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Survival of commercial yeasts in the winery environment and their prevalence during spontaneous fermentations

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Abstract Inoculation of active dry yeasts during the wine-making process has become a common practice in most wine-producing regions; this practice may affect the diversity of the indigenous population of *Saccharomyces cerevisiae* in the winery. The aim of this work was to study the incidence of commercial yeasts in the experimental winery of Estación de Viticultura e Enoloxía de Galicia (EVEGA) and their ability to lead spontaneous fermentations. To do this, 64 spontaneous fermentations were carried out in the experimental cellar of EVEGA over a period of 7 years. Samples were taken from must and at the beginning, vigorous and final stages of fermentation. A representative number of yeast colonies was isolated from each sample. *S. cerevisiae* strains were characterised by analysis of mitochondrial DNA restriction patterns. The results showed that although more than 40 different strains of *S. cerevisiae* were identified, only 10 were found as the dominant strain or in codominance with other strains in spontaneous fermentations. The genetic profiles (mtDNA-RFLPs) of eight of these strains were similar to those of different commercial yeasts that had been previously used in the EVEGA cellar. The remaining two strains were autochthonous ones that were able to reach implantation frequencies as high of those of commercial yeasts. These results clearly indicated that commercial wine yeasts were perfectly adapted to survive in EVEGA cellar conditions, and they successfully competed with the indigenous strains of *S. cerevisiae*, even during spontaneous fermentations. On the other hand, autochthonous dominant strains that presented desirable oenological traits could be of interest to preserve wine typicity.

Keywords Spontaneous fermentations · Commercial yeasts · *S. cerevisiae* strains

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Introduction

Inoculation of commercial yeasts during the wine-making process has become a common practice in most wine-producing regions. Active dry yeasts (ADY) have been selected on the basis of their oenological properties, and they guarantee a successful vinification process through increased fermentation speed and reproducibility of wine characteristics. It is well known that wine quality is significantly affected by the strain of *Saccharomyces cerevisiae* conducting the fermentation; therefore, the use of ADY can contribute to the loss of some typical sensorial properties of wines. Consequently, there is increasing interest in selecting yeasts that allow the expression of particular characteristics of a specific area. In addition, there is a general belief that ADY strains are very well adapted to the winery environment, and their use may have a negative impact on the biodiversity of natural yeasts present in the winery.

Several studies from different wine-producing areas have approached this subject, and the results varied depending on several factors including sampling points, type of wineries, winemaking practices and frequency and diversity of commercial inocula. Thus, it has been reported that the use of selected yeasts reduced the biodiversity of non-*Saccharomyces* yeasts at the beginning of the vinification process [7, 9, 11]. Frezier and Dubourdieu [8] showed that some *S. cerevisiae* strains remained in the cellar from one harvest

to another and appeared in the next fermentations. Constantí et al. [8] also found that following the first grape harvest carried out in a newly established winery, certain *Saccharomyces* strains not only became resident in the cellar, but they proliferated and took over fermentations the next year. Ciani et al. [3] confirmed that *S. cerevisiae* strains colonising winery surfaces were the ones that carried out natural must fermentations. During a 6-year study, Beltrán et al. [1] observed that the use of ADY reduced the diversity and importance of the indigenous *S. cerevisiae* strains in the winery, although in this work the presence of non-*Saccharomyces* yeasts was not modified by the inoculation of commercial yeasts. Furthermore, it has been reported that some commercial strains tend to become resident in the wineries and take over spontaneous fermentations [1, 2, 6, 17]. However, more recent studies [16] indicated that the presence of previously commercial inocula was scarce or non-existent in some wineries. The latter finding was supported by Cocolin et al. [4], who reported a lack of colonisation of commercial cultures in a modern winery. However, in accordance with the former findings, Mercado et al. [12] found commercial strains during fermentation (at different percentages), but their presence on winery equipment was low.

Finally, regarding the presence of ADY in vineyards, Valero et al. [19] reported that permanent implantation of commercial strains in vineyards did not occur. Instead, these strains were subjected to the same natural fluctuations of periodic appearance/disappearance as autochthonous strains. Moreover, the dissemination of commercial strains was restricted to short distances and limited periods of time, and it was favoured by the presence of run-off water [19]. In addition, Comitini and Ciani [5] showed that wine grapes were not a favourable ecological niche for the development and colonization of *S. cerevisiae* species.

Preliminary studies carried out in the experimental winery of Estación de Viticultura e Enoloxía de Galicia (EVEGA) investigating the diversity of *S. cerevisiae* strains in spontaneous fermentations have shown the presence of commercial strains in these fermentations, even when they have not been inoculated [2]. The aim of this work was to study the incidence of commercial yeasts in the native population of *S. cerevisiae* strains in the experimental winery of EVEGA and their ability to lead spontaneous fermentations over a larger period (2002–2008).

Materials and methods

Fermentations

Several spontaneous fermentations were carried out in the experimental cellar of EVEGA over a period of 7 years (2002–2008); samples were not collected in 2007. Grapevine varieties used and their origin, number of fermentations and the number of yeast isolates tested are specified on Table 1.

Sampling and yeast isolation

Samples for yeast isolation were taken during the beginning, vigorous and final stages of fermentation. All samples were collected in sterile tubes, adequately diluted in sterile water and spread on WL nutrient agar [13]. Plates were incubated at 28°C for 48 h. Then, 20 colonies from each sample were randomly selected and isolated on YEPD [1% (w/v) yeast extract, 2% (w/v) peptone and 2% (w/v) glucose] for further characterisation.

Yeast identification

All isolated colonies were plated on Lysine Agar Medium (Scharlau Microbiology, Barcelona, Spain) to distinguish between *Saccharomyces* and non-*Saccharomyces* yeasts. *Saccharomyces* yeasts do not grow in this medium.

Isolates of *S. cerevisiae* were characterised at the strain level by analysis of random fragment length polymorphisms of mitochondrial DNA (mtDNA-RFLP) [14] using the restriction endonuclease *Hinf* I (Promega). Restriction fragments were separated by electrophoresis on a 0.8% (w/v) agarose gel in TAE buffer (40 mM Tris-acetate, 1 mM EDTA), visualised in a transilluminator and photographed.

Results and discussion

The diversity and implantation of *S. cerevisiae* strains in 64 spontaneous fermentations were studied over a period of 7 years. The genetic characterisation of yeast isolated at different stages of fermentation revealed the existence of up to 40 different *S. cerevisiae* strains (data not shown) from a total of 2,624 isolates tested. Despite this yeast diversity, only ten strains (Fig. 1) appeared as dominant yeasts in the fermentation processes. These results also show that dominant strains took over fermentations from the initial stages until the end of fermentation, prevailing against the remaining strains that were present in the must.

In addition, the distribution of leader strains over the period examined provided insight into the dynamics of dominant yeast populations in the winery (Fig. 2). For example, strain II dominated fermentations from 2002 to 2004. Strain III appeared in 2002 and 2004, but not in 2003. Almost all strains were present in one or more fermentations as the leader yeast in 2004 due to the larger number of fermentations studied that year. The results after 2004 indicated a succession of strains that dominated the fermentation. For

Table 1 Origin of grapevine varieties, number of fermentations carried out and number of *S. cerevisiae* isolates analysed in this study

Year	Grapevine variety	Origin	Number of fermentations	Number of isolates**
2002	Godello	Evega*	8	108
2003	Godello	Evega	3	98
	Treixadura	Evega	6	95
	Albariño	DO Rías Baixas	3	145
2004	Godello	Evega/DO Valdeorras	5/1	227
	Albariño	Evega/DO Rías Baixas	6/5	501
	Treixadura	Evega/DO Ribeiro	5/1	275
	Lado	Evega	1	46
	Branco Lexítimo	Betanzos	1	52
	Agudelo	Betanzos	1	33
2005	Godello	DO Valdeorras	1	72
	Albariño	DO Rías Baixas	5	321
	Treixadura	Evega/DO Ribeiro	1/1	132
	Lado	Evega	1	57
	Branco Lexítimo	Betanzos/Evega	1/1	100
	Agudelo	Betanzos	2	90
2006	Treixadura	Evega	1	77
2008	Godello	Evega	1	52
	Albariño	DO Rías Baixas	1	60
	Treixadura	DO Rías Baixas	2	83
Total			64	2,624

* Experimental vineyard of Estación de Viticultura e Enoloxía de Galicia

** This number indicates the number of isolates of *S. cerevisiae* analysed

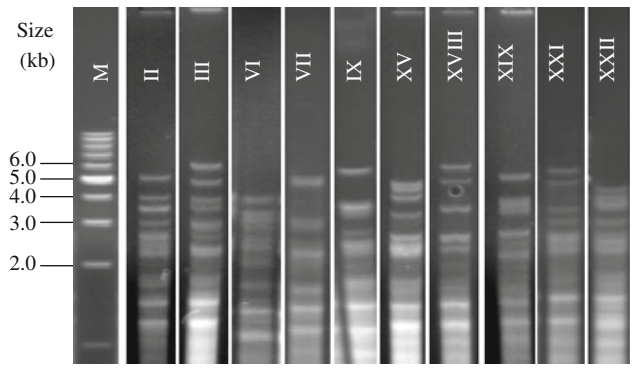

Fig. 1 Genetic profiles (mtDNA-RFLP) of dominant *S. cerevisiae* strains found in this study. Lane M is 1-kb molecular weight marker (Promega); lanes II, III, VI, VII, IX, XV, XVIII, XIX, XXI and XXII are genetic patterns described in the yeast collection from EVEGA

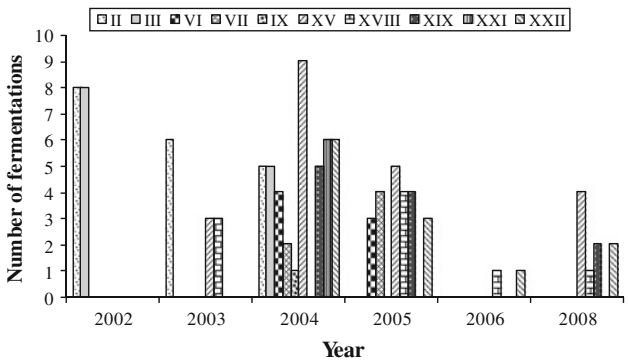

Fig. 2 Distribution of dominant *S. cerevisiae* strains in spontaneous fermentations carried out in the EVEGA cellar from 2002 to 2008

example, *S. cerevisiae* strains VI and VII had a relevant role in 2004 and 2005, but strains XV, XVIII, XIX and XXII took over fermentations from 2004 to 2008. These results highlight the fluctuation of dominant strains in an experimental winery over time. Furthermore, our findings are in agreement with those supporting the natural succession of yeast populations in vineyards [19].

The mtDNA-RFLP of dominant strains was compared to the genetic profile of commercial *Saccharomyces* strains that had been previously used in the EVEGA cellar. These results showed that the mtDNA-RFLP patterns of eight dominant strains were similar to those of ADY (Table 2). The remaining strains (VII and XXI) were autochthonous *S. cerevisiae* strains from the winery. The frequency of appearance of these strains varied widely, from 17 to 94%. As expected, the frequencies were lower when two or more strains appeared as dominant yeasts during must fermentation (codominance) and higher when only one strain was the main strain responsible for fermentation. This phenomenon of codominance is quite common in spontaneous fermentations carried out without starter yeast inoculation. The presence of several yeast strains contribute to wine complexity [10, 11, 15, 18].

The results described here indicate that commercial yeasts took over most of the spontaneous fermentations and

Table 2 Origin of dominant strains found in this study and the frequency of their appearance during fermentation

Genetic profile	Origin*	Number of fermentations as	
		Dominant strain (range of frequencies)	Codominant strain (range of frequencies)
II	ADY	**6** (55%)	**13** (30–38%)
III	ADY		**13** (24–35%)
VI	ADY	**4** (46–88%)	**3** (30–53%)
VII	Wild type	**2** (37–86%)	**4** (22–36%)
IX	ADY	**1** (59%)	
XV	ADY	**9** (47–94%)	**13** (17–57%)
XVIII	ADY	**3** (56%)	**7** (17–30%)
XIX	ADY	**1** (70%)	**6** (18–58%)
XXI	Wild type		**6** (38%)
XXII	ADY		**12** (23–38%)

* ADY (active dry yeast), commercial yeast previously used in the EVEGA cellar; wild-type, autochthonous *S. cerevisiae* strain isolated in the EVEGA cellar

disagree with those reported by Santamaría et al. [16], who found that the presence of ADY was scarce or non-existent in both winery ecosystems and spontaneous fermentations. However, our data corroborate those related to yeast ecology during fermentation, which report a significant impact of the use of commercial starters in wineries [1, 6, 12, 17]. The yeast population from winery equipment was not tested in this work; therefore, future investigations are necessary to clarify this point, especially because the information available on this subject is contradictory. Some studies have indicated that the use of commercial *Saccharomyces* strains has no impact on the resident yeast population in the winery, likely because environmental pressure under these conditions is not as selective for *Saccharomyces* as pressure under fermentation conditions [4, 16]. In contrast, other authors have reported that ADY used for inoculation remained in the cellar and appeared in non-inoculated vinifications during the following years [2, 6]; this effect was especially important after using the same ADY for several years. In support of further study in this area, Ciani et al. [3] found that under real vinification conditions, the *S. cerevisiae* strains colonising the winery surfaces were the ones that carried out the natural must fermentations in a long-established industrial winery.

These discrepancies regarding the implantation of commercial yeasts could be due to the adaptability of these strains to the specific winery conditions and the amount and frequency of yeasts inoculated. The data reported here were obtained in an experimental winery where different ADYs are used routinely every year, and some strains are being inoculated over subsequent years. Based on this fact, we can conclude that the commercial wine yeasts used in the EVEGA cellar were perfectly adapted to the conditions of winemaking in this cellar, and they successfully competed with the indigenous yeast strains even during spontaneous fermentations.

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