OENO One
VINE AND WINE OPEN ACCESS JOURNAL
By IVES International Viticulture & Enology Society

DOI:10.20870/oeno-one.2022.56.2.4660
OPEN ACCESS

# Yeast diversity in Cabernet-Sauvignon and Merlot grapes grown in the highlands of Southern Brazil

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*Associate editor:*
Patricia Taillandier

*Received:*
26 March 2021

*Accepted:*
7 April 2022

*Published:*
3 May 2022

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## ABSTRACT

Considering the influence of natural yeasts on wine production and the organoleptic properties of the final product, the objective of the present study was to evaluate the diversity of yeasts in Cabernet-Sauvignon and Merlot (*Vitis vinifera* L.) grown in the highlands of the Northeast region of Rio Grande do Sul State, "Serra Gaúcha", Brazil. Grape samples were collected from commercial vineyards between February and March in the 2017, 2018 and 2019 vintages. One hundred sixty-six isolated yeasts were classified at the species level by D1/D2 domain of 26S rRNA sequencing. A total of 31 yeast species were identified. The most prevalent species were *Hanseniaspora uvarum, Issatchenkia terricola, Saturnispora diversa and Starmerella bacillaris*. These same yeasts were the most frequent regardless of the year evaluated. The results indicated that there is a great diversity of yeast species in grapes cultivated in the highlands of Southern Brazil. However, the yeast communities remain similar in CabernetSauvignon and Merlot grapes the analysed parameters (grape variety/vintage) are not interfering in the yeast populations found in the region highlands of Rio Grande do Sul, "Serra Gaúcha", Brazil. This study enabled the knowledge of the yeast populations present in the region and their variations during the harvesting of wine grapes, showing that there is a majority pattern of species independent of the harvest.

**KEYWORDS:** grapes, yeast diversity, variety, vintage

## INTRODUCTION

Grape must and berry surface is a rich environment for various yeast species (Cioch-Skoneczny *et al.,* 2021). Worldwide surveys indicate that sound grapes and freshly crushed grape juice harbour a diversity of yeast species, including representatives of the genus *Hanseniaspora, Metschnikovia, Brettanomyces, Candida, Debaryomyces, Issatchenkia, Kluyveromyces, Pichia, Saccharomyces, Saccharomycopsis, Saccharomycodes, Torulaspora, Zygosaccharomyces*, among others (Bezerra-Bussoli *et al.,* 2013; Jolly *et al.,* 2014). On the berry surface, yeast population and diversity are affected by many intrinsic and extrinsic factors like the ripening stage, grape variety, geographic region, climatic conditions, agricultural practices, and the health status of grapes (Mortimer and Polsinelli, 1999; Barata *et al.,* 2012). It is generally agreed that the surfaces of mature grapes have yeast populations in the order of $10^3$–$10^5$ colony-formed units (CFU/g), but these can reach more than $10^6$ CFU/g in damaged or fungal infected berries (Mortimer and Polsinelli, 1999; Barata *et al.,* 2012).

Wine results from a complex biochemical process that starts with grape harvesting, followed by alcoholic and malolactic fermentation, wine ageing and bottling (Romano *et al.,* 2003). Different microorganisms, and particularly yeasts, are involved and play a key role in the production of wine, mostly in alcoholic fermentation (Padilla *et al.,* 2016). Wine fermentation can either be spontaneous, conducted by yeasts present on the grapes and in the winery, or inoculated with commercial strains of *Saccharomyces*. In the traditional winemaking process, autochthonous non-*Saccharomyces* yeasts start the alcoholic fermentation and are progressively substituted by *Saccharomyces* that finish wine production. The influence of non-*Saccharomyces* yeasts on wines can either be positive or negative depending on yeast species and their population dynamics during the wine-making process (Fleet, 2008; Morata *et al.,* 2020). The addition of sulphite and the inoculation with selected commercial yeast minimize the influence of wild yeasts, but it may not necessarily prevent the growth and metabolic activity of wild yeasts (Lambrechts and Pretorius, 2000; Andorrà *et al.,* 2019).

In recent years, the demand for natural fermentation wines has increased, so it is important to know the microbiota of yeasts present in grapes. Some strains of apiculate yeast (*Hanseniaspora ssp.*) can bring undesirable characteristics such as accentuated acetic acid production. In addition to apiculate yeasts and fermentative yeasts, deteriorating yeasts such as *Brettanomyces intermedia* and *Pichia membranifaciens* may appear after fermentation, causing serious defects in the final product (Romano *et al.*, 2003; Ramírez-Castrillón *et al.,* 2017). More and more, the regional microbiota has been assessed for the production of wines, bringing local characteristics to the final product and defining a fermentative "*terroir*".

The role of the different species and strains of yeasts present on grapes regarding the final product is dependent on the winemaking techniques and the metabolic characteristics of the yeasts present. Species and native strains have become particularly interesting within the context of "terroir", which involves both the characteristics of cultivars, edaphoclimatic, and those associated with fermentative processes, including microorganisms, which together determine the peculiarities of a wine product (Bokulich *et al.,* 2014; Moschetti *et al*., 2016; Grangeteau *et al.,* 2017; Börlin *et al.,* 2020).

According to the Brazilian Wine Institute, in 2019, the highlands of the northeast region of Rio Grande do Sul, known as the "Serra Gaúcha" region, produced 41.6 million litres of wine, accounting for approximately 85 % of the Brazilian wine production (IBRAVIN, 2019). This mountainous region (400–800 m), located between 28° 30'S and 29° 30'S and 50° 45'W and 52° 00'W, has a peculiar subtropical highland climate (Cfb—Köppen classification) defined as wet temperate and warm with temperate nights (Tonietto and Carbonneau, 1999). The *Vitis vinifera* red varieties most cultivated in this region are Cabernet-Sauvignon and Merlot, representing approximately 50 % of fine red wines produced (de Mello and Machado, 2018). Despite the social and economic importance of viticulture in southern Brazil, there are few studies on the diversity and ecology of yeasts in this ecosystem (Ramírez-Castrillón *et al.,* 2014; Silva *et al.,* 2016; Ramírez-Castrillón *et al.,* 2017).

Considering the influence of natural yeasts on wine production and their contribution to the organoleptic properties of the final product, the objective of the present study was to evaluate the diversity of yeasts in Cabernet-Sauvignon and Merlot (*Vitis vinifera* L.) grapes, the most important red varieties used for the production of fine wines in the highlands of the Northeast region of Rio Grande do Sul State, Brazil.

## MATERIAL AND METHODS

### 1. Grape sampling

Grape samples were collected during harvest (between February and March) in 2017, 2018 and 2019 vintages. Two varieties, Cabernet-Sauvignon and Merlot (*Vitis vinifera* L.), were sampled from 6 representative vineyards in the Northeast region of Rio Grande do Sul State ("Serra Gaúcha"), Southern Brazil (Figure 1). Samples were collected in the same vineyards for three consecutive years (2017, 2018 and 2019). The vineyards (5 to 7 years old) were conducted in trellis systems, producing between 8 and 12 T/ha. All the vineyards adopted conventional management.

The region is localized at 29°S and 51°W and characterized as highlands at an elevation of 600–800 m, with an annual average rainfall of 1700 mm, an average temperature of 17.2 °*C* and a relative air humidity of 76 %. Two kg of grapes were randomly and aseptically collected from at least 10 vines of each variety and vineyard. Samples were immediately transported to the laboratory, where healthy and undamaged grapes were selected and processed. All samples

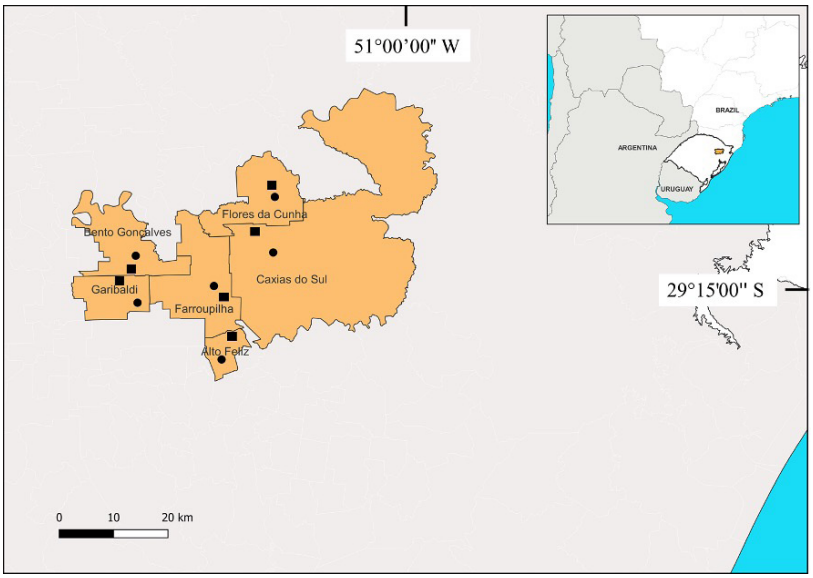



**FIGURE 1.** Six sample collection locations in the "Serra Gaúcha" region, Brazil.

(■) vineyard location (●) counties.

collected did not show any visible fungal contaminants or changes, having good sanitary quality.

## 2. Yeast isolation and enumeration

For each sample, one kg of grapes was homogenised in a blender (30 s). Homogenised samples were diluted ($10^{-1}$ to $10^{-5}$) in saline solution (0.9 % w/v NaCl). Aliquots of 0.1 ml from the original and serially diluted samples were plated in triplicate on Wallerstein Laboratory nutrient agar (WL; Oxoid, England) supplemented with 10 mg/L gentamicin and 100 mg/L cefoxitin to inhibit bacterial growth. Plates were incubated at 28 °C for 4 days. Yeast isolation and enumeration were carried out on plates from the dilutions that contained between 30 and 300 colonies. Colonies with different characteristics (colour, texture, size, shape, margin) were scored, isolated and purified through two rounds of streaking on WL medium. A minimum of six colonies representative of colony accords its morphology were purified and stored in glycerol 20 % (v/v) at –80 °C for further analysis (Li *et al.,* 2010; Bagheri *et al.,* 2015).

The prevalence of species for each sample was calculated by the presence or absence of the species in each sample. The frequency of species in each sample was calculated by the number of colonies forming units (CFUs) of each species divided by the total number of CFUs (Brysch-Herzberg and Seidel, 2015).

## 3. Chemical analysis

Reducing sugar content, titratable acidity, Brixº and pH of grape musts were determined according to the standard methods recommended by OIV (2009). All experiments were conducted in triplicate.

## 4. Molecular identification of yeast isolates

The identification of yeast isolates (a total of 498 isolates, three of each colony morphotype per grape sample) at the species level was carried out by the forward and reverse sequencing of the D1/D2 domain of the 26S rDNA gene (Kurtzman and Robnett, 1998).

Isolates were grown in 2 ml of YEPD for 24 h at 28 °C and their genomic DNA was extracted using a 200 mM lithium acetate solution + 1 % SDS, as described by Lõoke *et al.* (2011). The 26S rDNA D1/D2 region was amplified by a PCR using the primers NL1 (5′-GCATATCAATAAGCGGAGGAAAAG-3′) and NL4 (5′-GGTCCGTGTTTCAAGACGG-3′) according to Kurtzman and Robnett (1998). The amplified products were then purified by the enzymatic method ExoSAP with Exo1 (exonuclease 1) and SAP (Shrimp alkaline phosphatase) and sequenced using the ABI BigDye terminator sequencing kit on a 3500 Series Genetic Analyzer (ThermoFischer®).

Nucleotide sequences were assessed and compared using the nBLAST algorithm (Basic Local Alignment Search Tool) with the sequences deposited in the NCBI data bank (http://www.ncbi.nlm.nih.gov). A cut-off of 99 % identity with the sequences of type strains was used to identify the isolates (Ramírez-Castrillón *et al.,* 2017).

## 5. Statistical analysis of biodiversity

The diversity of yeast species was estimated using the average of each grape variety per year, using the Shannon-Wiener ecological index (*H'*) (Shannon, 2001). The Shannon index was calculated as follows:

$$H' = -\sum_{i=1}^{s} pi \ln (pi)$$

where *S* is the species richness (number of species found) and $pi = n1/N$ is the relative abundance of species, calculated as the proportion of individuals ($n$) of species $i$ with respect to the total of individuals (N).

**TABLE 1.** Physical-chemical analysis of musts and the total number of yeast populations.

| Year | Variety | °Brix | pH | Total acidity (MEq/L) | Yeast counts (CFU/ml) |
|---|---|---|---|---|---|
| 2017 | C. Sauvignon | 16.9 ± 0.6$^{c}$ | 3.3 ± 0.4$^{a}$ | 74 ± 46$^{ab}$ | 2.7 x 10$^{4}$- 5.9 x 10$^{5a}$ |
| | Merlot | 19.8 ± 0.2$^{ab}$ | 3.5 ± 0.3$^{a}$ | 70 ± 10$^{a}$ | 1.1 x 10$^{5}$ - 2.4 x 10$^{5a}$ |
| 2018 | C. Sauvignon | 19.0 ± 0.8$^{ab}$ | 3.2 ± 0.4$^{a}$ | 74 ± 26$^{a}$ | 1.9 x 10$^{5}$ - 4.2 x 10$^{5a}$ |
| | Merlot | 19.1 ± 0.9$^{a}$ | 3.4 ± 0.2$^{a}$ | 70 ± 20$^{a}$ | 1.2 x 10$^{5}$ - 3.0 x 10$^{6a}$ |
| 2019 | C. Sauvignon | 17.9 ± 0.9$^{bc}$ | 3.3 ± 0.3$^{a}$ | 100 ± 32$^{b}$ | 2.7 x 10$^{4}$- 7.2 x 10$^{5a}$ |
| | Merlot | 19.5 ± 1.0a | 3.3 ± 0.4$^{a}$ | 67 ± 23$^{a}$ | 1.2 x 10$^{4}$ - 2.6 x 10$^{5a}$ |

*All treatments were realized in triplicate.

*Different letters within the same column indicate significant differences at $P < 0.05$ by the Tukey test.

Moreover, Simpson's diversity index ($1 - D$) was employed and was calculated as follows (Simpson, 1949):

$$1 - D = 1 - \sum_{i=1}^{s} (pi)^2 = 1 \sum_{i=1}^{s} \left( \frac{N(N-1)}{N(N-1)} \right)$$

where $D$ is the Simpson's index and $S$ and $pi$ as described above.

The EstimateS software was used to calculate the rarefaction curve (Colwell and Elsensohn, 2014). In addition, the Jaccard similarity index was calculated and Bray–Curtis (Magurran, 1988), with 9,999 permutations.

One-way analysis of variance (ANOVA) was used to determine whether the must samples presented statistically significant differences. The analysis was performed using the PAST 4.01 software, using species frequencies to separate samples according to varieties and vintage.

Differences in yeast populations between grape varieties and vintage were tested by PERMANOVA (multivariate permutational analysis of variance using distance matrices) (Anderson, 2001) using PAST 4.03 (2020) software, available in https://past.en.lo4d.com/windows.

# RESULTS

## 1. Physicochemical and microbiological analysis

Samples were collected from 6 vineyards (Figure 1) each year during the harvest and we evaluated ºBrix, pH, total acidity and total yeast population (Table 1).

Factors such as the slope of the terrain, the degree of ripening of the grapes can interfere in the analysis of °Brix, pH and total acidity, as shown in the table above. Yeast populations ranged from $10^4$–$10^6$ CFU / ml. The Merlot variety presented the largest population of yeasts ($3.0 \times 10^6$) in 2018, while the smallest population ($1.2 \times 10^4$) was found in 2019, also in the Merlot variety. For the Cabernet-Sauvignon variety, the largest population was found in 2019, $7.2 \times 10^5$. In general, according to ANOVA analysis, no significant differences were found between the yeast populations according to varieties or years.

## 2. Prevalence and frequency of isolated yeasts

Considering the three years, vines and varieties, 31 yeast species belonging to 6 families were identified.

The Saccharomycetaceae family was the one with the largest number of species (*Candida apicola, Candida azyma, Candida bentonensis, Candida californica, Candida fructus, Candida oleophila, Candida railenensis, Issatchenkia terricola, Issatchenkia occidentalis, Hanseniaspora opuntiae, Hanseniaspora uvarum, Nakazawaea ishiwadae, Nakazawaea pomicola, Pichia kluyveri, Pichia manshurica, Pichia sporocuriosa, Saccharomyces cerevisiae, Saturnispora diversa, Starmerella bacillaris, Torulaspora delbrueckii* and *Zygosaccharomyces bailii*). Moreover, the Saccharomycopsidaceae family was represented by two species (*Saccharomycopsis crataegensis* and *Saccharomycopsis vini*), the Trichomonascaceae family by (*Wickerhamiella azyma* and *Zygoascus meyerae*) and the Wickerhamomycetaceae family by two species of Wickerhamomyces (*W. ciferrii* and *W. sydowiorum*). The Debaryomycetaceae, Dothioraceae and Sporidiobolaceae families had just one representative each: *Meyerozyma caribbica, Aerobasidium pullulans, Rhodotorula mucilaginosa,* respectively.

In general, the most prevalent species in the years evaluated were *Hanseniaspora uvarum*, *Issatchenkia terricola*, *Saturnispora diversa* and *Starmerella bacillaris*. When we compare the prevalence between years, that is, between harvests, 2017 was the year that we found the largest number of species, 19, compared to 14 in 2018 and 17 in 2019. Some species appeared only in 2017 as *Candida apicola*, *Candida fructus*, *Candida oleophila*, *Meyerozyma carabbica*, *Rhodutorula mucilaginosa*, *Wickerhamomyces ciferri*, *W. sydowiorum* and *Zygoascus meyerae*. In 2018, some species were prevalent only this year, such as *Candida azyma*, *Candida railenensis, Nakazawaea ishiwadae*, *N. pomicola*, *Pichia guillermondii* and *P. kluyveri*. We had only two species that were prevalent only in 2019, *Pichia manshurica* and *Torulaspora delbrueckii*.

However, it should be noted that some species are rare and were found only in one of the two cultivars. *Aerobacidium*

**TABLE 2.** Prevalence of isolated species of grape must (Cabernet-Sauvignon and Merlot) during the years. As indicated in the scale at the bottom of the table, the shades of blue reflect the prevalence of each species.

| Variety | Cabernet-Sauvignon | | | Merlot | | | Prevalence per variety | | Prevalence for years | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Years | 2017 | 2018 | 2019 | 2017 | 2018 | 2019 | Cabernet-Sauvignon | Merlot | 2017 | 2018 | 2019 |
| Nº grape samples | 6 | 6 | 6 | 6 | 6 | 6 | | | | | |
| Yeast species | | | | | | | | | | | |
| *Aerobacidium pullulans* | | | | | | | | | | | |
| *Candida apícola* | | | | | | | | | | | |
| *Candida azyma* | | | | | | | | | | | |
| *Candida bentonensis* | | | | | | | | | | | |
| *Candida californica* | | | | | | | | | | | |
| *Candida fructus* | | | | | | | | | | | |
| *Candida oleophila* | | | | | | | | | | | |
| *Candida railenensis* | | | | | | | | | | | |
| *Hanseniaspora opuntiae* | | | | | | | | | | | |
| *Hanseniaspora uvarum* | | | | | | | | | | | |
| *Issatchenkia occidentalis* | | | | | | | | | | | |
| *Issatchenkia terricola* | | | | | | | | | | | |
| *Meyerozyma caribbica* | | | | | | | | | | | |
| *Nakazawaea ishiwadae* | | | | | | | | | | | |
| *Nakazawaea pomicola* | | | | | | | | | | | |
| *Pichia guillermondii* | | | | | | | | | | | |
| *Pichia kluyveri* | | | | | | | | | | | |
| *Pichia manshurica* | | | | | | | | | | | |
| *Pichia sporocuriosa* | | | | | | | | | | | |
| *Rhodotorula mucilaginosa* | | | | | | | | | | | |
| *Saccharomyces cerevisiea* | | | | | | | | | | | |
| *Saccharomycopsis crataegensis* | | | | | | | | | | | |
| *Saccharomycopsis vini* | | | | | | | | | | | |
| *Saturnispara diversa* | | | | | | | | | | | |
| *Starmerella bacillaris* | | | | | | | | | | | |
| *Torulaspora delbrueckii* | | | | | | | | | | | |
| *Wickerhamiella azyma* | | | | | | | | | | | |
| *Wickerhamomyces ciferrii* | | | | | | | | | | | |
| *Wickerhamomyces sydowiorum* | | | | | | | | | | | |
| *Zygoascus meyerae* | | | | | | | | | | | |
| *Zygosaccharomyces bailii* | | | | | | | | | | | |
| **Prevalence scale** | 10 % | | | | | 50 % | | | | | 100 % |

*pullulans*, *Candida bentonensis, Candida oleophila*, *Candida railenensis*, *Meyerozyma caribbica*, *Nakazawaea pomicola*, *Pichia guillermondii*, *Wickerhamomyces ciferrii*, *Wickerhamomyces sydowiorum* and *Zygoascus meyerae*, ware found only in Merlot variety, while *Nakazawaea ishiwadae*, *Pichia kluyveri*, *Pichia manshurica*, *Rhodotorula mucilaginosa* and *Torulaspora delbrueckii*, were found only in Cabernet-Sauvignon variety.

However, the relative abundance (Figure 2) of these species was different from their prevalence, varying according to the year and the grape variety. In 2017, the most frequent species for Cabernet-Sauvignon were *Hanseniaspora uvarum* (45 %), *Issatchenkia terricola* (33 %) and *Saturnispora diversa* (16.25 %). In 2018, the species *Hanseniaspora uvarum* (68.4 %), *Saturnispora diversa* (14.8 %), *Starmerella bacillaris* (8.6 %) and *Issatchenkia terricola* (5.2 %) were more frequent for Cabernet-Sauvignon. In 2019, *Starmerella bacillaris*, *Hanseniaspora uvarum,* and *Saturnispora diversa* presented similar frequencies (about ± 25 %). *Hanseniaspora uvarum Starmerella bacillaris*, *Saturnispora diversa* and *Hanseniaspora opuntiae* presented similar frequencies, ranging from 15 to 19 %, in 2017 for the Merlot variety. In the two subsequent years, 2018 and 2019, some species also had similar populations, *Starmerella bacillaris* and *Hanseniaspora uvarum* (± 47 and 39 %, respectively) of all the yeast populations found. The results showed that the

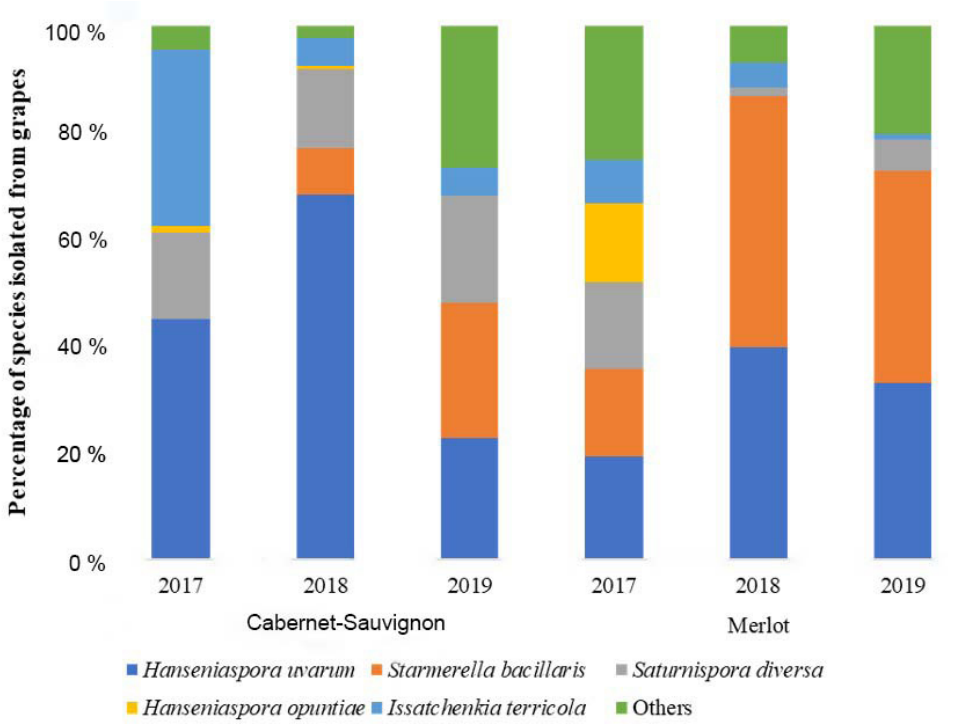



**FIGURE 2.** Relative abundance of the appearance of the most prevalent yeast species in Cabernet-Sauvignon and Merlot grapes in three consecutive years (2017, 2018 and 2019).

number of yeast species or biodiversity, that is, the number of isolated species, remains almost constant over the years and in the different grape varieties.

### 3. Yeast biodiversity and similarity indexes

The proportion of different species in both grape varieties and each year evaluated allowed the calculation of biodiversity indices: Shannon (H′) and Simpson (1-D), indices that measure species richness in a given environment. The general results showed that the Merlot variety in 2017 had the highest biodiversity indexes (H′ = 2.22 and 1-D = 0.87). The Cabernet-Sauvignon variety had its highest rates in 2019 (H′ = 1.97 and 1-D = 0.84) (Table 3). In contrast, 2018 was the year with the lowest indices, both for Cabernet-Sauvignon (H '= 1.06 and 1-D = 0.50) and for Merlot (H' = 0.61 and 1-D = 0.30).

The general richness of species was compared by constructing the rarefaction curve, which revealed a greater species richness than found in this work (Figure 3).

The similarity of the samples was analysed in pairs, using the Jaccard and Bray–Curtis indices (Table 4). The values of both indices range from 0 to 1, with 0 representing no similarity between two samples and 1 meaning no difference. Cabernet-Sauvignon 2019 samples and Merlot 2019 samples showed similarities between all comparisons, from 0.5556 by Jaccard and 0.4944 by Bray–Curtis. The samples that had the most dissimilarity was the comparison between Merlot 2017 and Merlot 2018 (0.2174 Jaccard and 0.1144 Bray–Curtis).

**TABLE 3.** Yeast biodiversity indices in *Vitis vinifera* grapes must (Cabernet-Sauvignon and Merlot) in three consecutive years

| Variety | Years | Biodiversity Indexes | |
|---|---|---|---|
| | | H′ | 1 - D |
| Cabernet-Sauvignon | 2017 | 1.25 | 0.66 |
| | 2018 | 1.06 | 0.50 |
| | 2019 | 1.97 | 0.84 |
| Merlot | 2017 | 2.22 | 0.87 |
| | 2018 | 0.61 | 0.30 |
| | 2019 | 1.46 | 0.67 |

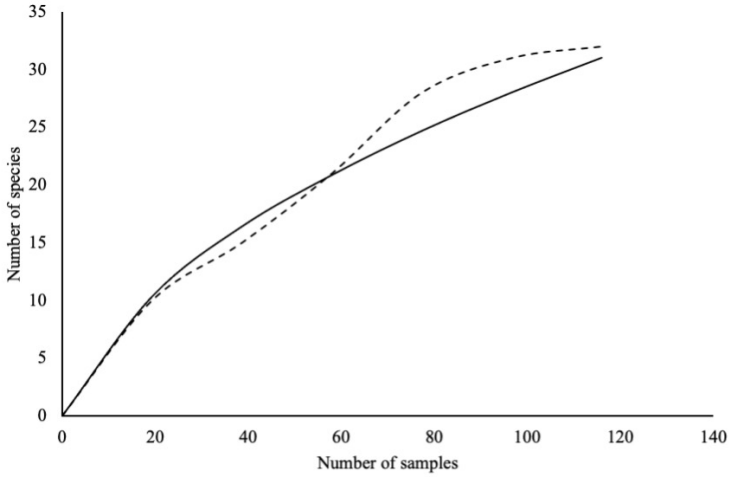



**FIGURE 3.** Rarefaction curve of expected yeast species richness. The number of analysed samples (31) is indicated by a vertical dashed line.

Statistical analysis using PERMANOVA (Table 5) proved that there is no difference in yeast population (species richness and frequency) between varieties and vintage.

## DISCUSSION

The sugar content and acidity are closely related to the ripening stage of the grapes, where the higher the sugar content, the lower the fixed acidity in the grapes. The difference shown in our study, in relation to the degree of ripeness of the grapes is closely linked to the climatic conditions during the harvest period. In 2017, according to the Brazilian Agricultural Research Corporation (Borges and Tonietto, 2017), the grape maturation period was quite rainy (about 285 mm of rain), mainly in January and February (harvest period in the southern hemisphere), which meant that the grapes harvested during this period, Merlot in mid-February and Cabernet-Sauvignon mid-March, did not reach the full stage of maturation. In 2018, unlike the previous year, climatic conditions were favourable for a better ripening of the grapes, with rainfall below average in the region (Borges and Tonietto, 2018) and in the last year evaluated, 2019, the climatic conditions were within the standard for the region (Borges *et al.,* 2019).

For the isolation methodology we used, different yeast species can have the same morphologies and the less abundant species are less likely to be isolated than the more frequent ones. A random isolation approach (considering the morphological assumptions of colonies mentioned above) was associated with a greater number of isolates, offering a greater probability of finding the rare species. Thus, there may be a bias in the calculation of prevalence, frequency and all other indices/calculations performed.

The presence of several species of yeasts has been reported in several wine regions of the world (Barata *et al.,* 2012; Brysch-Herzberg and Seidel, 2015; Cachón *et al*., 2019; Mendoza *et al*., 2019). Moreover, species such as *Hanseinaspora uvarum*, *Issatckenkia terrícola*, *Saturnispora diversa* and *Starmerella bacillaris* are reported to be among the most prevalent and frequent in grapes and must (Li *et al.,* 2010; Baffi *et al.,* 2011; Wang *et al.,* 2015), as well as in our work. On the other hand, some species are rarer to be found,

**TABLE 4.** Comparison of the yeast population in the different years and the variety by the measurement analysis by Jaccard and Bray–Curtis indices of similarity.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Bray–Curtis | | | | | | | |
| Variety | | Cabernet-Sauvignon | | | Merlot | | |
| | Years | 2017 | 2018 | 2019 | 2017 | 2018 | 2019 |
| Cabernet-Sauvignon | 2017 | | 0.3952 | 0.4311 | 0.4614 | 0.1208 | 0.4787 |
| | 2018 | 0.3529 | | 0.4628 | 0.3754 | 0.3988 | 0.3697 |
| | 2019 | 0.4118 | 0.3889 | | 0.4433 | 0.1984 | 0.4944 |
| Merlot | 2017 | 0.3333 | 0.3810 | 0.3043 | | 0.1144 | 0.4860 |
| | 2018 | 0.2941 | 0.4375 | 0.5000 | 0.2174 | | 0.1484 |
| | 2019 | 0.4444 | 0.5000 | 0.5556 | 0.3333 | 0.5294 | |
| Jaccard | | | | | | | |

**TABLE 5.** Comparison of the yeast population present in Cabernet-Sauvignon and Merlot grape musts and three consecutive vintages by distance measurement analysis (PERMANOVA).

| PERMANOVA | | | | | | |
|---|---|---|---|---|---|---|
| | Me-17 | CS-17 | Me-18 | CS-18 | Me-19 | CS-19 |
| Me-17 | | 0.8007 | 0.3210 | 0.1131 | 0.4922 | 0.7988 |
| CS-17 | 0.2313 | | 0.1392 | 0.3061 | 0.5829 | 0.8990 |
| Me-18 | 1.0280 | 1.9210 | | 0.6268 | 0.0589 | 0.6259 |
| CS-18 | 1.6010 | 2.7840 | 0.2587 | | 0.0823 | 0.4980 |
| Me-19 | 0.9122 | 0.3613 | 4.0720 | 4.5070 | | 0.4206 |
| CS-19 | 0.2623 | 0.2706 | 0.7012 | 0.9611 | 0.7443 | |

F-values are shown below the diagonal and the level of significance (p-values) and the Bonferroni correction is shown above. Me—Merlot; CS—Cabernet-Sauvignon.

such as *Aureobasidium pullulans*, *Candida apicola*, *Pichia kluyveri*, *Rhodotorula mucilaginosa*, *Saccharomyces cerevisiae* (Li *et al.,* 2010; Brysch-Herzberg and Seidel, 2015; Wang *et al.,* 2015; Canhón *et al.,* 2019).

Baffi *et al.* (2011), in a study, carried out on *Vitis labrusca* grapes in Brazil, observed that the most frequent yeasts were *Hanseniaspora uvarum* (34 %), *Issatckenkia occidentalis* (24 %) and *Issatckenkia terricola* (9 %), data also corroborating with our work. These yeasts are present in greater numbers, representing over 40 % of the population, in healthy grapes and are part of the normal microbiota of grapes (Wang *et al.,* 2015; Canhón *et al.,* 2019). Furthermore, the predominance of these yeasts in must can cause unpleasant aromas to wine and, therefore, knowing the microbiota present in grapes is important to generate high-quality products (Pretorius, 2016; Morata *et al.,* 2020).

The presence of yeasts on the surface of grape berries can vary in prevalence and frequency depending on agrochemical treatments (Cordero-Bueso *et al.,* 2011; Milanovi *et al.*, 2013; Grangeteau *et al.,* 2017; Ciani and Comitini, 2019). However, many other factors can influence the occurrence and complexity of the yeast community on the surface of the grape: biotic factors, including microbial interactions such as competition and symbiosis or antimicrobial and enzymatic activity; abiotic factors, such as climatic conditions, geographical location and age, size and variety of the vine, and also phytochemical treatments (Grangeteau *et al.,* 2017; Canhón *et al.,* 2019; Ciani and Comitini, 2019).

It is commonly agreed that the grape/must is a microenvironment rich in biodiversity (Canhón *et al.,* 2019). The Shannon diversity index (H') that takes into account the richness of species present in the grape/must environment is relatively high (≥ 1.00), as reported in studies (Baghere *et al.,* 2015; Canhón *et al.,* 2019; Drumonde-Neves *et al.,* 2017), also confirmed in our study although we found a sample with an index (H') smaller than 1.00. On the other hand, the frequency of individuals of each species is an important factor for the Simpson index (1-D), in this context, some authors report that there is a direct correlation between these two indices and the species richness (Drumonde-Neves *et al.,* 2017; Canhón *et al*., 2019).

For Drumonde-Neves *et al.* (2017), the greatest number of yeasts and the greatest biodiversity is related to climatic conditions such as there being little rain during the maturation period of the grapes. Despite this, our work did not find a direct relationship to this statement because, for the Merlot variety, the year with the greatest biodiversity was a rainy harvest (Borges and Tonietto, 2017), corroborating the study by Longo *et al.* (1991). For the Cabernet-Sauvignon variety, 2019 was the year with the highest yeast diversity, considered a year with climatic conditions within the normal range for the region (Borges *et al.,* 2019).

Finally, the similarity indices of Jaccard and Bray–Curtis, showed similar values in the harvest/variety comparisons, corroborating with the PERMANOVA analysis did not show significant differences in population frequency and species richness between harvests and grape varieties. Thus, our results partially corroborate with the work carried out by Canhón *et al.* (2019), where the grape variety does not have an important impact on yeast diversity, at least among the studied varieties. Previous studies report that the microbial community is related to climatic conditions and viticultural practices (Barata *et al.,* 2012). Canhón *et al.* (2019) indicate that there is a biogeographic yeast pattern; however, more harvests would be needed to confirm these findings.

## CONCLUSION

In conclusion, our study showed that there is a great diversity of yeast species in the northeast region of Rio Grande do Sul. Thirty-one species were found in the region and the most prevalent species in the region were *Hanseniaspora uvarum*, *Issatchenkia terricola*, *Starmerella bacillaris* and *Saturnispora diversa*. The yeast communities remain similar in Cabernet-Sauvignon and Merlot grapes as the analysed parameters (grape variety/vintage) do not present any significant impact on the diversity indexes of the yeast populations found in the region highlands of Rio Grande do Sul, Serra Gaúcha, Brazil.

## ACKNOWLEDGEMENTS

This research was supported by the University of Caxias do Sul and the authors address their thanks to "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)" and to "Conselho Nacional de Desenvolvimento Científco e Tecnológico (CNPq)" for fellowship support.

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