# STUDY OF FERMENTATION INHIBITORS TOLERANCE OF YEASTS ISOLATED FROM MEXICAN ECOSYSTEMS

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**Introduction.** Second-generation bioethanol production requires fermenting organisms able to convert lignocellulose-derived sugars to ethanol in the presence of inhibitory compounds released during biomass pretreatment and hydrolysis. Fermentation inhibitors mainly include weak acids, furans and phenolic compounds. *Saccharomyces cerevisiae* has traditionally been used in the industrial production of ethanol from sugarcane and starch. *S. cerevisiae* is unable to ferment pentose; however, both laboratory and industrial strains of *S. cerevisiae* have been successfully genetically engineered to ferment pentoses. In this study, the tolerance of autochthonous yeasts, isolated from henequen and mezcal musts (Table 1) to lignocellulose-derived fermentation inhibitors was investigated.

**Methods.** 96 wells microplates were used to perform yeasts anaerobic growth in YNB medium containing 10 g/L of glucose [(1)]. Initial pH was adjusted to 5.5 and growth was monitored by $OD_{595\ nm}$ measurements. The inhibitor cocktail was added to achieve initial concentrations of 100, 75, 50 and 25% (v/v). A 100% concentration consisted of 3.5 g/L formic acid, 4.5 g/L acetic acid, 2.9 g/L furfural, 3.8 g/L 5-hydroxymethylfurfural, 0.15 g/L cinnamic acid and 0.18 g/L coniferylaldehyde [(2)].

**Table 1.** Yeasts isolates tested in this work.

| Isolate | Procedency |
|---|---|
| *S. cerevisiae* C110 | Henequen |
| *S. cerevisiae* D135A5 | Mezcal |
| *S. cerevisiae* D145A13 | Mezcal |
| *Zygosaccharomyces bisporus* D14FA | Mezcal |
| *S. cerevisiae* M13SA2 | Mezcal |

**Results.** *S. cerevisiae* C110 and D135A5 showed the highest tolerance and presented the same final $OD_{595\ nm}$ in the presence of 0 to 100% of the cocktail. *S. cerevisiae* D145A13 growth was affected from a 75% cocktail concentration. *Z. bisporus* D14FA growth decreased with cocktail concentration above 50%. *S. cerevisiae* M13SA2 seemed to be less tolerant and presented a decreased growth from a 50% concentration. In the presence of only furans, the isolates generally reached the same final $OD_{595\ nm}$ at all concentrations tested. However longer lag phase and decreased growth rate were detected. For only phenolic compounds, the isolates reached lower $OD_{595\ nm}$ at all concentrations tested (Table 2) and also showed a longer lag phase and decreased growth rate. The isolates were practically unaffected by acids.

**Table 2.** $OD_{595\ nm}$ after 10 h of growth in the presence of phenolic compounds.

| Isolate | Concentration of phenolic compounds (%) | | | | |
|---|---|---|---|---|---|
| | 0 | 25 | 50 | 75 | 100 |
| C110 | 0.9 | 0.8 | 0.5 | 0.5 | 0.35 |
| D135A5 | 0.95 | 0.7 | 0.5 | 0.35 | 0.3 |
| D145A13 | 1.2 | 0.7 | 0.55 | 0.55 | 0.3 |
| D14FA | 1.3 | 1.2 | 0.8 | 0.5 | 0.35 |
| M13SA2 | 0.9 | 0.6 | 0.4 | 0.33 | 0.3 |

**Conclusions.** The isolates showed significant differences in inhibitor tolerance and growth inhibition by classes of inhibitors. Phenolic compounds were the most toxic, followed by furans. Isolates probably adapted or detoxified furans during growth. The acids presented almost no effect in the tested concentrations. The most tolerant isolates could be used as starting material to develop genetically engineered strains for the production of second-generation bioethanol.

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