

YEASTS ISOLATED FROM PULQUE, THE TRADITIONAL
BEVERAGE OF MEXICO
(NATURAL OR WHITE PULQUE AND OAT CURED PULQUE)

by Teófilo Herrera*
Angélica Calderón-Villagómez*

LEVADURAS AISLADAS DEL PULQUE, LA BEBIDA
TRADICIONAL DE MEXICO
(PULQUE BLANCO O NATURAL Y PULQUE CURADO DE AVENA)

RESUMEN

Se hace una breve historia del pulque, bebida preparada por fermentación del jugo del agave o maguey (*Agave salmiana* Otto y otras especies del mismo género) tanto del aspecto etnológico como de los trabajos microbiológicos previos, y se registran las levaduras aisladas de ambos tipos de pulque utilizados en el presente estudio: pulque blanco o natural y pulque curado de avena, así como de la mezcla de avena y los otros ingredientes que se añadieron al primer tipo de pulque para obtener la última bebida mencionada. Se identificaron las siguientes especies de levaduras: *Candida colliculosa*, *C. rugopelliculosa*, *C. rugosa*, *C. valida*, *Kluyveromyces marxianus* var. *bulgaricus*, *K. marxianus* var. *lactis*, *Saccharomyces cerevisiae* raza *aceti* y *S. cerevisiae* raza *globosus*, todas ellas presentes en el pulque natural o blanco; las mismas fueron aisladas del pulque curado de avena excepto *C. colliculosa*, *C. valida* y *K. marxianus* var. *lactis*, en tanto que, de la mezcla de avena y los otros ingredientes, sólo fueron aisladas las dos variedades citadas de *Kluyveromyces marxianus*.

* Departamento de Botánica, Instituto de Biología, UNAM, Apdo. Postal 70-233, México, D.F. 04510.

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SUMMARY

A brief history of the ethnological and microbiological aspects of pulque, beverage prepared by fermentation of the agave or "maguey" juice (*Agave salmiana* Otto and other species of the same genus) is made. Yeasts were isolated and registered from both types of pulque studied: white or natural pulque and oat cured pulque, and also from a mixture of oat and other ingredients added to the first type of pulque to obtain the last mentioned beverage. The following species of yeasts were identified: *Candida colliculosa*, *C. rugopelliculosa*, *C. rugosa*, *C. valida*, *Kluyveromyces marxianus* var. *bulgaricus*, *K. marxianus* var. *lactis*, *Saccharomyces cerevisiae* race *aceti* and *S. cerevisiae* race *globosus*, all of them present in the white or natural pulque; the same were isolated from the oat-cured pulque except *C. colliculosa*, *C. valida* and *K. marxianus* var. *lactis*, and only the two varieties of *Kluyveromyces marxianus* were isolated from the mixture of oat and other ingredients.

INTRODUCTION

Much work has been done about the knowledge of pulque and the importance of this beverage in Mexico since prehispanic times. In this country it is still considered of national importance because it is highly appreciated among people of low economic level and therefore unable to have enough food rich in proteins and B-complex vitamins, both types of substances present in pulque. This beverage is prepared by fermentation of the agave or "maguey" juice (*Agave salmiana* Otto and other species of the same genus) which soon presents in abundance bacteria and yeasts in a peculiar succession, many species of them studied since the end of last century up to now (Del Río-Estrada, 1947; Ulloa *et al.*, 1987).

Pulque is a beverage closely linked to prehispanic mythology and historic events in the central part of Mexico, particularly those related to the Toltec, Aztec and other indigenous groups developed with the influence of the Nahuatl Culture. Mayahuel was the woman considered as the discoverer of the agave sugary juice or aguamiel ("honeywater") and later worshiped as the goddess of the agave or "maguey", as well as the mother of numerous gods of drunkenness, the Centzontotchtin; one of them was Pantecatli who discovered a weed used to speed up the fermentation. This plant, called "ocpatli" (which means medicine of the "octli", nahuatl true name of the beverage later called pulque by the Spaniards) is not well known but probably several botanical species were used for

this purpose in prehispanic times (Martín del Campo, 1938; Goncalves de Lima, 1978). The so called "cured pulque" could be a reminiscence of the "ocpatli", but using now several types of cereals, seeds and fruits (oat, rice, nuts, red prickly pear, guava fruit, pineapple, lemon and banana are some of them), weeds such as celery, (to get a beverage of peculiar flavor), and also to improve the "old or sick pulque". Notwithstanding, the "ocpatli" was added to induce the fermentation of the "honeywater", and the complementary ingredients to get the "cured pulque" are added generally near the end of the fermentation of the natural or white pulque, and so, it is possible to start a second fermentation if the product is not consumed soon after its preparation.

Some preceding works on the microbiology of pulque are indicated in the following paragraphs in order to give an idea of the approaches concerning the researches on this fermented beverage.

Leopoldo Rio de la Loza (1864) was the Mexican chemist who observed for the first time a drop of pulque under the microscope, but he thought that numerous corpuscles of albuminous substances were present in this liquid, and not considered those corpuscles as microorganisms.

José Barragán (1870) made the first description of yeasts from pulque and identified them as a species of *Cryptococcus* similar to *C. cerevisiae*.

Angel Gaviño (1896) isolated a pink yeasts, probably a species of *Rhodotorula*, and also (1901) the yeast which he called *Saccharomyces* from pulque or *S. cerevisiae agavica*.

Antonio J. Carbajal (1901, 1912) isolated and studied two species of yeasts from pulque. He identified one of them as *Saccharomyces cerevisiae agavica silvestre*, which he considered constant, and the other as *Torula rosada*, a contaminant according to him.

Alexandre Guilliermond (1917) published a paper on yeasts from pulque, identifying them in the genera *Pichia* and *Saccharomyces*.

Paul Lindner (1926, 1932), from the Institute of Fermentations of Berlin, came to Mexico in the second decade of the present century to study the practical and scientific importance of pulque and the possibility to improve this beverage, from which he made important researches on bacteria, but payed little attention to yeasts.

Manuel Ruiz-Oronoz (1953) published several papers on yeasts from pulque, and between 1936-1942 described the following as new species: *Saccharomyces carbajali*, *Pichia barragani*, *Torulopsis*

hydromelitis, *T. aquamellis* and *Rhodotorula incarnata*.

Alfredo Sánchez-Marroquín, Carlos del Río Estrada and Celsa Celis (1949) worked on metabolic aspects of *Saccharomyces carbajali*, and later on, of the species mentioned above, the first author considered *S. carbajali* as *S. cerevisiae*, *P. barragani* as *P. membranaefaciens*, and *T. hydromelitis* as *Candida parapsilosis*.

Patricia Lappe, Miguel Ulloa and Teófilo Herrera (1989) published the paper "Study of five species of yeasts from pulque and the comparison of the microbiota of this beverage with that of others similar of the world". The following species were identified: *Saccharomyces cerevisiae* Meyen ex Hansen, *Pichia membranaefaciens* Hansen, and its asexual state *Candida valida* (Leberle) van Uden et Buckley, *Kluyveromyces marxianus* (Hansen) van der Walt var. *bulgaricus* (Santa María) Johansen et van der Walt, *Pichia carsonii* Phaff et Knapp, and *Candida guilliermondii* (Castellani) Langeron et Guerra.

The main purpose of this study was to isolated and identify the yeasts of an "oat cured pulque", to compare them with the yeasts involved in the fermentation of a natural or white pulque, and to find out what species of yeasts are present, which of them are the same and which different in both types of pulque. Yeasts of the ingredients used to cure the pulque were also isolated and identified.

MATERIALS AND METHODS

Natural or white pulque from Jalatlaco, State of Mexico (100 ml), was used throughout the study (part 1).

A mixture (part 2) of oat (64 g), evaporated milk (40 ml), sugar (50 g), vanilla extract (2 drops) and brandy (10 ml) was added to part 1 to obtain and "oat cured pulque", in which the fermentation continued with more vigor, or a new fermentation started once the parts 1 and 2 were mixed.

Isolates of yeasts were made at the same time from both types of pulque and from the mixture mentioned above, at different periods of fermentation, with the consideration of a possible succession of yeasts, spreading 0.5 or 0.1 ml of each of the three sources studied, on glucose-yeast extract-peptone-agar plates with the aid of a sterile L shaped road. The isolates from white pulque were made at the periods of 0, 24 and 48 hrs in relation to isolates from cured pulque, even though the first mentioned pulque had several hours of fermentation when the experiment started,

according to the traditional procedure of preparation of cured pulques.

Yeasts were isolated and identified, mainly, following Barnett *et al.* (1979), Kreger-van Rij (1984), Meyer *et al.* (1984), van der Walt and Johannsen (1984), van der Walt and Yarrow (1984), and Yarrow (1984).

DISCUSSION

Of the six species studied, one of them with two varieties and other with two races, as shown in table 1, were present in white pulque, while only *Kluyveromyces marxianus* with the two varieties studied were recovered from the oat mixed with other ingredients. In the oat cured pulque the species of the white pulque persisted in different proportions except *Candida colliculosa*, *C. valida* and *K. marxianus* var. *lactis*; only *K. marxianus* var. *bulgaricus* was common to the three sources studied. Results suggest that species and number of yeasts change in a peculiar succession during the process of fermentation from white to cured pulque. More studies should be done to know which of the species isolated in this research are constant and which are not, as well as their possible succession, according to different geographic areas and manners of preparation of white pulque and the great variety of cured pulque.

TABLE 1. DETERMINED YEASTS ISOLATED DURING THE PROCESS OF MAKING THE OAT CURED PULQUI

Species of Yeasts	White pulque			Mixture of oat and other ingredients			Oat cured pulque		
	0	24	48 hrs	0	24	48 hrs	0	24	48 hrs
<u>Candida colliculosa</u>	2**	2	-	-	-	-	-	-	-
<u>Candida rugopelliculosa</u>	4	4	2	-	-	-	4	4	4
<u>Candida rugosa</u>	2	2	-	-	-	-	4	2	-
<u>Candida valida</u>	2	-	-	-	-	-	-	-	-
<u>Kluyveromyces</u>									
<u>marxianus</u> var.									
<u>bulgaricus</u>	2	2	-	2	2	-	4	2	-
<u>Kluyveromyces</u>									
<u>marxianus</u> var.									
<u>lactis</u>	2	2	-	4	4	-	-	-	-
<u>Saccharomyces</u>									
<u>cerevisiae</u> race									
<u>aceti</u>	2	-	-	-	-	-	2	-	-
<u>Saccharomyces</u>									
<u>cerevisiae</u> race									
<u>globosus</u>	2	-	-	-	-	-	4	2	-

The same species were isolated in the agar plates, spreading 0.5 or 0.1 ml of the studied materials.

* The numbers indicate the strains studied.

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