

BRIEF ARTICLE
MYCOBIOTA OF THE TIBI GRAINS USED TO FERMENT
PULQUE IN MEXICO

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ARTÍCULO BREVE
MICOBIOTA DE LOS TIBICOS UTILIZADOS
PARA FERMENTAR PULQUE EN MÉXICO

ABSTRACT

A different manner of fermenting the sap of *Agave* to get pulque in Mexico, which is by inoculating it with tibi grains, is herein first reported. From such grains, which are microbiogloae made up of a polysaccharide matrix containing bacteria and yeasts in symbiosis, as well as from the fermented liquid or pulque, 14 yeasts isolates were obtained and identified as the following species: *Candida valida* (3), *Pichia membranaefaciens* (4), and *Saccharomyces cerevisiae* race *capensis* (7). The two former species had already been regularly found in pulque from different localities of Mexico, as well as in the tibi grains used as a starter culture to ferment other sugary solutions to prepare "tepache" and "vinagre de tibicos" (respectively, slightly alcoholic beverage and vinegar prepared from fruit juices inoculated with the so called sugary kefir grains or tibi grains). The race *capensis* of *S. cerevisiae* is registered for the first time for pulque, but not so the typical species, which is an essential microbial element of both the alcoholic beverage and tibi grains.

KEY WORDS: Pulque; tibi grains; yeasts; fermentation.

RESUMEN

Se reporta aquí una manera diferente de fermentar la savia de *Agave* para obtener pulque en México, que es por inoculación con tibicos. Tanto de estos tibicos de pulque, microbiogleas constituidas por una matriz de polisacáridos en los que se hallan embebidas bacterias y levaduras en simbiosis, como del

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líquido fermentado o pulque, se obtuvieron 14 aislamientos de levaduras, las cuales fueron identificadas como pertenecientes a las siguientes especies: *Candida valida* (3), *Pichia membranaefaciens* (4) y *Saccharomyces cerevisiae* raza *capensis* (7). Las dos primeras especies han sido regularmente aisladas del pulque de diferentes localidades de México, así como de los tibicos que se emplean como cultivo iniciador para la fermentación de jugos de frutas y soluciones azucaradas en la obtención de tepache (bebida de bajo contenido de alcohol) y de vinagre de tibicos. La raza *capensis* de *S. cerevisiae* se registra por primera vez para el pulque, no así la especie típica, que es un componente microbiano esencial tanto de esta bebida alcohólica como de los tibicos.

PALABRAS CLAVE: Pulque; tibicos; levaduras; fermentación.

Pulque, a Spanish word derived from the Náhuatl "polihuhqui", meaning spoiled (Robelo, 1948), is a white, slimy, and alcoholic beverage prepared by fermentation of aguamiel (water honey), which is the sugary sap or mead of several *Agave* species. The Aztecs named this beverage "metoctli" (maguey wine) or "iztacoctli" (white wine), while polihuhqui was the name they reserved for the beverage that had undergone spoilage, as it frequently happened (Lappe and Ulloa, 1993).

The process of making pulque, which has traditionally persisted up to now, varies somewhat among different ethnic groups. It is generally obtained as follows: *Agave* sap (or aguamiel) is extracted by suction from mature plants, whose floral stem has been cut to make a cavity (castration of the floral bud), by using a dry gourd or calabash named acocote. First the sap is carried in calfskin containers to wooden tubs named tinacales. Afterwards a spontaneous or inoculum-induced fermentation is carried out by addition of fermented pulque as starter. Fermentation is followed up to 8-30 days, depending on both the environmental conditions and preference of consumers. Finally pulque, consumed either white or cured with various fruits or vegetables, is produced.

Recently, in a locality of Cuautitlán, State of Mexico, another way of making pulque was incidentally found; fermentation was being induced by addition to aguamiel of what the makers of the beverage named tibicos del pulque, i. e. tibi grains of pulque (Fig. 1). These grains or microbiogloae are irregular both in size, from a few millimeters up to 1 or 2 cm, and shape, since they are either plate-like, rolled-up, or convoluted, of a creamy color, and of a soft, somewhat gelatinous consistency. They are made up of a matrix, probably consisting of dextrans, which are the polysaccharides found in the so called sugary kefir grains, named tibicos in Mexico (Rubio Monroy *et al.*, 1993), within which bacteria and yeasts are embedded, the latter either isolated or forming chains of pseudomycelium (Fig. 2). These tibi grains of pulque are very similar to the milky kefir grains, and to the so called búlgaros in Mexico. Both are also microbiogloae composed of mixed microbial associations used as starter cultures for the preparation of various fermented milk products, such as kefir. The latter, an acidic fermented cows milk, slightly alcoholic and effervescent, is prepared at a large scale in the former Soviet Union, and nowadays it is popular in Western Europe and the United States (Steinkraus 1983; Marshall *et al.*, 1984).

Since elaboration of Mexican pulque with tibi grains of pulque to induce a fermentation process has not been previously reported in the scientific literature, and no studies have been done on the yeast microbiota associated with both the microbiogloae and the fermented substrate, we decided to isolate and identify the yeasts associated with the tibi grains of pulque and of the pulque fermented with them.

Tibi grains of pulque were collected from the fermented liquid with a domestic wire sieve, and washed three times with sterile distilled water. Small fragments were then taken aseptically from the outer and inner portions of the grains, and inoculated by streaking with a transfer needle onto five glucose-yeast extract-peptone-agar (GYPA) plates for each portion of the microbiogloae. The medium was acidified to pH 3.8 with hydrochloric acid before it solidified (Lachance *et al.*, 1988). Five plates of the same culture medium were also inoculated with the fermented liquid or pulque, by streaking. From the pure yeast colonies, 14 isolates were obtained, which were transferred into all the solid and liquid culture media necessary to study their morphological, physiological, and biochemical characteristics to identify the species, following the methods of van der Walt and Yarrow (1984), the keys of Kreger-van Rij (1984), and the generic descriptions of Kurtzman (1984) and Yarrow (1984).

Table 1 shows both the source and number of the yeast isolates, as well as the corresponding species. *Pichia membranaefaciens* Hansen and *Candida valida* (Leb.) van Uden *et* Buckley (Figs. 3-4), which were found in both pulque and the tibi grains used to ferment it, had previously been reported as yeasts frequently isolated from pulque samples from various localities of Mexico (Lappe *et al.*, 1993), as well as from tibi grains (generally referred to as sugary kefir grains) cultivated in aqueous brown sugar solutions and diluted fruit juices (Ulloa *et al.*, 1987; Pidoux, 1989; Rubio Monroy *et al.*, 1993), and from the so called búlgaros, which are propagated in cows milk (Ulloa and Lappe, 1993). Both yeast species develop branched pseudomycelia which, as in other yeasts found in sugary kefir grains or tibicos, such as *Candida lambica* (Lindner *et* Genoud) van Uden *et* Buckley, and *Pichia kluyveri* Redford *ex* Kudriavsev, form nets firmly adhered to the grain surface. This explains why these yeasts can be isolated from the outer region of the grains even after several washings (Pidoux, 1989).

As for *Saccharomyces cerevisiae* Meyen *ex* Hansen, this species has been regularly found in pulque (Lappe and Ulloa, 1993), in tibicos and in their fermentation substrates (Hesseltine, 1965; Ulloa *et al.*, 1987; Rubio Monroy *et al.*, 1993), in kefir grains (La Rivière, 1963; Rosi, 1978), and in búlgaros (Ulloa and Lappe, 1993). However *S. cerevisiae* race *capensis* Meyen *ex* Hansen is herein registered for the first time for pulque and tibi grains of pulque; this physiological race had only been isolated from peelings, most, and juice of grapes, and differs from the typical species only in its inability to ferment galactose and maltose (van der Walt, 1970). However, probably this race had not been registered before for pulque, because, in order to identify it and distinguish it from the typical species, is necessary to do additional fermentation tests (Yarrow, 1984).

In the present study, only the yeasts involved in the microbial composition of the tibi grains of pulque and of the pulque fermented with them were studied. Identification of the bacteria present in them remains to be done. And, since the tibi grains of pulque are very similar to the kefir grains and to búlgaros, as far as macroscopic appearance, consistency, color, and size, is concerned, it becomes necessary to undertake further studies, such as light and electronic microscopy, as well as chemical studies, in order to determine the microstructure, the microbial distribution, and composition of the matrix. It would then be possible to know whether or not these tibi grains of pulque are the same sort of microbioglocae as the kefir grains or búlgaros, which instead of being propagated in the usual milky substrates were grown in *Agave* sap, or if they are more related to the sugary kefir grains or tibicos, but which were in this case grown in the sugary sap of *Agave* to get pulque. It will be necessary to study the bacteria of these grains because, in all of them, the lactic bacteria are responsible for the synthesis of the polysaccharides making up the matrix. For sugary kefir grains, *Lactobacillus hilgardii* Douglas and Cruess [previously known as *L. brevis* (Orla-Jensen) Bergey *et al.*] is the polymer-synthesizing microorganism (Pidoux, 1989), which has also been reported, along with other lactic acid bacteria (i. e. *L. kefir* Kandler and Kunath), for kefir and kefir grains. From kefir grains, the following yeasts have been found: *Candida holmi* (Jorgen) Meyen ex Hansen, *C. kefir* (Beijerinck) van Uden *et* Buckley, *Saccharomyces exiguus* Rees ex Hansen, *Kluyveromyces marxianus* var. *marxianus* (Hansen) van der Walt, *S. cerevisiae*, and *Torulaspora delbrueckii* (Lindner) Lindner.

Eventhough elaboration of pulque with the participation of tibi grains was noted as an isolated case in Cuautitlán, State of Mexico, and that this modality of making pulque is not a widespread practice among pulque makers in the country, it seemed interesting to publish this brief article, considering the potential importance of using the tibi grains of pulque as a starter culture. As in the case of the fermented milk products made with kefir grains (in Europe and USA) or with búlgaros (in Mexico), the utilization of these microbioglocae as starter cultures could be probably a better way of obtaining fermented products with acceptable and constant quality, and reduced hygienic risks, since the lactic acid and other metabolites produced during fermentation rapidly create a specific ecological niche that favors the growth and establishment of desirable microorganisms (such as the ones composing the sugary kefir grains and the kefir grains). Sugary kefir grains and kefir grains are recovered by filtration once the fermentation process has been performed; after washing they can be reused in subsequent fermentations in so far as appropriate hygienic conditions are maintained during the fermentation. Because of this, and due to the fact that the grains can be dehydrated and stored, retaining their viability, these starter cultures represent a practical means of inducing the fermentation of sugary solutions and of milk. In a similar way, the tibi grains of pulque could be utilized at large scale to induce the fermentation of *Agave* sap to get pulque, because this process is cheap, rapid, and does not require rigorous aseptic conditions. During pulque production good sanitary practices are not observed and so the product is quite variable both in taste and hygienic properties. Therefore, the use of

a stable starter culture, such as the tibi grains of pulque could represent a means of obtaining a fermented product with the desired characteristics, including its improved state of healthfulness, as in the case of kefir.

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Table 1. Yeasts isolated from pulque and the tibi grains used in its fermentation.

| Source | Yeast species (number of isolates) | | |
|-------------------------------|---------------------------------------|--|-------------------------------------|
| | <i>Candida valida</i> | <i>Pichia membranaefaciens race capensis</i> | <i>Saccharomyces cerevisiae</i> |
| Pulque | 1 | 2 | 2 |
| Outer portion of the grain | 2 | | 3 |
| Inner portion of the grain | | 2 | 2 |
| | 3 | 4 | 7 |

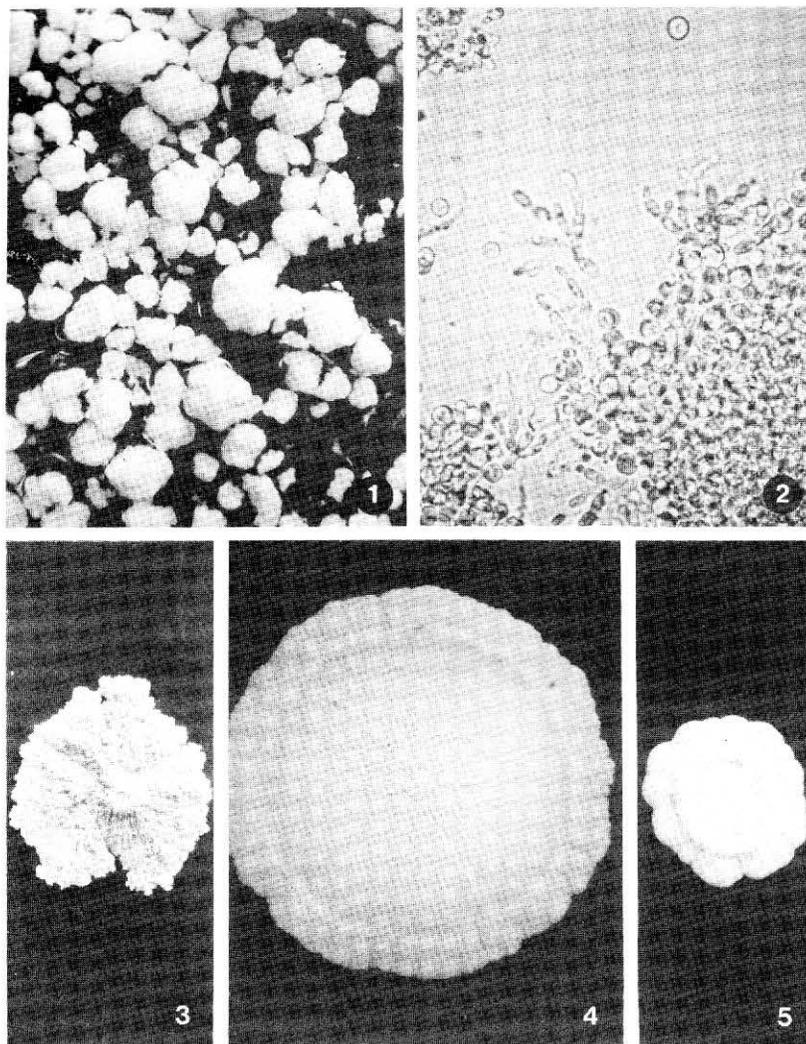


Fig. 1-5 1: Tibi grains used to ferment the *Agave* sap to obtain pulque, X 3. 2: Portion of a tibi grain squashed and mounted in water. Its biomass consists of yeasts and bacterial cells, X 600. 3-5: Giant colonies of 30 days on GYP, X 3, correspondig to *Pichia membranaefaciens*, *Candida valida*, and *Saccharomyces cerevisiae* race *capensis*.