



STRESS RESISTANCE OF SACCHAROMYCES AND NON-SACCHAROMYCES STRAINS OBTAINED FROM MEZCAL

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Introduction. During fermentation process, yeast cells are subjected to several fluctuating stress conditions, such as changes in osmolarity, temperature, oxidative stress and pH (1), being the main effect of these stresses the inhibition of both growth and different transport systems that combined with nutrient depletion contributes to possible fermentative problems (stuck fermentations).

Accordingly, in this study we assessed the resistances to osmotic (fructose), ethanol and temperature stresses of 6 strains of *Saccharomyces* (2) and 12 strains of non-*Saccharomyces* yeasts obtained from mezcal from Tamaulipas, and named as LCBG-code, plus the fructophilic control strain Fermichamp.

Methods. The ethanol (0, 2, 5, 8, 9, 10, 11, 12, 13, 14 and 15% v/v) and temperature (10, 15, 20, 30 and 40 °C) resistances were determined by formation of colonies on solid YPD medium inoculated by micro-drop. For osmotic (high fructose concentration) stress analysis, the base agar medium (YP) supplementing with increasing concentrations of fructose (2 to 90 % (w/w) and was analyzed as the logarithmic growth obtained at each condition.

Results. In the case of ethanol tolerance at different carbon sources. for the Saccharomyces cerevisiae group (Table 1), the strains could belong to any of three groups, being 15 % maximum ethanol concentration tolerated by this specie plus the strain Fermichamp; the control non-Saccharomyces strains tolerated up to 8 % ethanol, and some non-Saccharomyces strains were highly sensitive and only tolerated up to 5 % ethanol (data not shown). Also, due to the observed maximum inhibitory behavior observed when ethanol and fructose were present in the medium the maximum tolerance to the latter was assessed also in plates containing increasing concentrations of fructose. As can be observed (Fig. 1), for a group of contrasting S. cerevisiae strains, they showed a wide range of growth inhibition by fructose.

Table 1. Maximal ethanol tolerance with different carbon
sources of some of the S. cerevisiae mezcal strains
evidenced by their capacity to grow (data in log base)

Strain	Maximum ethanol (% EtOH, v/v) with quantificable growth		
	YPD-EtOH	YPD-EtOH	YPD-EtOH
	(5%)	(8%)	(15%)
Fermichamp	7.04	6.08	5.52
LCBG-3Y4	7.03	6.71	5.33
LCBG-3Y8	7.03	6.54	5.22
LCBG-3Y5	7.00	6.77	5.65
LCBG-3D4	7.04	6.36	4.20

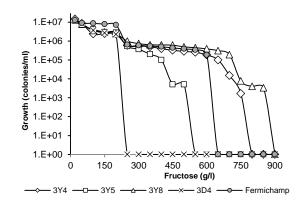


Fig.1 Cell viability as a response to increasing fructose concentrations for contrasting *S. cerevisiae* strains.

Conclusions. The wide stress resistances showed by the mezcal yeasts, not only among species but also intraspecifically reflect the high phenotypic diversity and complexity of this traditional fermentation, and confirms its importance as a source of interesting novel strains.

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References.

1. Gibson BR, Lawrence SJ, Leclaire JPR, Powell CD, Smart KA. (2007). *FEMS Microbiol Rev* 31(): 535-569.

2. Oliva Hernández AA., Taillandier P, Reséndez Pérez D, Narváez Zapata JA, Larralde Corona CP. (2013). *Antonie van Leeuwenhoek J Microbiol* 103(1): DOI 10.1007/s10482-012-9865-1