



## MOLECULAR IDENTIFICATION OF YEAST SPECIES ASSOCIATED WITH THE PRODUCTION OF TEQUILA

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**Key words:** yeast species, molecular identification, tequila

**Introduction.** The process of production of tequila involves 4 major steps: cooking, grinding, fermentation with yeast and distilled agave juice. Fermentation is performed with yeast of *Saccharomyces cerevisiae* or sometimes it performs a natural fermentation or spontaneous without an inoculum. Despite being a very important economical activity, there is little information on the different yeast species associated with this fermentation.

The objective of this work is the molecular identification of strains isolated from tequila environments.

**Methods.** Yeast strains were isolated from fields or agave juice of *Agave tequilana* Weber var. azul of Jalisco state. The molecular identification of the strains was carried out by means of the RFLPs of the ITS-5.8S genetic region (1). In some cases, to confirm the correct identification the sequence was compared with those deposited in the type strains.

**Results.** The species with the sizes of the PCR products and their patterns of restriction with enzymes *Hha* I, *Hae* III and *Hinf* I of the isolated yeast are shown in the Table 1. The patterns of restriction were compared (<http://www.yeast-id.com/>), and found that correspond to *Candida ethanolica*, *Clavispora lusitanae*, *Issatchenkia orientalis*, *Kluyveromyces* spp., *Pichia guilliermondii*, and *Saccharomyces cerevisiae*.

The yeast species *Clavispora lusitanae*, *Kluyveromyces* spp., *Candida ethanolica* and *Saccharomyces cerevisiae* have been isolated from tequila environments, fresh cooked or fermented plants of *Agave tequilana* for the production of tequila (2, 3) or *Agave salmiana* for the production of mezcal (3, 4, 5). On the other hand, *I. orientalis* and *P. guilliermondii* have been isolated from Togwa, a fermented food of Tanzania, sourdough bread, fermented fruit, but not reported in a tequila environment.

The frequency of the restriction patterns of the yeast species is shown in Fig. 1.

Table 1. Nucleotide fragment length of 5.8S-ITS profiles of yeast isolated from tequila.

Species	Ap (pb)	Hha I	Hae III	Hinf I
<i>C. lusitanae</i>	380	220+80	380	190+180
<i>Kluyveromyces</i> spp.	700	280+190+180+80	620+70	270+190+130+50
<i>S. cerevisiae</i>	850	375+340+130	320+230+175+125	380+370+120
<i>I. orientalis</i>	500	200+180+50	360+75	230+160+140
<i>P. guilliermondii</i>	600	290+250	390+120+60	300+275
<i>C. ethanolica</i>	450	150+90+80+50	320+90	240+190

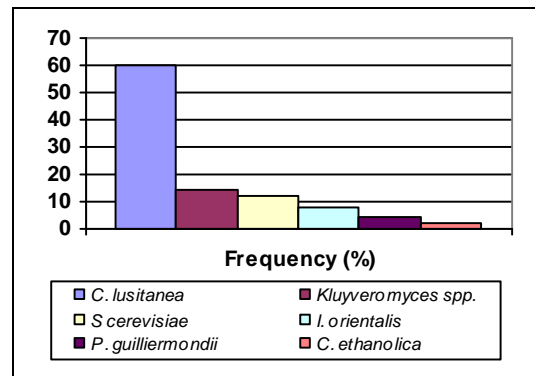


Fig.1 Frequency (%) of the yeast species isolated from a tequila environment.

**Conclusions.** The predominant species of yeast in a tequila environment was *Clavispora lusitanae*.

**Acknowledgements.** This work was supported by a grant from COECYTJAL-UDG 2010 (05-2010-1-979). J. L. acknowledges a fellowship "alumnos sobresalientes" from UDG.

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