ; van der Klei, Ida J. . The *Penicillium chrysogenum* *aclA* gene encodes a broad-substrate-specificity acyl-coenzyme A ligase involved in activation of adipic acid, a side-chain precursor for cephem antibiotics. *Fungal Genetics and Biology*, v. 47, p. 33-42, 2010.
3. Rocha, Saul N ; Abrahao-Neto, Jose ; Cerdan, Maria E ; Gonzalez-Siso, Maria I ; Gombert, Andreas K . Heterologous expression of glucose oxidase in the yeast *Kluyveromyces marxianus*. *Microbial Cell Factories*, v. 9, p. 4, 2010.
4. Basso, Thiago Olitta ; Dario, Marcelo Goulart ; TONSO, Aldo ; Stambuk, Boris Ugarte ; Gombert, Andreas Karoly . Insufficient uracil supply in fully aerobic chemostat cultures of *Saccharomyces cerevisiae* leads to respiro-fermentative metabolism and double nutrient-limitation. *Biotechnology Letters*, v. 32, p. 973-977, 2010.
5. Harris, Diana M. ; Westerlaken, Ilja ; Schipper, Dick ; van der Krogt, Zita A. ; GOMBERT, A. K. ; Sutherland, John ; Raamsdonk, Leonie M. ; van den Berg, Marco A. ; Bovenberg, Roel A.L. ; Pronk, Jack T. . Engineering of *Penicillium chrysogenum* for fermentative production of a novel carbamoylated cephem antibiotic precursor. *Metabolic Engineering*, v. 11, p. 125-137, 2009.

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