TOLERANCE AND ADAPTATION OF A FLOCCULENT STRAIN OF *SACCHAROMYCES CEREVISIAE* TO HIGH ACETIC ACID CONCENTRATIONS

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Introduction. Bioethanol produced from renewable resources, as lignocellulosic biomass from agricultural residues, is an attractive alternative to fossil fuels**.** One of the main fermentation inhibitors generated during the pretreatment and hydrolysis of biomass is acetic acid, which originates from the degradation of hemicellulose and lignin [(1)]. Acetic acid induces inhibition of the fermentation and intracellular acidification. The present study evaluated the tolerance and adaptation of a flocculent strain of *Saccharomyces cerevisiae* to high concentrations of acetic acid in order to generate isolates able to maintain ethanol production in the presence of high concentrations of acetic acid.

Methods. A flocculent *S. cerevisiae* strain isolated from a spent sulfite liquor plant was used. Adaptation to acetic acid was performed by sequential transfer of cultures to fermentation medium with increasing concentration of this acid. Fermentation medium contained, per liter: 1 g yeast extract, 1.6 g $(NH_4)_2SO_4$, 0.5 NaCl, 1.4 KH_2PO_4 and 20 g glucose. Colony morphology of adapted strains was observed in WL nutrient medium. Glucose and ethanol were determined in a YSI 2700 biochemical analyzer, acetic acid with an enzymatic kit (R-Biopharm) and biomass by cell dry weight determination.

Results. The effect of acetic acid on the non-adapted strain was studied (Table 1). Ethanol production was affected from 2.5 g/L of acetic acid. From 7.5 g/L of this acid, ethanol production was strongly inhibited and almost no ethanol was produced above 10 g/L. Interestingly, this strain consumed significant amounts of acetic acid. However, consumption of acetic acid decreased at increasing concentrations of this acid.
The parental strain was sequentially adapted to 2.5, 5 and 7.5 g/L of acetic acid. Cultures were spread onto WL nutrient medium and different colony morphologies were observed at the three concentrations of acetic acid tested (Fig. 1). The WL solid medium contains bromocresol green, a pH indicator. Yellow color indicates pH below 3.8 while green color indicates pH 3.8 to 5.4.

Table 1. Effect of acetic acid (AA) on specific growth rate, ethanol (ETOH) production and glucose (GLC) consumption.

Initial AA (g/L)	μ *(h^{-1})	Produced ETOH (g/L)	Consumed GLC (g/L)**	Consumed AA **(%)
0	0.31± 0.20	12.85 ± 0.30	19.85 ± 0.30	NA
2.5	0.28 ± 0.20	7.74 ± 0.40	11.19 ± 0.20	67.2
5	0.26 ± 0.20	6.95 ± 0.20	10.5 ± 0.20	59
7.5	0.25 ± 0.20	3.79 ± 0.30	9.48 ± 0.20	58.4
10	0.16 ± 0.20	1.80 ± 0.40	8.65 ± 0.40	55
12.5	0.16 ± 0.40	0.51 ± 0.10	7.20 ± 0.30	45.6
15	0.16 ± 0.10	0.43 ± 0.10	6.85 ± 0.50	34

All the adapted strains were able to convert glucose almost stoichiometrically to ethanol in the presence of 2.5, 5 or 7.5 g/L of acetic acid after 5 days of incubation, except a strain presenting the c) morphology which only converted half of the glucose to ethanol.

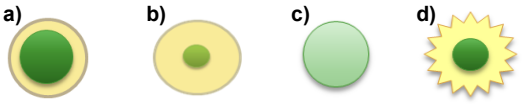

Fig.1 Colony morphologies observed after adaptation.

Conclusions. Low ethanol yields on glucose were observed in the presence of acetic acid for the parental strain. Adaptation to increasing concentrations of acetic acid resulted in evolved populations able to produce stoichiometric amounts of ethanol at high acetic acid concentrations.

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