ALCOHOL PRODUCTION BY WILD STRAINS *Saccharomyces cerevisiae* AND *Candida stellata* ISOLATED FROM TEQUILA FACTORIES

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Introduction. The lignocellulosic biomass is very profuse in nature and it has a great potential to produce a new generation of liquid fuels by fermenting the sugars contained in the cellulose and hemicellulose fractions (1). For the process of obtaining bioethanol to be economically feasible it requires that microorganisms have the ability to ferment most of the sugars present in the substrate (2).
The aim of this research was to establish the more suitable conditions for glucose fermentation in synthetic medium and to obtain a preliminary profile of carbon source assimilation.

Materials and methods. As part of the yeast physiological characterization a 2^3 factorial design was performed on YPD culture broth (20 g/L glucose, 10 g/L yeast extract, and 20 g/L peptone of casein). A preliminary test of sugars assimilation, based on the optical density measurement, was done in YNB minimal medium. The statistical analysis was done using the Statgraphics Centurion XV software.

Results and discussion. MG and L4B yeasts were identified as *Saccharomyces cerevisiae* and *Candida stellata*, respectively. The average values of both growth and alcohol production kinetic parameters, at better strains conditions, are show in Table 1.

Table 1. Growth and production parameters of *S. cerevisiae* MG and *C. stellata* L4B strains, at better conditions

Strain	Dry weight (g/L)	μmax (h^{-1})	DT (h)
MG	2.77	0.403	1.73
L4B	4.55	0.573	1.22
Strain	**Ethanol (g/L)**	**Pmax (g/L.h)**	**Efficiency (%)**
MG	9.33	0.884	94.9
L4B	9.24	1.232	99.0

The best growth conditions for both strains were 30°C, 5.5 pH, and 250 rpm. Maximal alcohol production was achieved at 40°C, a pH value of 4.5, and 250 rpm for the L4B strain whereas the MG strain required no agitation. According to the obtained results and the statistical analysis it was shown that the factors temperature, agitation, pH, and their interactions had a significant effect on the growth and production responses.

The Pmax Pareto chart shows that for the MG strain (Fig. 1 A), the temperature exerted the greatest influence; while for the L4B strain agitation and temperature, in that order, were the strongest influencing factors (Fig. 1 B).

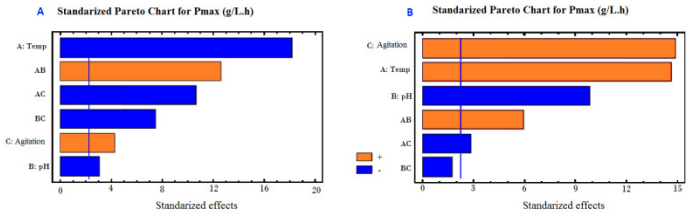

Fig. 1. Pareto chart for maximum productivity for *S. cerevisiae* MG (A) and *C. stellata* L4B (B).

Regarding the carbohydrate assimilation profile, it was observed that both strains preferentially assimilate sucrose (Fig 2A and 2B), although growth of the L4B strain in this source is statistically equal to that achieved in fructose, glucose, and mannose (Fig 2B). Both MG and L4B strains grew poorly on cellobiose and arabinose, another less assimilated sugar by the MG strain was manosse, and for the L4B strain was rhamnose.

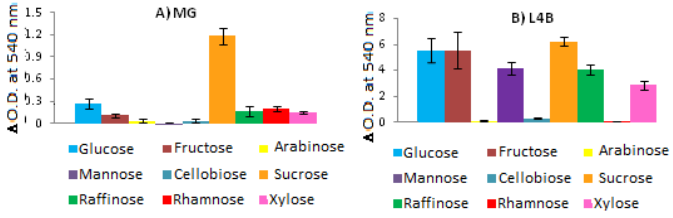

Fig. 2. Carbohydrate assimilation preliminary profile of yeasts *S. cerevisiae* MG (A) and *C. stellata* L4B (B).

The order of sugar preference for the L4B strain was: sucrose, glucose, fructose, mannose, raffinose, and xylose. While for the MG strain was sucrose, glucose, rhamnose, raffinose, xylose, and fructose.

Conclusions. All factors tested had a significant effect on the growth and yield parameters for both strains. Under the best conditions for each strain there was a significant difference in the maximum productivity. The L4B strain produced alcohol faster than MG, and additionally, this strain was capable to growth abundantly in 6 of the 9 substrates tested, compared to the MG strain.

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