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FERMENTATIVE METABOLISM OF YEAST STRAINS UNDER INDUCED STRESS CONDITIONS

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ABSTRACT

Saccharomyces cerevisiae can undergo changes in its replicative pattern and morphology according to the environmental conditions. In the present work, three different yeast strains – S288c, CA11 and PE-2 - were grown in YPD agar and broth, where different stress-inducing compounds were added, including ethanol, 1-butanol, isopropanol, 2-methyl-2-butanol, 2-furaldehyde, 5-hydroxymethyl-furfural and cyclic-adenosine-monophosphate. The three strains showed different fermentation rates, under the same fermentation media, being higher for PE-2 strain. Different fermentation condition also led to different fermentation rates, using the same strain. The presence of 1-butanol and amylic alcohols, as well as furans in the growth media showed to be the most stressful environments for yeasts growth. This was confirmed by the low fermentation rates under these conditions, by the chemical information and in certain cases by morphology changes mainly by hyphae or filaments formation.

INTRODUCTION

The yeast is one of the most studied microorganisms, due to the large ability to adapt, grow and be manipulated under laboratory conditions and to its essential role in fermentation processes in different industrial activities. The fermentation process, despite its massive use in the food industry, cannot be totally controlled. The means of control available are external to the process and are centered primarily in the selection of strains and optimization and control of the process conditions. *Saccharomyces cerevisiae* is a dimorphic yeast with the ability to change its morphology according to the environment. Under stress conditions it can modify from a unicellular to a filamentous form (Casalone et al. 2005), and different strains and/or growth conditions lead to different responses (Palková 2004; Casalone et al. 2005).
In this work, we used three different *S. cerevisiae* strains - S288c, a standard laboratory strain unable to form pseudohyphae (Liu et al. 1996); PE-2, a strain with a high tolerance to ethanol widely used in the industry (Pereira et al. 2011); and Ca-11, a flocculant strain isolated from Brazilian *cachaça* fermentations (Pereira et al. 2010). The three strains were grown in YPD agar (YPDa) and YPD broth (YPDb), in the presence and absence of stress inducers, and compared in terms of fermentation profiles, chemical composition and morphological changes.

MATERIAL AND METHODS

S. cerevisiae strains - S288c, CA11 and PE-2 - were obtained from the microbiological collection of the IBB at the University of Minho. The incubation was performed in YPDb medium (Sigma) during 12 hours at 30 ºC under constant agitation (150 rpm). Fermentations were performed in both solid and liquid medium, where different growth conditions were induced. The three strains were inoculated in the surface of YPD agar media (20 μl), during 96h, and in 50 ml YPDb (10^6 cells/ml), during 24h, without any stress condition (control) and with: 1 % (v/v) ethanol (Riedel-de-Han), 1 % (v/v) isoamyl alcohol (SAFC), 1 % (v/v) 1-propanol (Sigma Aldrich), 1 % (v/v) 2-methyl-2-butanol (Sigma Aldrich) under 30 ºC. Microscopy analysis were performed for morphological changes observation for both solid and liquid medium and YPDb medium fermentations were controlled by optical density, cells concentration, pH and CO_2 production and major fermentation metabolites were measured using HPLC and GC-FID techniques.

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DISCUSSION

Morphological changes

The presence of 1-butanol and the amylic alcohols induced cells morphology changes by pseudo-hyphae and filaments production. Morphological changes were more evident in solid media when compared to liquid.

Fermentation profiles

The three studied *S. cerevisiae* strains showed different fermentation rates under the same fermentation condition (Figure 1a), being higher for PE-2 (1.17 g/L.h), followed by CA11 (0.98 g/L.h) and S288c (0.93 g/L.h), i.e., the industrial strain is more adapted to the used conditions, comparing to the others. The presence of 1-butanol, 2-furaldehyde and 5-HMF showed distinct profiles with lower fermentation rates (Figure 1b),: 0.59, 0.31 and 0.31 g/L.h, respectively, comparing to the rest (≈ 0.9 g/L.h), using S288c.

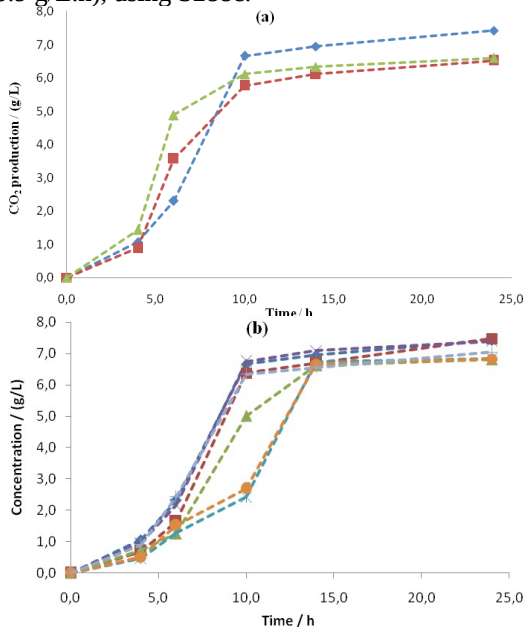

Figure 1: CO_2 production during fermentation in: **(a)** YPDb control, using: (♦) S288c, (■) CA11 and (▲) PE-2; and **(b)** YPDb using S288c in: (♦) control, (■) ethanol, (▲) 1-butanol, (×) isopropanol, (+) 2-methyl-2-butanol, (*) 2-furaldehyde, and (●) 5-HMF.

Chemical information

Singular value decomposition methodology of chemical information obtained by HPLC and GC-FID, confirmed that the presence of 1-butanol, 2-furaldehyde and 5-HMF leads to very distinct behavior. Samples in time 0h of fermentations under stress seem to have similar chemical composition, and different from the control. Fermentation with ethanol seems to approach, (after 10 hours) to the control, throughout fermentation time.

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