# ANALYSIS OF THE BACTERIAL DIVERSITY PRESENT IN AGUAMIEL AND PULQUE SAMPLES FROM THE MIXTEC OAXACAN HIGHLANDS

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**Introduction.** Analysis of the microbial diversity in traditional fermented foods such as fermented beverages, doughs and vegetables has revealed the presence of a remarkable lactic acid bacteria (LAB) diversity involved in the development of their characteristic sensorial properties. This allows the possibility to identify desired or non-desired microorganisms in order to improve the production process or final sensorial properties. Pulque is a traditional Mexican fermented, non-distilled alcoholic beverage produced by the fermentation of the sap (aguamiel), extracted from several maguey (Agave) species produced mainly in the Mexican Central Plateau. Pulque is probably the oldest and most traditional Mexican alcoholic beverage, prepared and consumed since pre-Hispanic times. Due to its great historical, religious, social, medical, and economical importance it is the most widely studied beverage from the anthropological and scientific points of view (1, 2). Studies on the bacterial diversity present in pulque samples from different geographical origin have revealed the presence of remarkable LAB diversity, including several non-previously reported species (3). However, these studies have been performed in pulque samples from the Mexican Central Plateau.
The aim of this contribution is to explore the bacterial diversity present in pulque samples from Oaxaca State, México.

**Methods.** Aguamiel and pulque samples were collected from three local producers in the Municipality of Villa de Tamazulapan del Progreso, Oaxaca State (17° 40′ 0″ N, 97° 34′ 0″ W). Aliquots of each sample were plated on standard count plate agar, MRS plates under aerobic and anaerobic conditions, and MRS plates supplemented with 2% sucrose (APTS) (1). Grown colonies were isolated, purified and maintained on 25% glycerol at -70°C. Total DNA was extracted and used for PCR amplification of 16S rDNA (1.5 kb); ARDRA fingerprinting, identification of unique ARDRA types and sequencing of 16S rDNA were performed as reported previously (3). Obtained sequences were identified by comparing against the non-redundant GenBank database.

**Results.** Identity obtained by 16S rDNA sequence comparison against the non-redundant GenBank database of isolated microorganisms for each sampled site is shown in Table 1. All analyzed sequences showed sequence identity against the closest match in the database between 95% to 100%, with the exception of two isolates identified as *Leuconostoc mesenteroides* from sample 2, which showed 91 % of sequence identity.

**Table 1.** Identity based on 16S rDNA sequence analysis of isolated bacteria from three producers

| Identity (Genus) | Number of isolated microorganisms | | | | | |
|---|---|---|---|---|---|---|
| | Sample 1 | | Sample 2 | | Sample 3 | |
| | AM | PQ | AM | PQ | AM | PQ |
| *Bacillus* | 1 | 3 | 0 | 1 | 1 | 0 |
| *Lactobacillus* | 2 | 0 | 3 | 0 | 0 | 0 |
| *Lactococcus* | 1 | 0 | 0 | 0 | 1 | 0 |
| *Leuconostoc* | 12 | 3 | 3 | 6 | 2 | 2 |
| *Staphylococcus* | 1 | 2 | 5 | 2 | 0 | 0 |
| *Uncultured bacteria* | 0 | 1 | 1 | 0 | 0 | 0 |

AM = Aguamiel, PQ = Pulque.

Bacterial diversity detected in samples collected from three analyzed samples showed the presence of Gram-positive bacteria including *Bacillus, Staphylococcus* and a remarkable diversity of homofermentative and heterofermnetative LAB. Most abundant LAB detected among analyzed samples was *Leuconostoc mesenteroides.* Interestingly, an isolated strain with a closest sequence match with a non-cultivated bacteria was detected both in sample 1 (pulque) and sample 2 (aguamiel).
Obtained results shown that *L., mesenteroides* was the most abundant LAB detected analyzed samples. This result was contrary to that obtained previously for the analysis of the bacterial diversity of pulque samples from the Central Mexico Plateau, showing that *L. mesenteroides* was not one of the most abundant LAB present in the analyzed samples (2).

**Conclusions.** In this contribution we report for the first time the bacterial diversity present in pulque samples from different producers indicating the presence of a common conserved bacterial diversity conformed by Gram-positive bacteria. Among them, *L. mesenteroides* was identified as the most abundant LAB.

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