ISOLATION AND IDENTIFICATION OF YEASTS FROM SWEET POTATO POZOL, A TRADITIONAL FERMENTED MEXICAN BEVERAGE

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Introduction. Pozol is a traditional, non-alcoholic, fermented maize beverage prepared in Southeastern Mexico. Different microorganisms have been isolated from this spontaneous fermentation, including lactic acid and non-lactic acid bacteria as well as fungi and yeasts [(1)]. The maize is soaked and cooked in an alkaline solution to remove the pericarp, stone-grounded, shaped into balls, wrapped into banana leafs and allowed to ferment for 0.5 to 4 days. It is sometimes supplemented with cooked sweet potato, cocoa or chili. Cooked sweet potato has a high carbohydrate, minerals and vitamins content. Here, yeasts were isolated from sweet potato pozol and preliminarily identified by sequencing of their internal transcribed spacers (ITS) regions.

Methods. The samples were obtained from 20-24 hours fermented sweet potato pozol purchased from three different producers in the rural market of Macuspana, Tabasco. Yeasts and molds were first grown in PDA medium. Yeasts were then purified by repeated streaking in WL nutrient agar (Fluka). Genomic DNA of the isolates was extracted and purified using the ZR Fungal/Bacterial DNA MiniPrep kit (Zymo Research). The ITS1 and ITS4 primers were used to amplify the ITS of the isolates. A preliminary screening of the isolates was performed by Restriction Fragment Length Polymorphism (RFLP) with the restriction endonucleases *Hae*III and *Hin*fI [(2)]. Selected PCR products were then sequenced and compared using Blast at NCBI.

Results. Eleven different yeast isolates were selected in WL solid medium and designated Pz1, Pz2, Pz3, Pz4, Pz5, Pz6, Pz8, Pz9, Pz10, Pz11 and Pz13. In this medium, different strains present variations in colonies shape, size, edges and color. The medium contains bromocresol green which is taken up differently by different strains. As a consequence, colonies vary in color from white/cream through pale green to deep green. Color distribution inside the colonies also varies. Bromocresol green is yellow when the pH is 3.8 or below and green if the pH is greater than 3.8 and less than 5.4. The obtained RFLP patterns are shown in Figure 1.

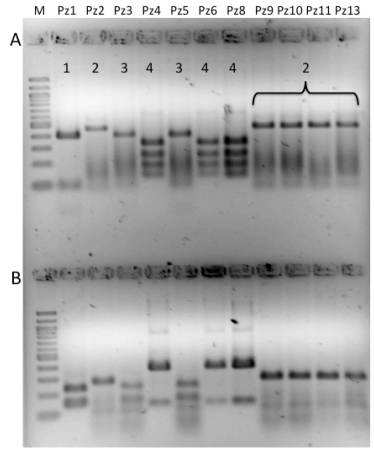

Fig.1 RFLP patterns of the isolates obtained with *HaeIII* (A) and *Hin*fI (B). M is the 100 bp molecular weight marker.

RFLP pattern 1 corresponded to isolate Pz1; pattern 2 to Pz2, Pz9, Pz10, Pz11 and Pz13; pattern 3 to Pz3 and Pz5 and pattern 4 to Pz4, Pz6 and Pz8. Isolates corresponding to pattern 1 and 3 were 99 and 98% similar to *Pichia kudriavzevii*, respectively. Isolates from group 2 were 99% similar to *Candida tropicalis* and isolates from group 4 were distantly related to *Saccharomyces cerevisiae* (88%).

Conclusions. Yeasts as *P. kudriavzevii* [(3)] and *C. tropicalis* [(4)] have been identified as important for lignocellulosic bioethanol production since they are able to ferment both hexoses and pentoses sugars. *S. cerevisiae* is an industrial important yeast. The isolates obtained in this study should be further characterized in order to confirm their correct identification, especially for those distantly related to *S. cerevisiae*. Their biotechnological potential should be evaluated.

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References.

1. Wacher C, Cañas A, Bárzana E, Lappe P, Ulloa M, Owens JD (2000) Food Microbiol. (17): 251-256.
2. Esteve-Zarzoso B, Belloch C, Uruburu F, Querol A (1999) Int. J. Syst. Bacteriol. (49): 329-337.
3. Chan GF, Gan HM, Ling HL, Rashid NA. (2012) Eukaryot. Cell 11(10): 1300-1301.
4. Huang CF, Jiang YF, Guo GL, Hwang WS. (2011) Bioresour. Technol. 102(3): 3322-3329.