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Yeast species involved in artisanal cachaça fermentation in three stills with different technological levels in Pernambuco, Brazil

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ABSTRACT

Both the taste and aroma of cachaça, an alcoholic beverage produced by yeast fermentation of sugar cane, are influenced by yeast metabolites and volatiles. The knowledge of yeast population dynamics during the fermentation process will help to establish the basis for quality control of alcoholic beverage. In the present work, the population dynamics of three fermentation processes, with differing levels of technological sophistication, were studied. *Saccharomyces cerevisiae* was found to be the dominant species, but *Candida milleri* (*Candida humilis*), *Pichia caribbica*, *Pichia guilliermondii* and *Zygosaccharomyces fermentati* (*Lachancea fermentati*) were also significantly involved. In addition, four new yeast species that are not represented in NCBI/EMBL nucleotide database were found. These yeasts were classified as *Candida* sp., *Candida drosophilae*-like, *Candida ubatubensis*-like and *Zygosaccharomyces* sp. In fermentation trails at laboratory scale, all species were found to contribute to the production of volatiles. Thus it is probable that product quality is strongly dependent on population dynamics during the fermentation process.

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1. Introduction

Cachaça is an alcoholic beverage that is distilled from fermented sugar cane. 'Artisanal' cachaça is fermented and distilled in small copper stills, whereas 'industrial' cachaça is fermented and distilled in large stainless steel stills. In the artisanal product, fermentation is initiated by yeasts present in the substrate (Pataro et al., 2000) whereas the industrial process is usually initiated using commercial yeasts such as baker's yeast. Previous work has demonstrated the succession of wild yeasts during spontaneous cachaça fermentation. *Saccharomyces cerevisiae* is the dominant species (Morais et al., 1997; Pataro et al., 1998) but many non-*S. cerevisiae* yeasts have also been isolated during the fermentation process (Schwan et al., 2001). As a consequence artisanal cachaças normally have additional sensory qualities due to the metabolites and volatiles unique to each yeast species present during the fermentation process. Oliveira et al. (2004) showed that the presence of non-*S. cerevisiae* species in the process could lead to more acetic acid and glycerol formation during cachaça fermentation, but they first minimized the importance of those yeasts due to their low fitness compared to *S. cerevisiae*. That study was corroborated by more accurated metabolic and sensorial analysis, but it was shown that the presence of *Schizosaccharomyces pombe* could be deleterious to the product (Oliveira et al., 2005). Moreover, it has been well documented that different *S. cerevisiae* autochthonous strains could be selected to produce cachaças with specific sensorial quality (Gomes et al., 2007). Thus, because these specific properties increase the market value, it is important to monitor yeast populations during fermentation both to detect the presence of undesired yeasts and to select native *S. cerevisiae* strains.

Whereas this type of analysis has been carried out on stills in Minas Gerais state (Morais et al., 1997; Pataro et al., 1998, 2000; Schwan et al., 2001; Guerra et al., 2001), no microbiological analysis has yet been done in Pernambuco. In the present work, we isolated and identified yeast species in three small stills that produce artisanal cachaça. Cachaça from still A has an international standard quality certification and cachaça from still B has been recently won awards at Brazilian meetings. Cachaça from still C is considered off-quality. We show that the presence of contaminant yeasts seems to be dependent on the management of the process, rather than the composition of the starter culture.

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2. Material and methods

2.1. Still description and yeast isolation

The three cachaça stills studied herein were classified according to their technological level of sophistication. Still A used a selected cane variety, filtered sugar cane juice and stainless tanks for fermentation. The process was monitored by controlling temperature and must pH, sucrose consumption and level of ethanol production. Fermentation began with the development of wild yeasts from the substrate. In the first stage, yeast inoculum was prepared by mixing 30 kg corn bran, 10 kg rice bran, 5 kg soybean bran and 100 L sugar cane juice diluted to 6°Brix (ca. 60 g sucrose/L). The mixture was allowed to ferment for cycles of 24 h at 33 °C without agitation, with addition of 100 L sugar cane juice at increasing concentrations (8°, 10°, 12°, 14° and 16°Brix) at the end of every cycle. After the last cycle, 500 L of the must was sent for distillation. The remaining 100 L was mixed with 500 L of 16°Brix cane juice and fermentation completed at 33 °C. A new inoculum was created every three months or at the point that the inoculum became infected by bacteria and lost its ability to initiate fermentation.

Samples were withdrawn during the preparation of the inoculum and from three batches at different periods during fermentation, diluted in sterile saline and seeded in WLN medium containing bromocresol green to 10–20 colonies per plate and yeast colonies were first classified by colony morphology (Silva-Filho et al., 2005a; De Souza-Liberal et al., 2007). Yeast cells were suspended in sterile saline and seeded in new plates to confirm purity.

Still B also used a selected cane variety, filtrated sugar cane juice and stainless tanks for fermentation. However, the process was not monitored. The fermentation was started with 50 kg of baker's yeast added to 500 L of 6°Brix cane juice. After 24 h fermentation at 33 °C another 500 L of 12°Brix cane juice was added. After fermentation ended, 500 L of fermented must was sent for distillation. New batches were started by adding 500 L of 12°Brix cane juice to the remaining 500 L of must. This process continued for three months. Samples were withdrawn at the beginning of the process and at different during it and treated as above.

Still C operated as a low-level fermentation process, had no process management and used iron-maiden fermentation tanks. The fermentation process and sampling procedures were similar to those described for the still B.

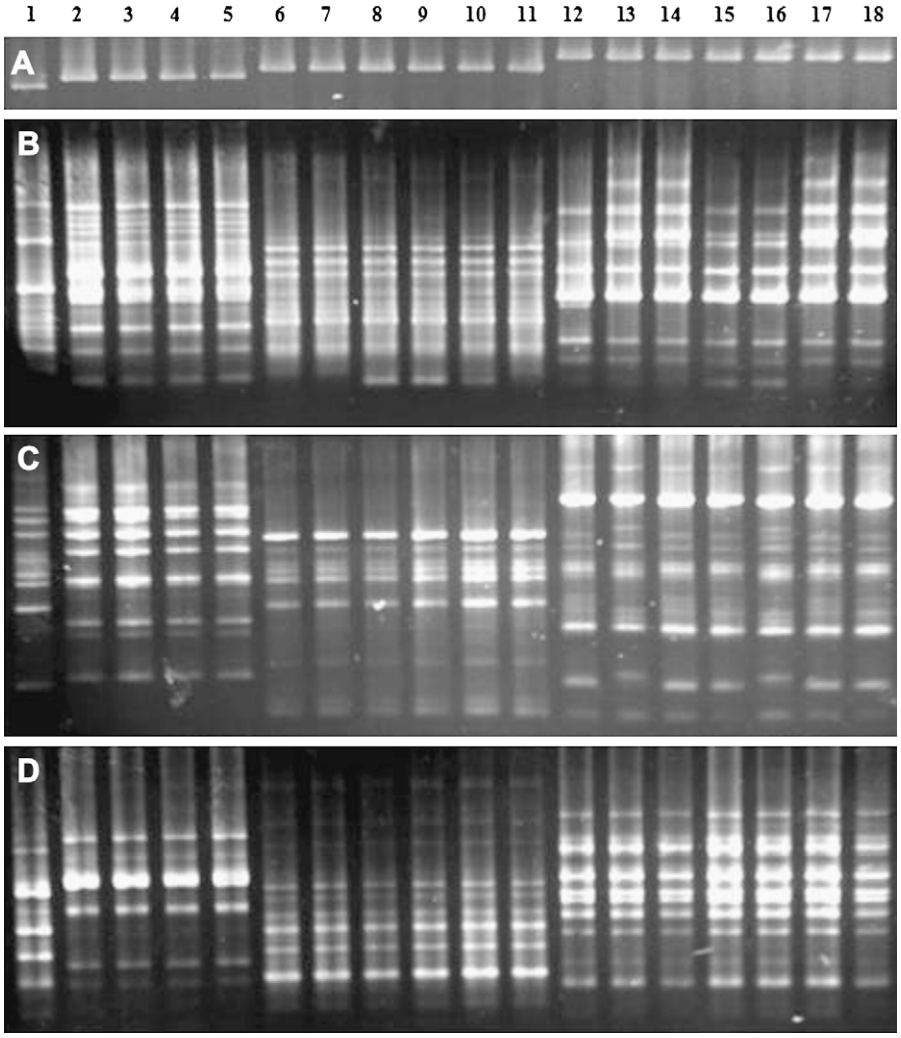

Fig. 1. Molecular profile of yeast species isolates from the still A for SSU-rRNA (panel A) and for the ISSR markers $(GTG)_5$ (panel B), $(GACA)_4$ (panel C) and M13 (panel D). Lane 1: yeast isolate representative of the ribotype r550 (*Candida* sp.); lanes 2–5: yeast isolates representative of the ribotype r600 (*P. caribbica*); lane 6–11: yeast isolate representative of the ribotype r700 (*C. milleri*); lane 12–18: yeast isolate representative of the ribotype r850 (*S. cerevisiae*).

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