



MOLECULAR IDENTIFICATION OF YEAST STRAINS FROM A TEQUILA FERMENTATION PROCESS

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Introduction. Tequila is a Mexican distilled beverage obtained from Agave. Yeasts from the fermentative process are responsible for the properties of the final product (1). The identification of yeast strains can be made based on the sequence of the internal transcribed spacer (ITS) region, which has the highest degree of variation from the DNA (2). The aim of this study was to identify and compare nine yeast strains from tequila fermentation by amplification and sequencing of the ITS-1 region of DNA.

Methods. Nine yeast strains isolated from tequila fermentative process were analyzed. DNA was isolated and amplified by PCR reaction using ITS1 and ITS4 primers (3). The PCR product was cloned using pGEM®-T Easy Vector Systems (Promega) and *E coli* DH5 α (Promega). DNA sequencing was performed in UAM-Iztapalapa. Sequences obtained were compared using NCBI database.

Results. Agarose gel with DNA bands correspondent to the amplified ITS region from 800-1000 bp. are shown in Fig. 1.

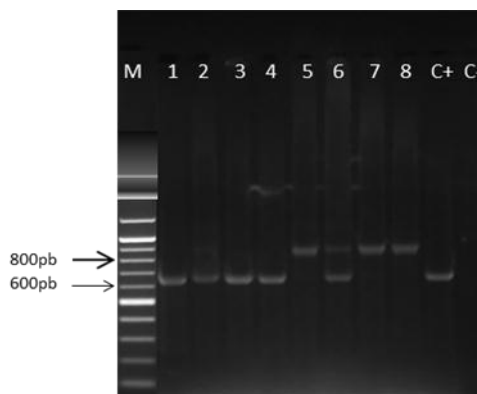


Fig.1 2% Agarose gels. MM: 100bp. ladder. 1 -10: Amplified ITS regions from yeast strains. c+: Positive control. C-: Negative control.

ITS sequences analyzed using BLAST from the NCBI data base are showed in Table 1. Strains 5, 7 and 9 were *Saccharomyces cerevisiae*, a commonly found yeast during Tequila production process (1). While strains 1, 2, 3, 4, 6 and 10 corresponded to *Meyerozyma guilliermondii* which is synonym of *Pichia guilliermondii* (4), a model organism widely used in fermentation process for xylitol production (5)

Table 1. Summary of the BLAST analysis results.

strain	organism	Length (bp)	% similarity
1	<i>M. guilliermondii</i>	574	99
2	<i>M. guilliermondii</i>	570	99
3	<i>M. guilliermondii</i>	568	99
4	<i>M. guilliermondii</i>	568	99
5	<i>S. cerevisiae</i>	571	100
6	<i>M. guilliermondii</i>	293	99
7	<i>S. cerevisiae</i>	434	99
9	<i>S. cerevisiae</i>	390	99
10	<i>M. guilliermondii</i>	586	99

Conclusions. The yeast isolated from tequila fermentative processes showed a low diversity. Besides, this is the first report from the tequila fermentation process where *M. guilliermondi* has been identified by molecular techniques.

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