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BIOTECHNOLOGY AND INDUSTRY - RESEARCH PAPER

Isolation of indigenous *Saccharomyces cerevisiae* strains isolated of fermented must from wine grapes in a tropical semiarid region of Brazil

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Abstract

The present study aimed to isolate, identify and characterize the yeasts of fermented must from grapes in the São Francisco Valley region, Brazil. The grapes were collected from four vineyards in the region and taken to the laboratory for must production and strain isolation. The yeasts were identified by sequencing the D1/D2 variable domains of the largest subunit of the rRNA gene. The *Saccharomyces cerevisiae* strains were differentiated using the mitochondrial DNA restriction technique (RFLP-mtDNA), and compared with the commercial strains used in the region for wine production. A total of 368 yeasts were isolated, 109 of which were non-*Saccharomyces* and 259 *S. cerevisiae*. Of these, 184 were indigenous and 75 commercial varieties. Among the indigenous strains, 22 RFLP-mtDNA and two commercial profiles were characterized. The must of the samples collected was appropriate substrate for identifying and isolating the non-*Saccharomyces* and *S. cerevisiae* strains and commercial yeasts. Given that the indigenous strains were more numerous than their commercial counterparts, which were selected in countries with a temperate climate, new studies should be conducted, testing the capacity of indigenous yeasts in producing quality wines, with typicality and regional identity, some of which may become commercially viable.

Keywords Mitochondrial DNA · *Saccharomyces cerevisiae* · Tropical wines · Grape · *Vitis vinifera* L.

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Introduction

Wine is produced in several regions of the world, in both temperate and tropical climates, such as the São Francisco Valley (SFV) in Northeastern Brazil. The SFV has a semi-arid tropical climate, where tropical wines have been produced since the 1980s, but has received little recognition for its products [1, 2].

Four million liters of fine wines (*Vitis vinifera* L. varieties) are produced every year on 400 hectares, cultivated in a pergola trellis and vertical shoot system. Production occurs in two Brazilian states, Pernambuco in the municipalities of Petrolina, Lagoa Grande and Santa Maria da Boa Vista, and Bahia, in Juazeiro, Casa Nova, Sobradinho and Curaçá [2]. The main characteristic that differentiates the SFV from other wine-producing regions in the world is the ability of grapevines to produce two crops per year, due to their capacity to stagger production. This occurs due to the absence of low temperatures, with an annual average of 26ºC, the use of irrigation, hormones that stimulate germination, and high indices of solar radiation [1]. Thus, the producer decides when to prune and harvest, normally as a function of market demand, physical structure, as well as the quality and typicality of the wine produced. The physicochemical and sensory composition of wines may vary as a function of the time of year in which the grapes are harvested and the wines produced.

The composition, quality and typicality of wines, anywhere in the world, depend on three primary factors: the climate and soil where the vineyards are located, as natural factors, and the human factor. Producers are responsible for selecting the variety used, rootstock, plant spacing, plant density, irrigation, nutrition, harvest date, and winemaking protocols, including the type of yeast [3–6]. Yeasts interfere significantly in final wine typicality, with a wide diversity depending on the type of wine, namely white, red and sparkling [7]. Thus, with the increase in studies, and greater understanding of the microbial community and its effects on wine fermentation, production may be optimized, and regional characteristics expressed, resulting in better management of the microorganisms present, that is, the microbial "terroir" influencing the final characteristics [8–10]. These influences were also identified by Setati et al. [11] when the microbiome associated with South African Cabernet Sauvignon grapes were compared at three adjacent vineyards that use different agronomic approaches.

The surface of grapes contains a limited number of yeasts, including oxidative metabolism species of the genus *Rhodotorula*, and some alcohol-sensitive species, primarily *Kloeckera apiculata* (99%) [12]. Other species are also found in smaller proportions, such as *Saccharomyces cerevisiae*, which increase rapidly as aeration occurs during the onset of winemaking, with a simultaneous rise in ethanol concentration, subsequently declining due to the mortality that takes place between the middle and final phases of fermentation [12]. In this respect, Sirén et al. [13] studied how microbial communities vary by vineyard, alcoholic fermentation, and between musts that successfully complete fermentation and those that become "stuck" in the process. Vicente et al. [14] studied how rare non-*Saccharomyces* yeasts could influence the mixed fermentation of wines by interacting with *S. cerevisiae* and found that they affected the co-culture growth parameters and chemical profile of the wine.

Morrison-Whittle and Goddard [15] used sequencing as a molecular technique to investigate and quantify the microbial community in vineyard soil, grape skin, juice and must. The skin microbiota increasingly resembled that of the must as fermentation progressed, varying between the regions studied. Lai et al. [16] explored the enological characteristics of non-*Saccharomyces* yeast strains and their influence on the complexity of aromatic compounds during the fermentation process and concluded that they were unable to maintain themselves until the end of fermentation because they did not tolerate high ethanol levels. However, they did obtain good ester production.

Wine production using indigenous *S. cerevisiae* strains as fermentation initiators is increasingly explored by producers seeking to provide typicality to their beverages [17–27]. However, wine producers in the SFV, as in most winemaking regions of Brazil and the world, use several commercial strains from other regions. There are no regional indigenous strains commercialized for wine production that could contribute to typicality. There are essentially only two large international companies that dominate the world yeast market, where the yeasts are isolated, selected and commercialized in France, Australia (South) and the USA, all of which have temperate climates. As such, studies are needed to show the viability of indigenous yeasts, mainly from new winemaking regions, such as the SFV, located in a tropical semiarid climate.

Ponzzes-Gomes et al. [28] selected five different indigenous *S. cerevisiae* strains in the fermented must of five grape varieties cultivated in the SFV. These five grapes varieties are also used to produce wines in the SFV and were collected in 2008 on only one farm in the region. However, in the present study, we expand the research to four different vineyards, and the grapes varieties Syrah, Tempranillo, Petit Verdot, and Chenin blanc, which were not sampled by Ponzzes-Gomes et al. [28]. We will provide an overview of the occurrence of indigenous *S. cerevisiae* strains with potential for winemaking in the SFV. Thus, the aim of the present study was to isolate, identify and characterize yeasts of fermented must from grapes used in wine production in the SFV region from four vineyards, in the implementation

phase of the geographic indication for fine still and sparkling wines, in addition indigenous yeasts are not yet used in the region, but wineries are interested in starting using, looking for the distinctiveness and typicality of the commercial products.

Materials and methods

Sampling and yeast isolation

Grape samples were harvested between 2011 and 2013 in the semiarid SFV region (Brazil) at four vineyards, located in different cities, in two states (Pernambuco and Bahia). The vineyards are located in Casa Nova, Bahia, and Lagoa Grande and Santa Maria da Boa Vista, Pernambuco. Red (Syrah, Tempranillo, Cabernet Sauvignon, Petit Verdot), and white grapes (Chenin blanc) were used in the present study. The grapes were harvested at ten sampling points (0.124 miles apart) of each vineyard. In 2011, samples of Syrah (November and December) and Tempranillo (December) grapes were collected from different vineyards (W2, W1 and W1, respectively). In December 2012, samples of Syrah and Chenin blanc grapes were collected from the same vineyard (W2), Petit Verdot grapes were collected from the different vineyard (W3) and Cabernet Sauvignon from a different one (W3 and W1). In 2013, Cabernet Sauvignon (February), Tempranillo and Chenin blanc grapes (March) were obtained from different vineyards (W1, W1 and W4, respectively).

Samples of two kilograms of grapes were aseptically collected and placed in sterile plastic bags, transported under refrigeration (5 ± 2ºC) and shipped for processing to the Applied Microbiology Laboratory of the Federal University of Sergipe, within 48 h of collection. Grapes from each sampling point were crushed and the grape juice fermented at 25 ± 2 °C in 750 ml bottles containing 500 ml of must, closed with gauze stoppers and manually shaken twice a day [29]. Spontaneous fermentation was monitored and when total sugars became stable (measured in °Brix with a portable refractometer model ATC Range 0–32%) and serial decimal dilutions were prepared using the same diluent. Aliquots (100 µL, in triplicate) of appropriate decimal dilutions were spread on YM agar (yeast malt agar: 1% glucose, 0.3% malt extract, 0.3% yeast extract, 0.5% peptone, 2.0% agar and 0.01% chloramphenicol) to obtain *S. cerevisiae* strains and other yeasts, in which dilutions were plated 10^{-4} and 10^{-6}, and on lysine agar (1.17% yeast carbon base, 0.056% lysine, 2.0% agar and 0.01% chloramphenicol) to isolate only non-*Saccharomyces* yeasts, in which dilutions were plated 10^{-2} and 10^{-4}.

The plates were incubated at 25 °C for three to seven days. After growth was noted, plates containing between 30 and 300 yeast colonies were examined. From each grape fermentation, when possible, at least five randomly selected colonies of the most prevalent yeast morphotype were purified by restreaking on the YM agar plates (with the aim of isolating *S. cerevisiae* yeasts). The yeasts that displayed different morphologies on the YM agar and lysine agar plates (with the aim of isolating non-*Saccharomyces* yeasts) were counted, selected for isolation, purification and subsequent identification. All isolates were stored in GYMP broth (2% glucose, 0.5% yeast extract, 0.5% malt extract, 0.2% Na_2PO_4) with 20% glycerol at -80 °C for subsequent identification.

Identification and molecular characterization of yeasts isolated from fermented must

The yeasts were grouped preliminarily according to various characteristics, including colony morphology and standard growth tests on different carbon and nitrogen sources [30], for *S. cerevisiae* yeast: Yeast Nitrogen Base (YNB) with glucose, sucrose, xylose, sorbitol, mannitol and with cycloheximide, and grown in Yeast Carbon Base (YCB) with lysine, and for non-*Saccharomyces*: YNB with glucose, L-sorbose, maltose, celebiose, melizitose, D-xylose, D-mannitol, M-inositol, L-arabinose; 10% NaCl, cycloheximide and YCB with nitrate. All isolates with identical morphological and physiological characteristics were grouped together and subjected to PCR fingerprinting using the Intron Splice Site primer EI-1 (5'-CTGGCTTGGTGTA TGT-3') [31]. Yeast strains with identical DNA banding patterns were grouped and putatively considered to belong to the same species [32]. At least 50% of the isolates from each EI-1 PCR group were subjected to sequence analysis of the internal transcribed spacer (ITS-5.8 S) region and the D1/D2 domains of the large subunit rRNA gene, as described below. Physiologically distinct strains with unique EI-1 PCR banding patterns were also selected for direct identification by sequencing of the ITS-5.8 S region and D1/D2 domains, which were PCR-amplified directly from whole cells, as previously described [33]. The primers used were ITS-1 (5'-TCCGTAGGTGAACCTTGCGG-3') and NL-4 (5'GGTCCGTGTTTCAAGACGG3'), as described by Lachance et al. [34].

Species identifications were performed by analysis of the sequences of the ITS-5.8 S region and the D1/D2 variable domains [33]. The amplified DNA was cleaned and sequenced in an ABI 3130 Genetic Analyzer automated sequencing system (Life Technologies) using BigDye v3.1 and POP7 polymer. The sequences were assembled, edited, and aligned with the program MEGA7 [35]. They were

compared with annotated yeast sequences in the GenBank database using the Basic Local Alignment Search Tool (BLAST at http://www.ncbi.nlm.nih.gov/) (supplementary material) [36].

Molecular differentiation of *S. Cerevisiae* strains

All isolates previously identified as *S. cerevisiae* were compared using restriction endonuclease analysis of mitochondrial DNA (mtDNA-RFLP) to distinguish among different *S. cerevisiae* strains, as described by Querol et al. [37–39]. The mitochondrial DNA was digested with *HinfI* restriction endonuclease (Invitrogen, Carlsbad, CA, USA). To determine if the strains found in this study were indigenous, the mitochondrial DNA restriction profiles of *S. cerevisiae* isolates were compared with the profiles of the six commercial *S. cerevisiae* strains commonly used in the São Francisco Valley region. Commercial strains tested were *S. cerevisiae* (Maurivin AWRI 796) denominated C1 in the present study; *S. cerevisiae* (var. *bayanus*) (Lalvin R2) (C2); *S. cerevisiae* (var. *bayanus*) (Mycoferm Cryo SP) (C3); *S. cerevisiae* (var. *bayanus*) (PDM Maurivin) (C4); *S. cerevisiae* (Mycoferm) (C5); *S. cerevisiae* (Fermol Rouge) (C6). Winery 1 (Casa Nova/BA) uses Maurivin AWRI 796 (C1), Mycoferm Cryo SP (C3) and Maurivin PDM (C4) commercial yeasts; Winery 2 (Lagoa Grande/PE) Maurivin AWRI 796 (C1), Maurivin PDM (C4) and Lalvin R2 (C2); Winery 3 (Santa Maria/PE) Maurivin PDM (C4); and Winery 4 (Lagoa Grande/PE) Mycoferm Cru 05 (C5).

Results and discussion

Molecular characterization of *S. Cerevisiae* strains

A total of 368 isolates were obtained, 259 of which were identified as *S. cerevisiae* and 109 as non-*Saccharomyces* yeasts. Analyses using the RFLP-mtDNA technique with *S. cerevisiae* isolates identified 184 as indigenous and 75 as commercial strains (Fig. 1). Figures 2 and 3 show the diversity of the mtDNA restriction profiles of indigenous and commercial *S. cerevisiae* strains. Twenty-two different mtDNA restriction profiles were found for the 184 indigenous *S. cerevisiae* strains (71% of the isolates of this species), a substantial number in a little studied region. The 75 commercial isolates (29% of *S. cerevisiae* isolates) were identified as belonging to two different commercial profiles (C1 and C3/C4). This technique was also applied with the six commercial *S. cerevisiae* yeasts, two of which (C3 and C4) exhibited the same mtDNA restriction profile (Fig. 2-B). Similar results were observed by Fernández-Espinar et al. [40] and Ponzzes-Gomes et al. [28]. These authors found that yeasts commercialized as different strains displayed the same band profile when compared using this molecular technique. The presence of commercial strains in spontaneous wine fermentation, as found in the present study, has been reported by a number of authors [41–44]. This may be due to dispersal in vineyards of yeasts commercially used in wineries through the disposal of winemaking remnants from processes such as destemming, pressing and crushing, aimed at returning nutrients to the soil. Ramírez et al. [45] found that vineyard soil is a natural source of grape contamination that may increase yeast biodiversity during

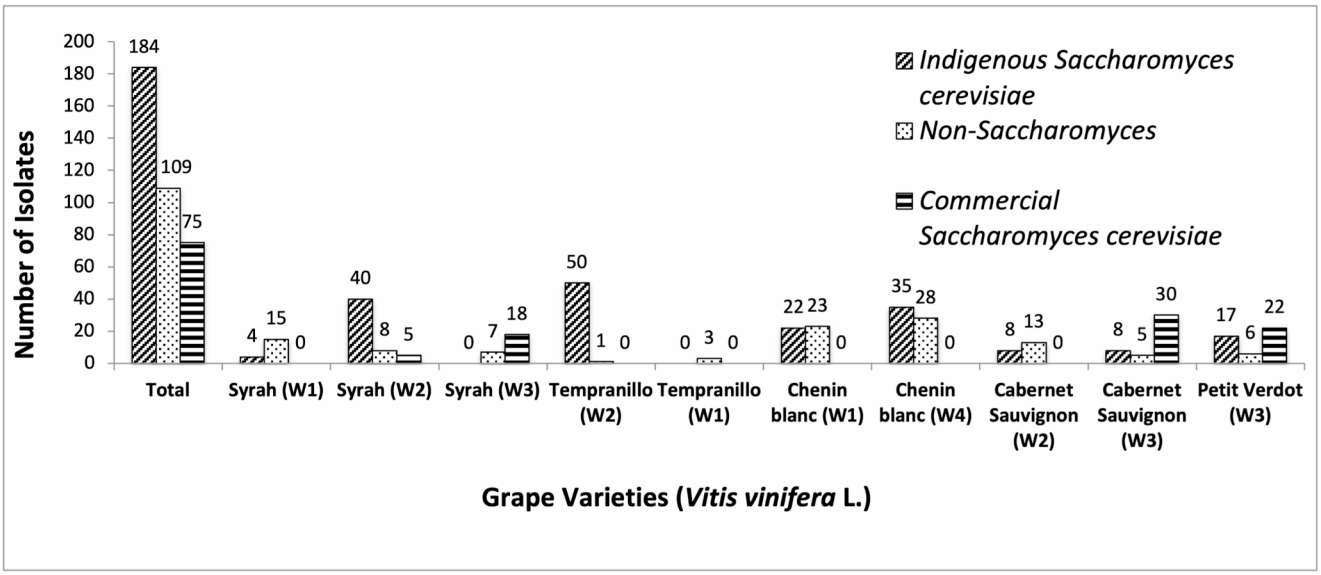

Fig. 1 Number of indigenous *Saccharomyces cerevisiae*, commercial *S. cerevisiae* and non-*Saccharomyces* yeast isolates in must fermented from grapes from each variety studied. Winery 1 (W1); Winery 2 (W2); Winery 3 (W3); Winery 4 (W4)

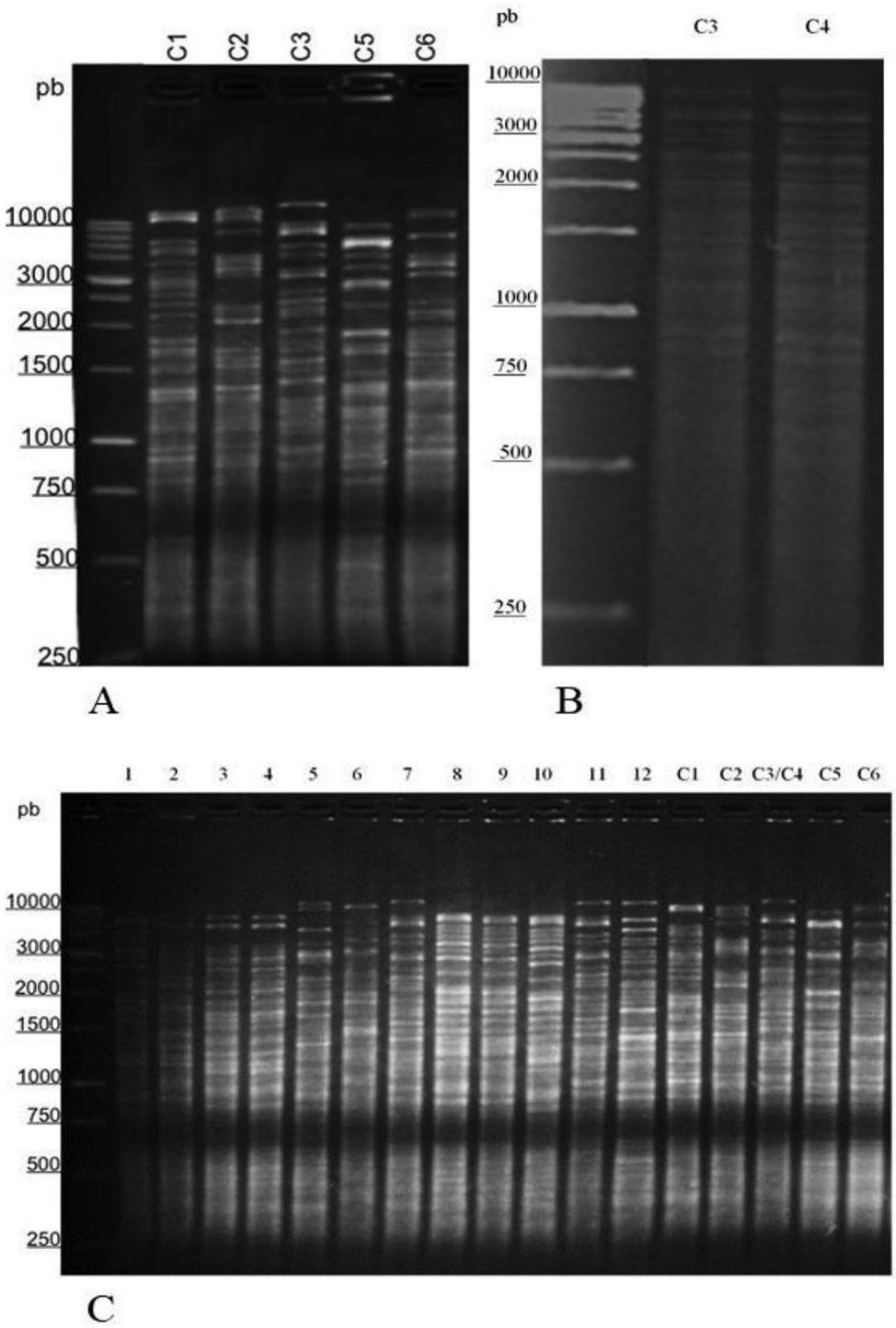

Fig. 2 Mitochondrial DNA restriction profiles of indigenous and commercial *Saccharomyces cerevisiae* strains for wine production in the SFV. Molecular marker 1 kb DNA ladder. Figure 4**A**: C1 (Maurivin AWRI 796), C2 (Lalvin R2), C3 (Mycoferm Crio SP), C4 (Maurivin PDM), C5 (Mycoferm Cru 05), and C6 (Fermol Rouge) correspond to commercial *S. cerevisiae* profiles P23, P24, P25, P25, P26 and P27, respectively. Figure 4**B**: depicts the two commercial *S. cerevisiae* strains (C3 and C4) that exhibited the same band profile. Figure 4**C**: 1 to 12 correspond to indigenous *S. cerevisiae* with profiles P1, P2, P2, P2, P3, P4, P5, P6, P6, P6, P5, P7, respectively

spontaneous fermentation and affect the organoleptic quality of the wine.

Table 1 shows the percentage of profiles per winery, where the yeasts were isolated, with winery 2 obtaining the highest (39.8%), followed by winery 3 (36.7%). However, 27% of this percentage corresponds to the isolated commercial strains.

According to Table 1, the highest percentage of mtDNA restriction profiles was exhibited by the commercial strain C1 (profile P23), which was 17.4% of the total number of *S. cerevisiae* isolates, followed by the indigenous strains of mtDNA profiles P6 and P12 (14 and 12% of the isolates, respectively) and the commercial strain C3/C4 (profile P25, 11.6%). Strains with mtDNA restriction profiles corresponding to commercial yeasts used in the region may be due to

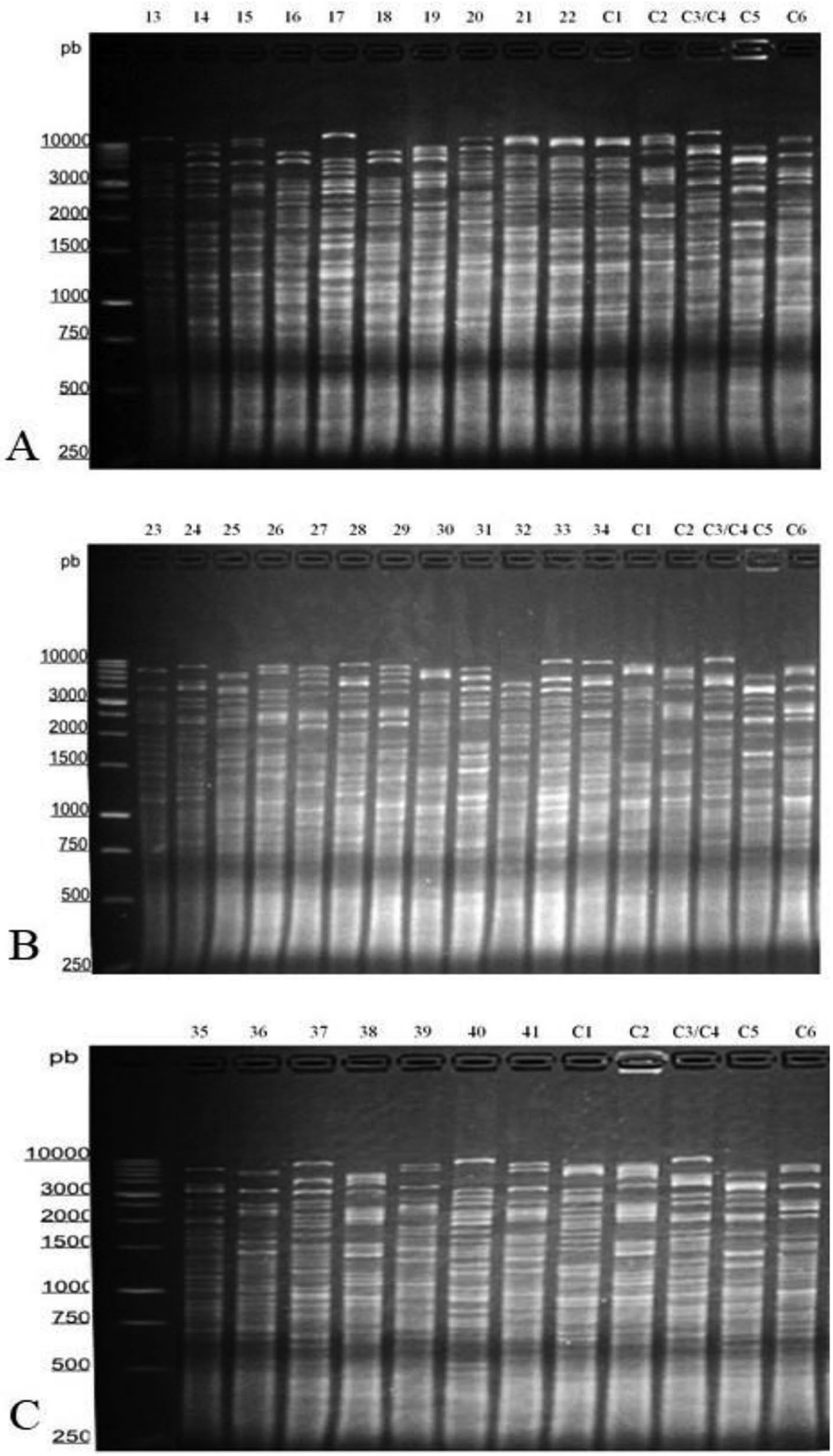

Fig. 3 Mitochondrial DNA restriction profiles of indigenous and commercial *Saccharomyces cerevisiae* strains isolated from the fermented must of grapes (*Vitis vinifera* L.) from the SFV. Molecular marker 1 kb DNA ladder. C1 (Maurivin, AWRI 796), C2 (Lalvin R2), C3 (Mycoferm Crio SP), C4 (Maurivin PDM), C5 (Mycoferm Cru 05), and C6 (Fermol Rouge) correspond to commercial *S. cerevisiae* profiles P23, P24, P25, P26, and P27, respectively; 13 to 22 represent *S. cerevisiae* profiles P8, P9, P10, P11, P8, P11, P12, P13, P23, and P23, respectively. Yeasts 21 and 22 correspond to the same commercial yeast profile C1 (P23); 23 to 34 to *S. cerevisiae* profiles P14, P15, P16, P17, P18, P15, P18, P23, P9, P19, P20, and P6, respectively. Yeast 30 represents the same profile as commercial yeast C1 (P23); 35 to 41 to *S. cerevisiae* profiles P25, P21, P7, P22, P3, P8, and P10, respectively. Yeast 35 corresponds to the same commercial yeast profile C3/C4 (P25)

Table 1 Number of isolates and percentage of mitochondrial DNA restriction profiles (RFLP-mtDNA) of indigenous and commercial *Saccharomyces cerevisiae* isolated in each winery of the São Francisco Valley (wineries 1–4). Profiles P1 and P22 correspond to the indigenous *S. Cerevisiae* isolates and P23 to P27 to the commercial *S. Cerevisiae* isolates (yeasts C1 to C6, respectively, with yeasts C3 and C4 exhibiting the same profile (P25)

Profiles	Winery 1	Winery 2	Winery 3	Winery 4	Profile total (%)	Number of isolates
P1	6.2	———	———	———	6.2	16
P2	3.5	———	———	———	3.5	9
P3	2.3	1.5	———	———	3.8	10
P4	1.9	———	———	———	1.9	15
P5	4.2	———	———	———	4.2	11
P6	14.7	———	———	———	14.7	38
P7	2.3	———	0.4	———	2.7	7
P8	0.4	3.5	———	———	3.9	10
P9	———	2.0	0.4	———	2.4	6
P10	0.4	1.1	———	———	1.5	4
P11	———	1.9	1.1	———	3	8
P12	———	———	———	12.0	12	31
P13	———	———	———	0.4	0.4	1
P14	———	———	———	1.1	1.1	3
P15	———	———	3.5	———	3.5	9
P16	———	———	0.8	———	0.8	2
P17	———	———	1.1	———	1.1	3
P18	———	———	0.8	———	0.8	2
P19	———	———	0.4	———	0.4	1
P20	———	———	0.4	———	0.4	1
P21	———	———	0.8	———	0.8	2
P22	1.9	———	———	———	1.9	5
P23	1.9	———	15.5	———	17.4	45
P24	———	———	———	———	0	0
P25	———	———	11.6	———	11.6	30
P26	———	———	———	———	0	0
P27	———	———	———	———	0	0
Total (%)	39.7	10	36.8	13.5	100	259
Number of isolates	26	103	95	35	259	

the disposal of winemaking waste, enabling these yeasts to colonize the grapes. The higher occurrence of commercial strain C1 suggests that it is well adapted to the vineyard conditions and one of the most widely used commercial strains in the region, being more prevalent than its indigenous counterparts. In this sense, it was expected that this C1 commercial strain would be the most prevalent among the commercial strains.

Capece et al. [19] studied the diversity of *S. cerevisiae* isolated after maturation of traditional Georgian wines. The authors found 23 different mtDNA restriction profiles in 70 indigenous *S. cerevisiae* isolates. In a study conducted in El Penedès, Spain, Esteve-Zarzoso et al. [46] found 22 mtDNA profiles in 68 *S. cerevisiae* isolates obtained from spontaneous wine fermentation, the same number of indigenous profiles found in the present study. However, the percentage of profiles identified in the indigenous *S. cerevisiae* isolates (32.35%) was higher than that obtained here (12%). Ponzzes-Gomes et al. [28] found 155 *S. cerevisiae* isolates of fermented must from SFV grapes and identified four different mtDNA restriction profiles belonging to indigenous strains, and one to a commercial *S. cerevisiae* strain. Of these, 71 corresponded to indigenous *S. cerevisiae* and 64 to the mtDNA restriction profile of commercial *S. cerevisiae*. These authors found fewer mtDNA profiles of indigenous *S. cerevisiae* than that observed in the present study. Ponzzes-Gomes et al. [28] isolated yeasts in 2008 from grapes collected at a vineyard that had been operating for about three years. This time period may not have been sufficient for colonization and adaptation of a larger number of indigenous *S. cerevisiae* strains in the grapes. According to Pretorius [47] and Schuller et al. [29], one of the factors that influences yeast microbiota in grapes is the age of the vine, which means it will have greater diversity. Schuller et al. [29] studied the ecology of *S. cerevisiae* strains from a white wine vineyard in Portugal, for three consecutive years. Of the 54 spontaneous fermentations studied, the authors obtained 1,620 *S. cerevisiae* isolates, with 297 different mtDNA profiles. The fact that these vineyards were already well established, and fermentations were monitored

in several different periods, may explain the number of distinct mtDNA profiles found in their study. Ortiz et al. [21] isolated 240 *Saccharomyces* spp. at different fermentation stages of wines produced at a family-run winery in Spain and characterized these yeasts using mtDNA restriction analysis. The authors found 21 different molecular profiles.

In the present study, Cabernet Sauvignon grapes exhibited the highest occurrence of different mtDNA restriction profiles for indigenous *S. cerevisiae* strains, with 10 distinct profiles (Figs. 2, 3 and 4), the most prevalent being profile P22, with 5 isolates. However, it displayed the lowest number of isolates (16 = 8.7%). Chenin blanc grapes showed six mtDNA profiles in the 57 *S. cerevisiae* isolates analyzed, the most numerous being P12, with 31 isolates, corresponding to more than half the isolates of this variety, suggesting that this yeast strain may be better adapted to colonize this grape in the SFV than the other *S. cerevisiae* strains. Syrah grapes displayed six different *S. cerevisiae* mtDNA profiles among the 44 isolates analyzed, the most prevalent being mtDNA profile P1, represented by 12 isolates. Tempranillo grapes exhibited five distinct *S. cerevisiae* mtDNA profiles among the 50 isolates analyzed, P6 being the most numerous with 30 isolates. Petit Verdot grapes had five different profiles in the 17 *S. cerevisiae* isolates found, P15 being the most prevalent with 7 isolates. The number of indigenous wild *S. cerevisiae* strains isolated from this region was high, and these yeasts could be further studied as selected starter strains for wine production in the SFV.

The highest number of isolates from commercial *S. cerevisiae* strains (75 of the 259 *S. cerevisiae* isolates studied) was found in winery 3, with 70 isolates, including 18 isolates (commercial strain C1) in the Syrah grapes, 22 (Commercial strain C1) in Petit Verdot grapes and 30 (commercial strains C3/C4) in Cabernet Sauvignon grapes. The fewest commercial *S. cerevisiae* isolates were found in winery 2, with 5 isolates, all representing the C1 commercial *S. cerevisiae* strain. No commercial *S. cerevisiae* strains were found in vineries W1 and W4, they probably were unable to adapt on the ground or the sampling was not sufficient to cover the range.

Identification of non-*Saccharomyces* yeasts

According to the sequencing results of region ITS-5.8 S and domains D1/D2 of the largest subunit rRNA gene, the 109 non-*Saccharomyces* isolates corresponded to the following species: *Pichia kudriavzevii* (77.1%), *P. membranifaciens* (3.7%), *P. occidentalis* (0.9%), *P. manshurica* (0.9%), *Meyerozyma caribbica* (1.8%), *M. guillermondii* (0.9%), *Zygosaccahromyces bailii* (1.8%), *Torulaspora delbrueckii* (0.9%) and *Schizosaccharomyces pombe* (12%) (Table 2). The proportion of these species varied in the different fermentation grape musts as well as in each vineyard where isolations occurred. *Pichia kudriavzevii* was isolated in all fermentation musts studied, with a high occurrence in vineyard 1(Table 2).

P. kudriavzevii and *S. pombe* were the predominant non-*Saccharomyces* species in this study. These yeasts grow at high temperatures and may be associated with the microclimate of the region studied. The predominant

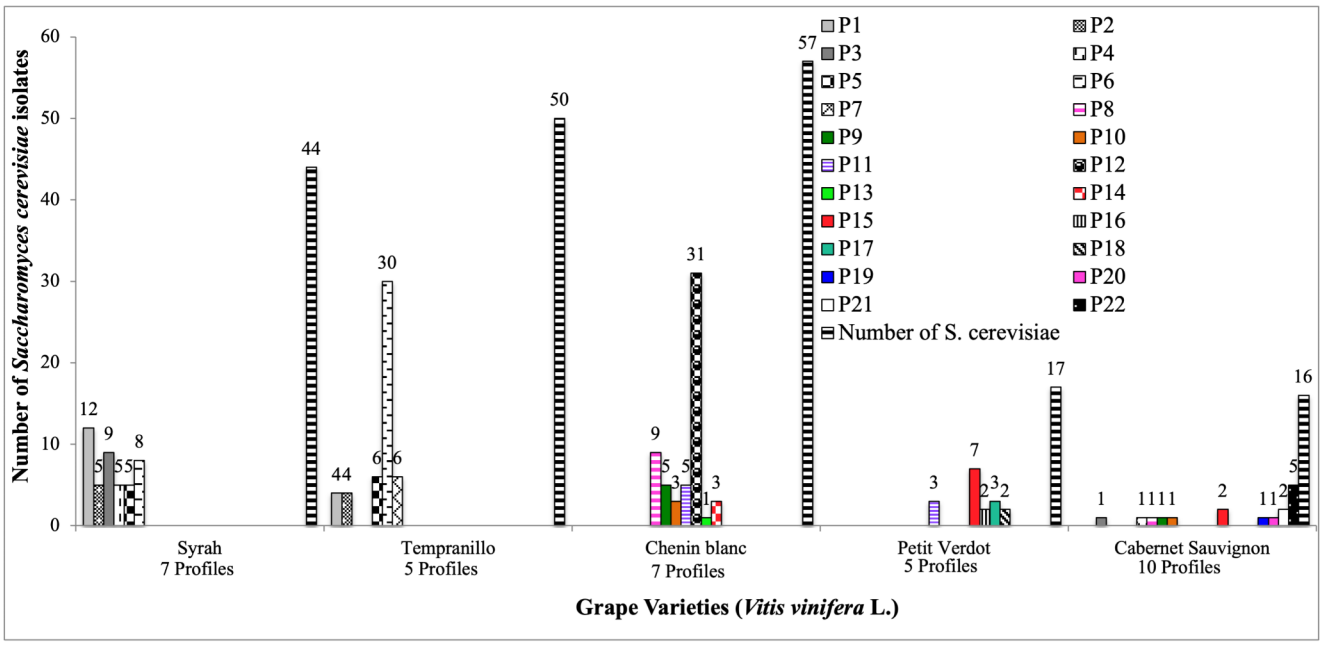

Fig. 4 Number of isolates from each mitochondrial DNA restriction profile of indigenous *Saccharomyces cerevisiae* from the fermented must of São Francisco Valley grapes, according to the varieties studied

Table 2 Percentage of each non-*Saccharomyces* species identified and isolated at each São Francisco Valley winery in this study (wineries 1–4)

Species	Winery 1	Winery 2	Winery 3	Winery 4	%
Pichia kudriavzevii	34.86	17.43	11.93	12.84	77.1
Pichia occidentalis	------	------	------	0.92	0.9
Pichia membranifaciens	------	0.92	2.75	------	3.7
Pichia manshurica	------	------	------	0.92	0.9
Meyerozyma caribbica	1.83	------	------	------	1.8
Meyerozyma guilliermondii	------	0.92	------	------	0.9
Zygosaccharomyces bailii	------	------	1.83	------	1.8
Torulaspora delbrueckii	0.92	------	------	------	0.9
Schizosaccharomyces pombe	0.92	------	8.26	2.75	11.9
% of species (per winery)	38.53	19.27	24.77	17.43	100.0

non-*Saccharomyces* species on the surface of the grapes, and consequently in the initial fermentation stages in traditional (cold) winemaking regions, are generally *Hanseniaspora uvarum* (the highest proportion) and species of the genera *Candida*, *Pichia*, *Rhodotorula* and *Kluyveromyces* [17, 22, 24, 48–50]. However, Ponzzes-Gomes et al. [28] isolated yeasts from grape musts of a winery in the SFV, and the prevalent non-*Saccharomyces* species were *Rhodotorula mucilaginosa*, *P. kudriavzevii*, *C. parapsilosis*, *M. guilliermondii*, *Wickerhamomyces anomalus*, *K. apis*, *P. manshurica*, *C. orthopsilosis* and *Starmerella bacillaris.*

The present study shows the occurrence of different species of non-*Saccharomyces* yeasts in the vineyards studied in relation to the previous study by of Ponzzes-Gomes et al. [28]. *Pichia kudriavzevii*, *P. manshurica*, and *M. guilliermondii* were the only species isolated in both studies. These differences may be related to the chemical composition of each grape variety and the different climate conditions of the regions where the vineyards are located. Moreover, the microclimate in the region of the vineyard may influence yeast composition.

This study demonstrated the presence of *S. cerevisiae*, non-*Saccharomyces* and indigenous yeasts, as well as commercial strains in the grapes collected in four SFV vineyards. Comparative trials should be conducted in future studies, inoculating commercial yeasts with must prepared without the addition of commercial strains, only their indigenous counterparts, in order to determine their influence on the physicochemical and sensory composition of the wines, with a view to assessing the quality and typicality of tropical wines from Northeastern Brazil.

Conclusion

The fermented must of the five grape varieties collected at four vineyards located in a semiarid climate in the SFV, Northeastern Brazil, were appropriate substrates to isolate non-*Saccharomyces* species, and commercial and indigenous *S. cerevisiae* strains. These indigenous strains were more numerous than the commercial strains used in the winemaking processes of the region, suggesting that they are able to colonize grapes efficiently.

Analysis of the mitochondrial DNA restriction profiles (RFLP-mtDNA) of the 259 *S. cerevisiae* isolates obtained showed the occurrence of 22 different molecular profiles in the indigenous *S. cerevisiae* strains and two profiles corresponding to the commercial strains used in the region. Thus, it was possible to detect the presence of commercial strains introduced in the vineyards of the region and differentiate them from their indigenous counterparts. In addition, two of the *S. cerevisiae* commercial strains used in wine production exhibited the same mtDNA restriction profile, despite being commercialized with different names by different manufacturers.

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Declarations

Conflict of interest The authors declare that there are no conflicts of interest regarding the publication of this paper.

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