XIII Congreso Nacional de Biotecnología y Bioingeniería
SIPAL
VII Simposio Internacional de Producción de Alcoholes y Levaduras

BACTERIAL DIVERSITY DURING THE FERMENTATION OF PULQUE, A MEXICAN TRADITIONAL NON-DISTILLED ALCOHOLIC BEVERAGE: DISCOVERY OF NEW MICROORGANISMS WITH BIOTECHNOLOGICAL PERSPECTIVES.

Adelfo Escalante[1], María Elena Rodríguez[1] and Martha Giles-Gómez[2].
[1]Departamento de Ingeniería Celular y Biocatálisis. Instituto de Biotecnología, Universidad Nacional Autónoma de México (UNAM). Av. Universidad 2001. Col. Chamilpa. Cuernavaca, Morelos. México. [2]Departamento de Biología, Facultad de Química, UNAM. Ciudad Universitaria, Coyoacán. México D. F. México.
adelfo@ibt.unam.mx

Keywords: Pulque, bacterial diversity, polyphasic approach

Pulque is a traditional Mexican alcoholic fermented beverage produced from the sap known as aguamiel, which is extracted from several species of maguey (*Agave americana, A. atrovirens, A. ferox, A. mapisaga, A. salmiana*). This beverage is currently produced and consumed mainly in the central states of Mexico. For its production, freshly collected aguamiel is transported traditionally in wood barrels or in bags made from young goat skins and transferred into large barrels where fermentation takes place. The fermentation process is begins by the addition of the seed (a portion of previously produced pulque). Fermentation time varies from a few hours to overnight, depending if the sap is collected at daybreak or at dusk. Traditionally, development of viscosity due to exopolysaccharide (EPS) synthesis has been the main criteria to determine the degree of fermentation (fresh or mature pulque). The final product is placed in wood barrels and distributed daily for sale and consumption, without the addition of any preservatives. The entire process is performed under non-aseptic conditions, therefore the mixture of microorganisms involved in the fermentation process are those naturally occurring in aguamiel and those incorporated during its collection, transport, inoculation and manipulation. Pulque production consist of three types of fermentations: acidic, alcoholic, and viscous, making this traditional beverage an interesting environment were microorganisms or genes with potential biotechnological applications, such as those encoding sugar transporters, hydrolytic enzymes, EPS, lactic acid or ethanol production, could be isolated (1).

Several studies have been performed to characterize the microbial diversity of aguamiel and pulque samples using traditional culture dependent and identification methods. Important industrial microorganisms isolated from various pulque samples, comprise several yeasts, such as the ethanol producing *Saccharomyces cerevisiae* and species of *Kluyveromyces* producers of inulinase. Isolated bacterial species include the alcohol producing *Zymomonas mobilis* and the dextran producing lactic acid bacteria (LAB) *Leuconostoc mesenteroides*. Among them, *S. cerevisiae, L. mesenteroides* and *Z. mobilis* have been proposed as essential microorganisms for the pulque fermentation process.

In Mexico, interest in alcoholic beverages obtained from Agave has increased in recent years due to their acceptance both in local and international markets. Among these beverages, tequila and in a minor proportion mezcal are of particular importance due to their economical impact and the complex microbial process involved in their production; therefore, it is possible to foresee that some of these traditional fermentation processes could become industrialized. In this work we present a polyphasic approach to study bacterial diversity of pulque and two important biotechnological applications: (A) Analysis of 16S rDNA clone libraries generated from total DNA of the bacterial community present in pulque samples collected in three different production locations in central Mexico. Clonal types were screened and grouped, on the basis of amplified 16S rDNA restriction analysis (ARDRA). 16S rDNA sequence analysis of unique ARDRA groups led us to identify for the first time bacterial groups not previously detected in pulque (1). (B) The bacterial community present in aguamiel and its dynamics during the fermentation of pulque using cultured and non-cultured dependent methods. The microbiological study was correlated with the sucrose, glucose, fructose and fermentation products concentration, as well as medium rheological behavior and scanning electron microscopy analyses in aguamiel and during pulque fermentation (2). (C) Isolation and characterization of two new EPS produced by the LAB *Leuconostoc citreum* and (D) Isolation and characterization of new LAB with potential probiotic capabilities.

This work was supported by Grants P46052-Z from the Consejo Nacional de Ciencia y Tecnología (CONACYT), México and PAPIIT-UNAM IN-205005-2 and IN213508.

References:

1. Escalante A, Rodríguez ME, Martínez A, López-Munguía A, Bolívar F, Gosset G. Characterization of bacterial diversity in Pulque, a traditional Mexican alcoholic fermented beverage, as determined by 16S rDNA analysis. (2004) *FEMS Microbiol Lett*. 235: 273-279.
2. Escalante A, Giles-Gómez M; Hernández G; Córdova-Aguilar MS, López-Munguía A, Gosset G, Bolívar B. Analysis of bacterial community during the fermentation of pulque, a traditional Mexican alcoholic beverage, using a polyphasic approach. (2008) *Int. J Food Microbiol*. 124:126-134.