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The Artisanal Production of Pulque, a Traditional Beverage of the Mexican Highlands

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Abstract Pulque is a traditional fermented alcoholic, acidic, viscous drink of the Mexican central highlands. Its production from the "aguamiel" (sap) of agave plants dates back ~1,500 years and continues to be made by artisanal methods. However, the variability of pulque's quality and its instability hamper its widespread distribution and consumption. Microbiological surveys of pulque from three ranches revealed tremendous variability in the types and quantity of the indigenous microbiota. The population of lactic acid bacteria ranged from 6×10^7 to 2×10^{11} CFU/mL. This variability might be attributed to the conditions on the ranches where the pulque was made. In an attempt to identify these sources of variability, the microbial populations of aguamiel and pulque from a single agave plant were determined. Surprisingly, the population size of the "unfermented" aguamiel and the pulque converged by the end of 3 weeks. The potential use of bacteriocinogenic LAB and known starter cultures to improve pulque properties are discussed.

Keywords Aguamiel · Pulque · Fermented beverage

Introduction

Pulque is an alcoholic drink produced in the mountainous regions of central Mexico (Fig. 1) by fermenting the sap of an agave plant. The consumption of this indigenous drink, which is acidic, alcoholic, and viscous, may go back to the Olmec times of 2,000 B.C.E, but certainly dates back to ~500 C.E. [3, 6]. These societies consumed it in religious ceremonies, for its nutritional and health-promoting properties, as well as for its alcoholic content. Pulque consumption peaked in the middle of the twentieth century at 500 million liters per year [9]. This has dramatically declined in the last 50 years for many reasons, including pulque's perception as a low-class, variable-quality beverage consumed primarily by the elderly. Pulque is now produced and consumed primarily in poor rural areas where wild agave cacti are an abundant, but largely untapped natural resource. Fortunately, the consumption of pulque is making a resurgence; it is served by many pulquerias in Mexico City that are frequented by young "hip" "trend-setting" people [2].

Many species of agave are used to produce pulque. When the plant is 5–10 years old, the floral bud is cut off by carving a hole in its central stem. This creates an inner cavity having a capacity of ~2 L (Fig. 1). The cavity surface is scrapped to facilitate the production of the liquid aguamiel sap. The cavity is "sealed" with rocks, leaves, or plastic bags to provide some degree of protection from animals, insects, and an environment that is otherwise septic. Farmers collect the aguamiel twice a day. The aguamiel is rich in sucrose, fructose, glucose, and polyfructans. Its fermentation to pulque takes 12–24 h.

Aguamiel is fermented to pulque in wood, clay, or more commonly, in plastic containers. The fermentation can be initiated by the resident microbiota, using a procedure similar to that of a fed-batch fermentation; aguamiel is added to the pulque barrel to maintain a constant volume as pulque is removed for consumption. Alternatively, the pulque from a previous batch is added to the fermentation

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vessel as inocula for the next batch. This later procedure is commonly referred to as "back-slopping" and may result in the selection of the strain most suited for the fermentation [9]. The fermentation takes place in the ranch environment with minimal process controls and at ambient temperatures ranging from 25 to 50 °C. It is complete in 12–24 h with a final alcohol concentration ranging from 3 to 6 %.

Pulque is fermented by the indigenous microorganisms associated to the sap of the agave plant. The diversity of microorganisms associated with pulque is complex [7, 8]. Pulque is a relatively stable fermentation product, which is not easily attacked by fungi and other bacteria; this may be due to the alcohol and acids in the pulque. Their production is attributed to *Zymomonas mobilis* and lactic acid bacteria (LAB), respectively. Sanchez-Marroquin [11] developed an effective starter culture consisting only of *S. cerevisiae, Z. mobilis, Lactobacillus* spp., and *L. mesenteroides* [5]. Interestingly, the first isolation of *Zymomonas mobilis* as a new bacterial species was from pulque [13]. *Z. mobilis*

Fig. 1 (*Clockwise*) The location of Tamazulapan (http://www.wikipedia.com); a farmer opening an agave plant; the cavity where aguamiel accumulates; and pulque fermenting in a clay jug

Table 1 Comparison of the microbial levels at three sites in the State of Oaxaca, as enumerated by culture methods using *Lactobacillus* MRS agar for lactic acid bacteria, MRS + 2 % sucrose for *Zymomonas* sp, and standard plate count agar for total counts

Type of bacteria and menstrum	Rancho San Jerónimo (CFU/mL)	Rancho Los Arcos (CFU/mL)	Rancho La Plazuela (CFU/mL)
Aguamiel			
Lactic acid bacteria (aerobic)	6.2×10^7	3.7×10^9	4.5×10^8
Lactic acid bacteria (anaerobic)	1.8×10^{10}	2.3×10^{10}	1.0×10^8
Zymomonas sp	3.5×10^8	6.8×10^9	n.d.
Total aerobic count	1.9×10^{11}	2.1×10^9	n.d.
Total anaerobic count	4.0×10^8	2.5×10^{10}	$>3.0 \times 10^{10}$
Pulque			
Lactic acid bacteria (aerobic)	7.1×10^7	8.3×10^7	2.7×10^{11}
Lactic acid bacteria (anaerobic)	n.d.	n.d.	9.2×10^8
Zymomonas sp	2.7×10^8	3.7×10^7	2.0×10^{11}
Total aerobic count	5.0×10^7	n.d.	8.0×10^{11}
Total anaerobic count	3.3×10^7	3.9×10^6	n.d.

n.d. not done

grows on glucose, fructose, and sucrose, the sugars in pulque. Growth on sucrose results in the formation of levan, a pre-biotic [12].

We hypothesize that, because the bacteria that produce alcohol, acid, and viscosity in pulque are the foundation of the product, knowledge of pulque's microbial ecology will provide an insight into the scope of issues that impact pulque quality. This knowledge could lead to the development of a defined inocula that would yield a higher quality and more consistent product.

Materials and Methods

Samples of pulque and aguamiel were obtained at three ranches in the town of Tamazulapan, Oaxaca State and transported to the laboratory on ice where they were held at refrigerated temperatures for less than 24 h before testing. Lactic acid bacteria and *Zymomonas* were enumerated on *Lactobacillus* MRS medium (Difco) [10] and *Lactobacillus* MRS + 2 % sucrose [4], respectively. Total aerobic and anaerobic counts were determined on Plate Count Agar (Difco) with the later incubated in GasPak jars (BBL, Cockeysville, MD). Plates were incubated at 37 °C for 24–48 h prior to counting the colonies. The gram stain and morphology of the isolates were confirmed microscopically. Each sample was analyzed in duplicate, but the experiment could not be repeated due to the changing conditions of the agave plants.

The chemical analyses of aguamiel and pulque samples were performed using the AOAC [1] methods for protein, carbohydrate content, reducing sugars, and ash. The total acidity of the pulque and aguamiel samples was determined based on the Mexican Norm NMX-V-042-1972.

Results and Discussion

The microbial profiles among the aguamiel samples from the three ranches, Rancho San Jeronimo (RSJ), Rancho Los Arcos (RLA), and Rancho La Palzuela (RLP), were very different (Table 1). In each of the ranches, the LAB and "total" populations were similar within an order of magnitude in both the aguamiel and pulque samples. However, there was a 100-fold difference in microbial populations

Fig. 2 Color change associated with the fermentation of aguamiel (*left*) to pulque (*right*)

Table 2 Changes in the microbiota of aguamiel and the pulque made from it, as a function of the time after the agave plant opening, enumerated by cultural methods using *Lactobacillus* MRS agar for lactic acid bacteria, MRS + 2% sucrose for *Zymomonas* sp, and standard plate count agar for total counts

Type of bacteria	CFU/mL (week 1[a])	CFU/mL (week 3)	CFU/mL (week 5)
Aguamiel			
Lactic acid bacteria (aerobic)	5.0×10^7	1.0×10^8	3.5×10^8
Lactic acid bacteria (anaerobic)	3.9×10^7	3.0×10^7	5.1×10^8
Zymomonas sp	3.8×10^7	3.7×10^6	2.5×10^8
Total aerobic count	7.5×10^7	8.2×10^9	2.3×10^8
Total anaerobic count	6.3×10^7	7.6×10^7	4.4×10^8
°Brix	16.0	11.6	4.0
pH	4.3	5.3	4.0
Pulque			
Lactic acid bacteria (aerobic)	8.5×10^9	4.2×10^7	3.5×10^7
Lactic acid bacteria (anaerobic)	8.8×10^{10}	3.0×10^7	3.7×10^7
Zymomonas sp	n.d.[b]	5.1×10^6	4.3×10^7
Total aerobic count	9.7×10^{10}	$>10^9$	$>10^9$
Total anaerobic count	7.2×10^{10}	3.0×10^7	2.2×10^7
°Brix	10.0	7.4	3.0
pH	3.7	3.8	3.7

[a] There is no aguamiel present at Week 0 when the agave has just been opened

[b] *n.d.* not done

Table 3 Chemical analyses of pulque and aguamiel samples

	Aguamiel Rancho los Arcos (week 3)	Aguamiel Rancho los Arcos (week 5)	Aguamiel Rancho San Jerónimo	Pulque Rancho San Jerónimo
Protein (%)	0.49 ± 0.02	0.43 ± 0.03	0.47 ± 0.01	0.48 ± 0.04
Carbohydrate content (%)*	6.60 ± 0.30	2.40 ± 0.11	4.48 ± 0.05	2.40 ± 0.03
Reducing sugars (%)*	2.26 ± 0.03	n.d.	2.04 ± 0.01	n.d.
Ash (%)*	0.54 ± 0.03	0.34 ± 0.03	0.60 ± 0.02	0.49 ± 0.02
Total acidity (g lactic acid/100 mL)	0.68 ± 0.01	0.67 ± 0.01	n.d.	1.32 ± 0.04

* %: w/w

n.d. not done

between at least two ranches, except for *Zymomonas* where there was a ten-fold difference. There were also large differences in the variability of microbial populations within a given ranch. RSJ had 10,000-fold differences among microbial populations, RLP 200-fold, and RLA 10-fold.

The populations of *Zymomonas*, lactic acid bacteria, and total plate counts in pulque were similar ($\sim 10^7$ cfu/mL) within a given ranch, but varied markedly ($\sim 10^7$ to $\sim 10^{11}$ cfu/mL) among ranches (Table 1). In the case of aguamiel, large variations in the populations were observed when making both comparisons, within a ranch and between ranches. Unexpectedly, unfermented aguamiel had higher microbial populations than the fermented beverage.

We learned that there are many sources of variability in pulque production. Aguamiel is collected from agave plants that grow in pastures were the animal's feces can easily contaminate the aguamiel. The agave cavities are opened using unsanitized tools. The cavity is sealed with rocks, agave leaves, or plastic shopping bags that exclude pests with varying degrees of efficiency, which in turn can affect the cavity temperature. The aguamiel is collected manually through a hollow plant stalk, by a hand-held cup, or by other ranch-specific methods.

To remove ranch-to-ranch variability from our analysis, we followed the microbial profile of the aguamiel and the pulque obtained from a single plant over a 5 week period. While the number of bacteria in the aguamiel increased over the 3 weeks after the agave was opened, the microbial populations in the pulque produced from this aguamiel did not increase (Table 2). This may be due to the uniformly high microbial populations in the fermented product. Surprisingly, the microbial load of the aguamiel reached that of the pulque within 3 weeks. This increase is even more evident from an analysis of the °Brix profile (Table 2). The sugar content of the aguamiel was reduced to a level very similar to that of the fermented samples. This suggests that fermentation occurred in the agave cavity itself. However, there were marked differences in the color of aguamiel and the pulque made from it (Fig. 2). This implies that aguamiel fermentation to pulque significantly influenced the aguamiel's physicochemical properties.

The carbohydrate concentration declined with the time after the plant was opened (Table 3). There was also a reduction in carbohydrate content and, more markedly, in reducing sugars content of an aguamiel sample (Rancho San Jerónimo) relative to pulque samples from the same ranch. These results are in accord with the sugar content profiles (°Brix) discussed above. The protein content was not affected by aguamiel fermentation. Ash concentration (i.e., mineral content) decreased as the fermentation took place both in the agave plant and in the process of pulque production (Table 3).

In the course of these studies, we became aware of the many variables that influence pulque production. Since these are uncontrolled, and perhaps uncontrollable, it is doubtful that a simple defined starter culture would become dominant. However, a culture having a selective advantage, such bacteriocin production, might help standardize the pulque fermentation. The probiotic properties long attributed to pulque's could also be investigated. But in this case, because it is so difficult to prove that a native culture is probiotic, it would be better to use a known probiotic culture such as *L. acidophilus* NCFM [6]. Preliminary studies demonstrated it grows well in aguamiel. All of these actions could be implemented on an artisanal level at the farm.

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