BRAZILIAN JOURNAL OF MICROBIOLOGY XXX (2018) XXX–XXX

BRAZILIAN JOURNAL OF MICROBIOLOGY

http://www.bjmicrobiol.com.br/

Food Microbiology

Saccharomyces cerevisiae populations and other yeasts associated with indigenous beers (*chicha*) of Ecuador

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ARTICLE INFO

Article history:
Received 20 July 2017
Accepted 7 January 2018
Available online xxx

Associate Editor: Adalberto Pessoa

Keywords:
Saccharomyces cerevisiae
mtDNA restriction patterns
Yeast populations
Chicha
Ecuadorian indigenous beers

ABSTRACT

Chicha, a type of beer made mainly with maize or cassava, is a traditional fermented beverage of the Andean region. There have only been a few studies on yeasts associated with *chicha* fermentation, and the species diversity occurring during the production of this beverage is not known. The objective of this study was to determine the biodiversity of yeasts in *chicha*, and to characterize the *Saccharomyces cerevisiae* populations associated with the production of *chicha de jora*, seven-grain *chicha*, *chicha de yuca*, and *chicha de morocho* in Ecuador. The molecular diversity of *S. cerevisiae* populations was determined by restriction polymorphism mitochondrial DNA (mtDNA) profiles. The beverages were characterized based on their physicochemical parameters. Twenty-six species were identified, and the most prevalent species were *S. cerevisiae* and *Torulaspora delbrueckii*. Other yeast species were isolated at low frequencies. Among 121 isolates of *S. cerevisiae*, 68 different mtDNA molecular profiles were identified. These results showed that *chichas* are fermented by a high number of different strains of *S. cerevisiae*. Some other species provided a minor contribution to the fermentation process. The *chicha* presented generally similar physicochemical parameters to those observed for other traditional fermented beverages, and can be considered as an acid fermented beverage.

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https://doi.org/10.1016/j.bjm.2018.01.002

Please cite this article in press as: Piló FB, et al. *Saccharomyces cerevisiae* populations and other yeasts associated with indigenous beers (*chicha*) of Ecuador. *Braz J Microbiol.* (2018), https://doi.org/10.1016/j.bjm.2018.01.002

Introduction

Chicha or maize beer could be regarded as the oldest beverage in Latin America. The name *chicha* possibly originates from the word *chichab*, from the original language spoken in the current territory of Panama, which means maize. Other theories suggest that the name is derived from the word *Chibcha*, a civilization that populated Colombia and Panama, or relate the word *chicha* to *Chichas*, an ethnicity present in southern Bolivia before the establishment of the Incas.[1]

Chicha is a clear, yellow, and frothy beverage present in the Andean region and in low-lying regions of Ecuador, Peru, Bolivia, Colombia, Brazil, and Argentina.[2] This traditional beverage is prepared mainly from maize, but currently, the name is considered generic and refers to a variety of beverages, fermented or not, prepared from various other materials, such as cassava, beans (such as rice, oats, and quinoa) and fruits (such as bananas). In Ecuador, the first reports of *chicha* production date back to 200 B.C., before the establishment of the Incas in the region.[1] This beverage was of great importance in traditional indigenous cultures, especially in the Incan culture, wherein it was also linked to festive ceremonies.[3]

In Ecuador, as in the rest of the Andean region, the most common maize *chicha* is *chicha de jora* (Fig. 1). This *chicha* is prepared from the yellow maize grain (*maíz amarillo*), which is malted (germinated and dried). For the preparation of malt, the grains are left in water for a day. This step is necessary to achieve the optimum grain moisture for germination. Subsequently, the water is drained and the maize is placed in baskets of stray to germinate over a period of 13 days. Once germinated, the maize is put into straw mats or plastic tarps under the sun for 2 days to dry completely, which stops the enzymatic activity within the grain. After drying, the beans are ground and the flour obtained is used for the preparation of *chicha*. For this, the *jora* flour is added to cold water and then this mixture is transferred to vessels with hot water, and boiled for approximately 20 min. After boiling, the mixture is strained and then placed in a container to ferment. The clay vessels, formerly used for boiling and fermentation, have been replaced by aluminium pots and plastic containers, respectively. The spent grain obtained after filtration is termed *afrecho* and serves as food for animals. The fermentation vessels are usually open. Usually after two days of spontaneous fermentation by indigenous microorganisms, the beverage is ready for consumption. Some producers typically boil *jora* flour with other ingredients, including *panela* (brown sugar in solid pieces). Others make a mixture of *panela* and herbs and then add this mixture to the *jora* flour and water. There are still those that add pieces of fruit and *panela* to the beverage, after filtering.

Other *chicha* beverages produced in Ecuador include *chicha de morocho*, made with white maize, and *chicha* produced with seven varieties of maize including *jora*, *maíz amarillo* (yellow maize), *maíz blanco* (white maize), *maíz negro* (black maize), *chulpi* (*chulpi* maize), *morocho* (*morocho* maize) and *cangil* (popcorn maize). Seven-grain *chicha* is produced in the town of Otavalo, in northern Ecuador, and is a very famous drink and appreciated throughout the country. The *yuca* (*cassava*, *Manihot esculenta*) is also an important raw material for the production of *chicha*.[4] This *chicha* is produced by the indigenous and mestizo population in the Amazon region of Ecuador.

Few studies have been performed to identify the yeast species in *chichas*. Vallejo et al.[5] isolated *Saccharomyces cerevisiae* as the single yeast species at the end of fermentation from 10 samples of *chicha de jora* collected from 10 different familiar traditional "chicherías" in the Cusco region of Peru. These authors suggested that this species was mainly responsible for alcoholic fermentation in these *chicha* samples. Rodríguez et al.[6] suggest that *Saccharomyces uvarum* is responsible for the traditional fermentation of apple *chicha* elaborated by aboriginal communities of Andean Patagonia (Argentina and Chile). Mendonza et al.[7] showed by high-throughput sequencing and culture-dependent approaches that *S. cerevisiae* was the dominant species in an Argentinian maize-based *chicha*. Other works on *chicha* fermentation linked bacterial populations to this process.[4,8,9] Despite the work of Vallejo et al.[5] and Mendonza et al.,[7] the yeast biodiversity associated with maize and cassava *chicha* production is almost unknown. In this work, *chichas* sold in bulk (Fig. 1), produced with different substrates and different fermentation times, were collected from markets, bars, restaurants, and in villages of Ecuador. The objective was to determine yeast species richness and to characterize the *S. cerevisiae* populations associated with the production of this beverage by restriction polymorphism mitochondrial DNA (mtDNA) analyses. In addition, the physicochemical parameters of the beverages were determined.

Materials and methods

Sampling

Forty-two *chicha* samples were collected from August to October of 2010 and April to September of 2012 in two regions of Ecuador: the Amazon region, within the *Yasuní* National Park (*Orellana* Province) and the Andean region, in the provinces of *Pichincha*, *Imbabura* and *Chimborazo*. The samples included two *chichas de yuca*, 34 *chichas de jora*, three seven-grain *chichas*, and two *chichas de morocho*. In these samples, the fermentation was considered finished by the producers, and the beverage was ready to drink. One sample of *chicha de jora* was sampled during successive fermentation times (0–5 days). The *chichas* were collected in sterile bottles of 100 mL, transported to the laboratory on ice, and processed the same day.

Yeast isolation and identification

Aliquots of 25 mL of each *chicha* were added to 225 mL of sterile 0.1% peptone water. For yeast isolation, 0.1 mL of appropriate decimal dilutions, in triplicate, was spread on yeast extract-malt extract (YMA: 1% glucose, 0.5% peptone, 0.3% malt extract, 0.3% yeast extract, 2% agar, and 0.02% chloramphenicol) and lysine (1.17% YCB, 0.056% lysine, 2% agar, and 0.02% chloramphenicol) agars. The YMA was utilized for the isolation of *Saccharomyces* and non-*Saccharomyces* yeasts while the lysine agar was utilized for the isolation of non-*Saccharomyces* yeasts. The plates were incubated at 25 °C for 5 days and the density of each different yeast morphotype

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