Letters in Applied Microbiology

Letters in Applied Microbiology ISSN 0266-8254

ORIGINAL ARTICLE

Diversity of *Saccharomyces cerevisiae* yeasts associated to spontaneous and inoculated fermenting grapes from Spanish vineyards

M. de Celis[1,†], J. Ruiz[1,†], M. Martín-Santamaría[1], A. Alonso[1], D. Marquina[1], E. Navascués[2], M.Á. Gómez-Flechoso[3], I. Belda[4,5,*] and A. Santos[1]

1 Department of Genetics, Physiology and Microbiology, Unit of Microbiology, Biology Faculty, Complutense University of Madrid, Madrid, Spain
2 Pago de Carraovejas, S.L. Camino de Carraovejas, Peñafiel, Valladolid, Spain
3 Department of Biodiversity, Ecology and Evolution, Unit of Biomathematics, Biology Faculty, Complutense University of Madrid, Madrid, Spain
4 Department of Biology, Geology, Physics & Inorganic Chemistry, Unit of Biodiversity and Conservation, Rey Juan Carlos University, Móstoles, Spain
5 Science Department, Biome Makers Spain, Valladolid, Spain

Significance and Impact of the Study: The aim of this study was to compare different viticulture and oenological practices to determine their influence on the composition and diversity of *Saccharomyces cerevisiae* strains in wine fermentations. The study shows that the use of autochthonous strains of *S. cerevisiae* as starters for wine fermentation could have an important incidence on *S. cerevisiae* strains diversity in surrounding vineyards. The use of autochthonous strains of *S. cerevisiae* reduced the detected number of *S. cerevisiae* strains, a fact that was not observed when allochthonous commercial strains were used. Furthermore, vineyards managed with organic practices showed intermediate to low levels of *S. cerevisiae* strain diversity, whereas conventional practices showed higher levels.

Keywords
autochthonous strains, farming practices, oenological practices, *Saccharomyces cerevisiae*, strain diversity.

Correspondence
I. Belda, Department of Biology, Geology, Physics & Inorganic Chemistry, Unit of Biodiversity and Conservation, Rey Juan Carlos University, 28933 Móstoles, Spain.
E-mail: ignacio.belda@urjc.es

***Present address**
Department of Biology, Geology, Physics & Inorganic Chemistry, Unit of Biodiversity and Conservation, Rey Juan Carlos University, Móstoles, Spain.

[†]These authors contributed equally to this work.

2019/0218: received 9 October 2018, revised 25 March 2019 and accepted 26 March 2019

doi:10.1111/lam.13155

Abstract

The use of commercial yeast strains is a common practice in winemaking leading to a predictable quality in wine production, avoiding stuck or sluggish fermentations. However, the use of commercial yeasts leads to a consequent reduction in autochthonous microbial diversity. In this study, 1047 isolates from three Spanish appellations of origin were checked for fingerprinting on interdelta polymorphisms and the strain composition and diversity analysed using an extensible open-source platform for processing and analysis of an in-house polymorphism database developed for this study. Ancient vineyards managed with organic practices showed intermediate to low levels of strains diversity indicating the existence of stable populations of *Saccharomyces cerevisiae* strains. A drastic reduction in the number of different *S. cerevisiae* strains was observed in vineyards with cellars using a selected autochthonous *S. cerevisiae* strain for winemaking. Contrary, the use of allochthonous commercial strains in wineries did not seem to affect the native *S. cerevisiae* strain composition and diversity.

Introduction

The transformation of grape must into wine is a complex process that involves the participation of several yeasts, being *Saccharomyces cerevisiae* the primarily responsible for alcoholic fermentation. The grape-associated yeasts continuously vary during wine fermentation, with different dominance patterns that are related to the changing

physical chemical environments that take place during wine production. Grape- and winery-associated yeasts can participate in wine fermentations influencing the organoleptic characteristics and quality of the wines produced (Romano *et al.* 2003; Ciani *et al.* 2010; Medina *et al.* 2013; Grangeteau *et al.* 2015; Belda *et al.* 2016, 2017a). Rich reservoirs of *S. cerevisiae* are found in damaged grape berries, winery equipment and cellar installations (Mortimer and Polsinelli 1999; Bokulich *et al.* 2013). Accordingly, yeast strains can reach the fermentation tanks from vineyards (with grapes and people acting as vectors) and the winery (Rosini *et al.* 1982; Fleet and Heard 1993; Martini 1993; Vaughan-Martini and Martini 1995; Martini *et al.* 1996; Beltrán *et al.* 2002). The same process in the opposite direction can also be accounted for making the transference of yeasts possible from the winery to the surrounding vineyards, influencing the native-autochthonous yeast communities (Cordero-Bueso *et al.* 2011a; Tello *et al.* 2012; Capozzi *et al.* 2015; Garofalo *et al.* 2015). Wineries are open systems without any specific biological containment systems where billions of yeast cells are produced every vintage. In this context, the constant dissemination of yeast to the environment closest to the cellar appears to be obvious. However, the persistence of these yeasts in natural niches and reservoirs such as soil, plant tissues, grapes, or the rhizosphere, is difficult to be precisely determined (Zarraonaindia *et al.* 2015; Knight and Goddard 2016; Belda *et al.* 2017b). These factors are of major interest taking into consideration that most wineries use massive quantities of yeasts in the form of Active Dry Yeast (Rosini *et al.* 1982; Martini *et al.* 1996; Pretorius 2000; Valero *et al.* 2005, 2007; Palková and Váchová 2006; Cubillos *et al.* 2009; Francesca *et al.* 2010; Goddard *et al.* 2010; Marqués *et al.* 2010; Salinas *et al.* 2010; Cordero-Bueso *et al.* 2011a; Schuller *et al.* 2012; Martiniuk *et al.* 2016).

During the last years, many wineries have decided to avoid the use of commercial *S. cerevisiae* strains as fermentation starters but to prefer the use of autochthonously selected strains, as an oenological strategy to combine the industrial benefits of inoculated fermentations and the genuineness and typicity of spontaneous fermentations conducted by native *S. cerevisiae* strains. The selected yeasts are better adapted to the vineyards, agricultural practices and the environment of the wine production region and therefore, they may easily persist and predominate over other yeast strains of the natural microbiota, contributing to the typical sensory properties of local wines. In consequence, the use of starter cultures based on selected autochthonous strains that could be better adapted to their own environment is a usual practice in winemaking, but its consequences in vineyard yeast diversity have scarcely been studied (Capece *et al.* 2010, 2012). Thus, considering the diversity of farming and oenological practices of the screened vineyards, the aim of this study was to investigate the diversity of *S. cerevisiae* strains in the correspondent wine-related environment.

Results and discussion

Interdelta analysis of *S. cerevisiae* diversity

Due to the low abundance of *S. cerevisiae* yeasts in vineyard soils, grapevine leaves and grapes, in this work *S. cerevisiae* isolates were obtained from initial, intermediate and advanced stages of grape must spontaneous fermentations. With the initial aim of checking a significant number of *S. cerevisiae* strains, 1047 yeast isolates were obtained from wine fermentations, of which 1016 resulted to be *S. cerevisiae* isolates (Table S1). In winery A (CF), a high number of strains were obtained (151 different interdelta polymorphism patterns from 370 isolates). By contrast, 100% of the isolates (177 yeast isolates) obtained from the micro-vinification samples of winery B (OF) had the same polymorphism pattern. Furthermore, this pattern was the same of the autochthonous *S. cerevisiae* strain used as inoculum in this cellar, indicating that this yeast was widely present in this vineyard (Fig. 2). Probably, due to its oenological characteristics (high-fermentation power, sulphite resistance, growth rate, etc. Data not shown) and its high presence in the vineyard, from which it was initially isolated and selected, this strain dominated all the developed micro-vinifications.

Forty polymorphism patterns (from a total of 380 isolates) were found in samples from winery C (OF), being the strain ScO-02 the most frequent (50·7%). This winery uses organic viticulture practices on old (pre-phylloxera) vine plants and fermentations are always spontaneously conducted. Under these highly constant, traditional and conserved conditions, *S. cerevisiae* strains' diversity was found to be relatively low. However, in winery D (CF), the most abundant strains were ScTL-07 (20·2%) and ScTL-08 (30·3%) of the 25 different fingerprint patterns found in the 120 isolates analysed in this case. One plausible explanation for these observations is that modern-conventional agronomic and oenological practices, that are commonly used in vineyards and wineries, could generate periodic external inputs (i.e. fungicides, chemical fertilizers, tillage, inoculation of ADY, coexistence of grape varieties, etc.) that, in turn, could generate changing conditions and disturbances in microbial niches having an impact on strains richness. Several studies have highlighted the importance of crop management practices in the equilibrium between species of microbial communities and soil functionality. Shifts in the environmental factors can strongly affect the microbial diversity and composition and

influence the ecosystem and its functionality. For example, crop rotation has been related with a diminished bacterial diversity of soils (Ashworth *et al.* 2017), whereas reduced tillage and organic fertilizers increase microbial diversity (Wang *et al.* 2017; Legrand *et al.* 2018).

Yeast population analysis

Biodiversity levels do not depend only on the number of taxonomic groups found, but also on the relative abundance and dominance of each. Usually, a high degree of dominance of a certain species/strain generates lower diversity values. Depending on the agronomic practices, differences in the composition and distribution of yeast populations in the vineyard have been described (Cordero-Bueso et al. 2011b), but certain enological practices applied in the winery may also affect to the surrounding vineyard yeast diversity. (Grangeteau *et al.* 2016). The four wineries studied in this work employ different farming and oenological practices that were consistent during the time-lapse study: wineries A and D apply conventional farming practices and use a wide spectrum of commercial yeast strains for inoculated fermentations at cellar. Winery B applies low-intervention practices (OF) and it uses its own selected autochthonous *S. cerevisiae* strain for wine fermentations. Finally, winery C applies OF practices and only performs spontaneous fermentations.

As shown in Fig. 1, winery A (CF) had the highest diversity of *S. cerevisiae* strains ($H'_A = 4{\cdot}47$) and the lowest concentration of dominance ($D_A = 0{\cdot}02$), whereas wineries C (OF) and D (CF) showed no significant differences in diversity of *S. cerevisiae* strains ($H'_C = 2{\cdot}16$ and $H'_D = 2{\cdot}42$). Furthermore, winery C presented a concentration of dominance higher than winery D ($D_C = 0{\cdot}28$ and $D_D = 0{\cdot}15$). Some studies reported that, compared with conventional farming, organic practices tend to achieve a higher and richer abundance of yeast species and at different levels implying higher microbial activity, biomass and diversity (Guerra *et al.* 1999; Cordero-Bueso *et al.* 2011b). Despite this, the highest diversity (attending to *S. cerevisiae* strains) was found in winery A. This was also observed in the rarefaction curves present in Fig. 1, that indicate that winery A shows a greater diversity potential when compared with wineries C and D and even more evident if compared with winery B. On the other hand, in winery B, only one autochthonous *S. cerevisiae* strain was isolated. As far as we are aware, this is the first study that obtains results as surprising as the results that we have obtained in vineyard B. The yeast strain used has been used continuously in winery B since 2009 as starter for alcoholic fermentations. We found substantial evidence in this cellar to suppose that the recurrent use of a selected yeast inoculum could lead to a notable decrease in the diversity of *S. cerevisiae* strains that appeared, not only in winery facilities, as it was also previously reported by Beltrán *et al.* (2002), but also in the surrounding vineyards. It has been described that selected yeast inocula can colonize vineyards closely located to the cellars and persist for a period of time (Valero *et al.* 2007; Tello *et al.* 2012; Grangeteau *et al.* 2017b). In winery B, the autochthonous strain used was isolated in the past from the same vineyard and it is probably more adapted to the particular environment surrounding the cellar, so possibly this is one of the reasons why the results obtained in cellar B are so surprising, not following the trend of the results reported by other authors. We assume that for these reasons, among others, this strain was isolated in 100% of the samples, both in controlled micro-vinifications and in several industrial spontaneous fermentations developed at cellar facilities. Recently, it has been showed that the same genotypes of *S. cerevisiae* are present in the local environment and ferments reinforcing the idea that the fungal communities present in the vineyard and its surroundings influence those present in the winery and vice versa (Goddard *et al.* 2010; Knight and Goddard 2016; Morrison-Whittle and Goddard 2018).

Spontaneous fermentations developed at industrial scale in cellar B

To confirm the results obtained in cellar B for *S. cerevisiae* strain diversity, an industrial scale study was carried out in this cellar. A very high level of implantation of the autochthonous strain used as inoculum in this cellar B was obtained (Figs 2 and 3). This strain was present in percentages ranging from 100% (six fermentations) to zero per cent (one fermentation), indicating that the strain is dominant in most fermentations and, therefore, its presence in this vineyard could be considered ubiquitous. Interestingly, the fermentation in which the strain was not detected, was developed with grapes obtained from a vineyard (named B3) that is situated more distant from the cellar (Fig. S3) and which was not studied in the 2015 vintage because this vineyard was recently owned by the winery.

The results obtained from industrial fermentations developed in cellar B also induced us to study additional variables (Fig. S3). Soil properties were variable in the three vineyard blocks tested around winery B (B1, B2 and B3). However, considering that blocks B2 and B3 have similar soils but a very different pattern of strains detected (Fig. 2) and also considering that vineyards B1 and B2 have different soil composition but a similar pattern of strains detected, the type of soil showed a limited incidence in the distribution of *S. cerevisiae* strains in the vineyards. However, it could also be hypothesized that

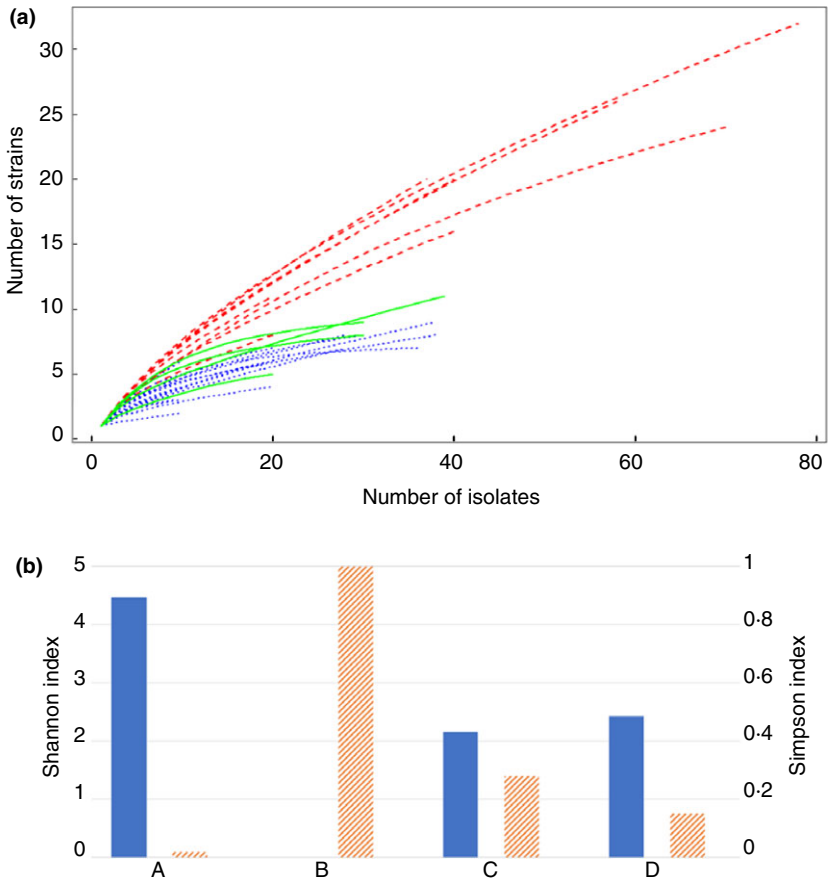

Figure 1 Strain diversity measurements. (a) rarefaction curves (number of strains *vs* number of isolates) for vineyard blocks from wineries A (red-discontinued lines), C (blue-dots), and D (green-lines) describing the observed number of strains as a function of the number of isolates per block. Winery B has been not included since only one strain was identified among the 177 isolates obtained from the six blocks sampled. (b) Shannon (blue-filled bars) and Simpson (orange-oblique lines) diversity indices calculated for the four studied wineries. [Colour figure can be viewed at wileyonlinelibrary.com]

the geographic orientation could have some importance in yeast distribution because blocks B1 and B2 were SW-exposed and B3 was NE-exposed (Fig. S3).

Saccharomyces cerevisiae strains diversity level in cellar C (OF) was not very different from vineyard D (CF). Vineyards of the cellar C are 100–200 years old pre-phylloxera vineyards, without the usage of yeasts starters at winery facilities and where organic management has been traditionally carried out. Recently, it has been robustly demonstrated that 40% fungal communities present in grape juice and ferments overlap with vineyard fungal communities and that 30% of species in ferments may also be found in local native forests, concluding that there is an overlap between microbial communities in natural habitats and managed agricultural ecosystems (Morrison-Whittle and Goddard 2018). It could be hypothesized that the agronomic and oenological practices developed in cellar C could create a homogeneous and stable vineyard ecosystem (absence of periodic stresses that can drive changes and diversification on the vineyard resident microbiota), in which the most adapted strains have been evolved and, thus, where the diversity of *S. cerevisiae* strains can be lower (Grangeteau *et al.* 2017a).

To summarize, we conclude that, as Tello *et al.* (2012) considered, microbial ecology studies are necessary to understand, and to properly size, the effect of anthropogenic factors on the natural biodiversity of agriculture systems (Capozzi *et al.* 2015; Garofalo *et al.* 2015; Morrison-Whittle and Goddard 2018). In this work, the effect of different viticulture and winemaking practices on vineyard *S. cerevisiae* strain diversity has been evaluated. We can highlight the effect of a continued use as inoculum of an autochthonous-selected *S. cerevisiae* strain in the surrounding microbiota, being able to completely dominate the *Saccharomyces* populations of vineyards around the winery. We can also conclude that no clear effects on *S. cerevisiae* diversity levels of vineyards can be associated with the conventional and organic farming practices considered in this study. However, some conventional vineyards showed extremely low H' values, since within the ambiguous term of 'conventional' we can also find crops subjected to extreme phytosanitary treatments.

Thus, this study allowed us to ascertain the effects that different winemaking and farming practices have in the diversity of *S. cerevisiae* strains inhabiting the vineyards and spontaneous fermentations. These results are of

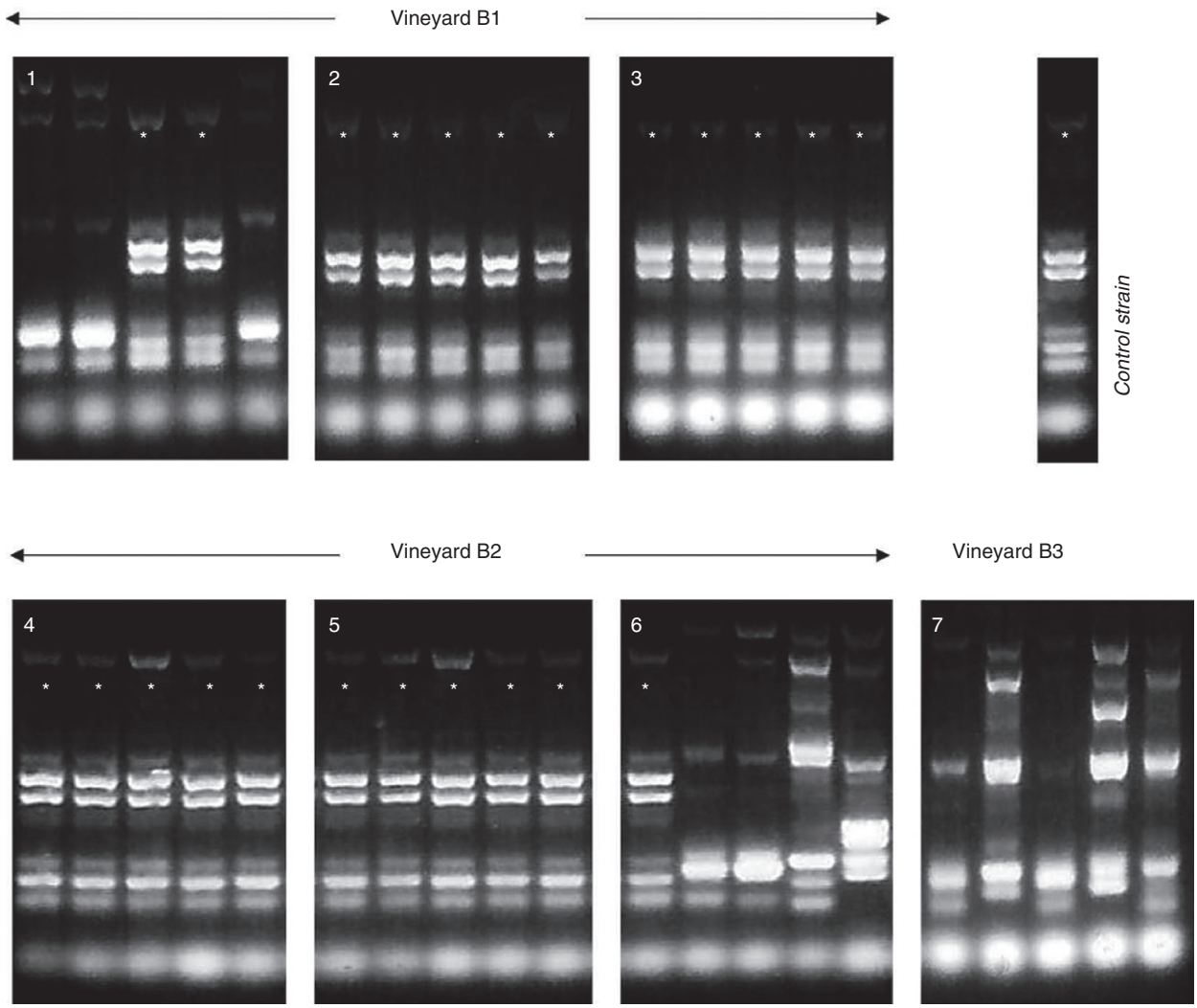

Figure 2 Interdelta electrophoretic patterns from isolates obtained from the seven spontaneous fermentations developed, at industrial scale, with grapes coming from three vineyard blocks of winery B. (*) Lanes that show the presence of the interdelta polymorphism corresponding to the autochthonous-selected *Saccharomyces cerevisiae* strain used as inoculum in this cellar (Control).

importance as they are involved in the definition of the technical-commercial concept of wine *terroir*. Finally, here we remark the interest of future microbiome-based studies for a better understanding of the influence of farming practices in soil microbial diversity and its relationship with crops sustainability.

Materials and methods

Sampling, spontaneous micro-vinifications, yeast isolation and culture media

Samples were obtained from four wineries of three Spanish Appellations of Origin (AO Ribera del Duero (2 cellars; named A and B), AO Rueda (cellar C) and AO Tierra de León (cellar D) (Table 1. Fig. S1, Table S1). Vineyards from wineries A and B are between 25 and 91 years old and winery C has ancient vineyards with pre-phylloxera vines between 100 and 200 years old and winery D had young (20–40 years old) vineyards. Winery B uses its own autochthonous yeast strain at cellular concentrations of about 10^6 CFU per ml as starter for wine fermentations since 2009.

Samples from vineyard soils, leaves and grapevine barks were taken for isolation purposes but, in these samples, only non-*Saccharomyces* yeasts were isolated. For that reason, the complete collection of *S. cerevisiae* strains of this study was obtained from different stages of spontaneous fermentations developed at cellar facilities. This collection was acquired by sampling 8, 6, 18 and 4 blocks from vineyards A, B, C and D respectively (Table 1). One kilogram/

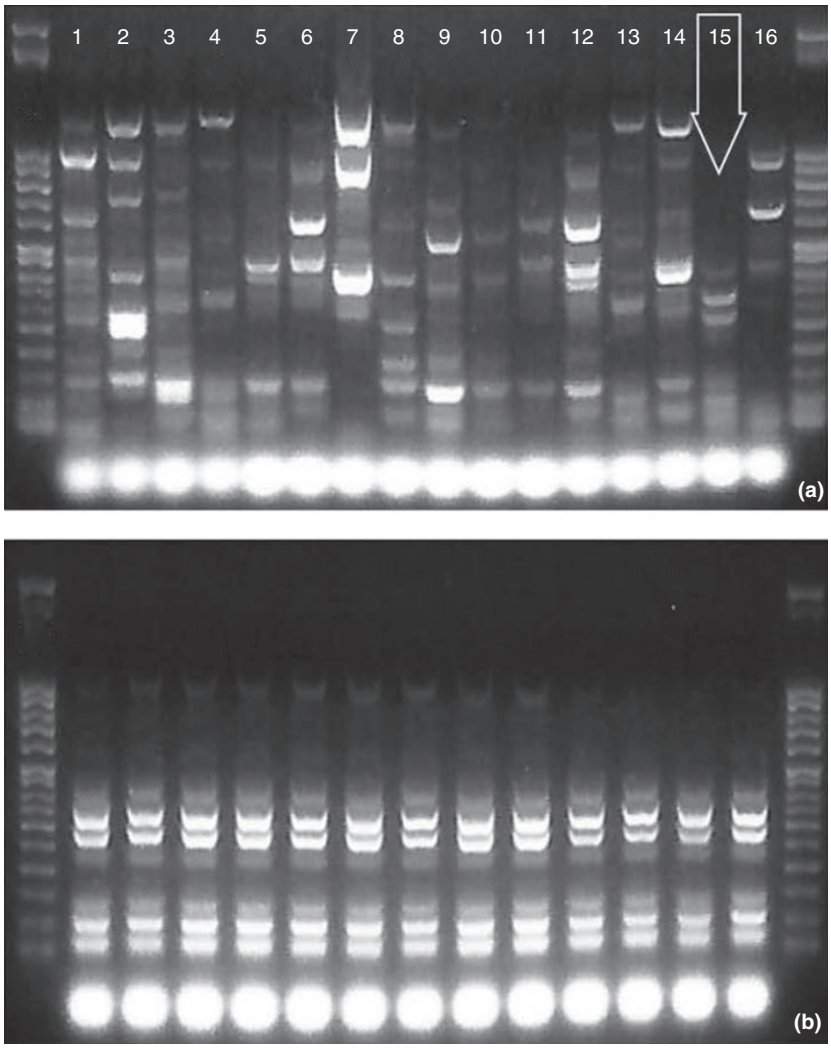

Figure 3 (a) Interdelta electrophoretic patterns of several different commercial *Saccharomyces cerevisiae* strains used for winemaking, including the autochthonous strain used in winery B (Arrow). (b) Interdelta electrophoretic patterns of several different isolates randomly obtained from winery B. Band patterns of the isolates corresponds to the pattern observed in lane 15 of the commercial strains, indicating that it is the same strain.

sample of grapes was collected, and then, destemmed and crushed in sterile conditions. Fifty grams of the mix obtained was subjected to spontaneous fermentation at a controlled temperature of 20°C in sterile flasks. The evolution of fermentations was followed by determining must density. Samples of 100 μl were taken at different fermentation stages (Table S1). After density values were constant for three days and glucose + fructose concentration values were lower than 4 g l^{-1} (sugar fermentation completed), the last sample of every fermentation was taken. Samples were serially diluted in saline buffer and spread on Sabouraud-chloramphenicol agar plates (Oxoid, Hampshire, UK). Plates were incubated at 28°C for 3 days for colony development. In most samples, 20

Table 1 General characteristics of the four Spanish wineries under study

Winery	Appellation of origin	Grape variety	Viticulture	ADY* inoculation	Number of blocks sampled	Number of isolates	*Saccharomyces cerevisiae* strains
A	Ribera del Duero	Tempranillo	Conventional	Allochthonous	8	370	151
B	Ribera del Duero	Tempranillo + Cabernet Sauvignon	Conventional	Autochthonous	6	177	1
C	Rueda	Verdejo	Organic	No (spontaneous)	18	380	40
D	Tierra de León	Prieto Picudo	Conventional	Allochthonous	4	120	25

*Active dry yeast.

colonies per sample were randomly taken and were re-isolated by repetitive streaking on Yeast Malt Agar (YMA) medium. Finally, yeast isolates were grown in pure culture and deposited in CYC (Complutense Yeast Collection, Complutense University of Madrid, Spain). The collection of *S. cerevisiae* strains was conserved at −80°C in the following cryoprotective medium: 1 g l^{-1} glucose, 15 g l^{-1} casein peptone, 5 g l^{-1} proteose peptone, 5 g l^{-1} sodium chloride, 2 g l^{-1} yeast extract, 1 g l^{-1} sodium citrate, 1 g l^{-1} sodium bisulphite and 150 g l^{-1} glycerol.

Genotyping

One thousand and forty-seven isolates from micro-vinifications: 547 isolates from AO Ribera del Duero (370 from cellar A and 177 from cellar B); 380 isolates from AO Rueda (cellar C) and 120 isolates from AO Tierra de León (cellar D) were checked for fingerprinting on interdelta polymorphisms by PCR amplification. Prior PCR amplification, genomic DNA was extracted using a standard procedure (Querol *et al.* 1992) and then diluted to a final DNA concentration of 100 ng μl^{-1}.

Amplification was carried out using ReadyMix™ Taq polymerase (Sigma-Aldrich, St. Louis, MO, USA), delta12 (5′-TCAACAATGGAATCCCAAC-3′) and delta21 (5′-CATCTTAACACCGTATATGA-3′) primers (Legras and Karst 2003). Amplification reactions were performed with an Eppendorf thermal cycler using the following programme: 4 min at 95°C followed by 35 cycles of 30 s at 95°C, 30 s at 46°C and 90 s at 72°C and a finishing step of 10 min at 72°C. For the delta1/delta2 primer pair, the annealing temperature was 42°C for the first five cycles and 45°C for the following cycles as described previously (Legras and Karst 2003). This technique allows the discrimination of *S. cerevisiae* isolates, as no amplicon would be generated for other *Saccharomyces* and non-*Saccharomyces* species (Legras and Karst 2003; Tristezza *et al.* 2009; Vigentini *et al.* 2015).

PCR products where later observed in agarose gels (16 g l^{-1} agarose, 150 ml TAE buffer, constant voltage at 90 V during 1 h) stained with GelRed® and analysed under an UV transilluminator. Molecular mass markers (DirectLoadTM 50 bp DNA Step Ladder) were used as inter-gel control.

Software development to generate spectra from electrophoresis images and analysis of spectra

The selection of the *S. cerevisiae* strains was achieved by differentiation of the band patterns detected using a new extensible open-source platform for processing and analysis software (open-source code available here: https://doi.org/10.17632/8gwjj4ybg8.1) (Fig. S2).

Industrial trials and study of implantation in cellar B

Taking into account the unexpected results obtained in cellar B, the diversity of *S. cerevisiae* strains was determined in spontaneous fermentations developed at cellar facilities. The study was carried out at industrial scale (10·000 kg per fermentation) using Tempranillo grapes obtained from three blocks (called FG1, FG2 and FG3) located in vineyards around the cellar (Fig. S3). Samples were processed for yeast isolation and checked for interdelta polymorphism in order to detect and determine the presence and persistence of the vineyard-associated yeast strain (Fig. S3).

Statistical analysis

Shannon (H') and Simpson (D) classical indexes were used to statistically evaluate the strain diversity of *S. cerevisiae* populations (Simpson 1949; Shannon 2001).

Acknowledgements

Funding for the research in this paper was provided by Agrovin S.A, under the framework of the MINECO-financed project IDI-20160102 and by Pago de Carraovejas S.L. MINECO-financed project IDI-20160750 (MINECO, Spanish Ministry of Economy, Industry and Competitiveness, Spain). Javier Ruiz acknowledges the Complutense University of Madrid for his doctoral grant (CT17/17 - CT18/17) and Ignacio Belda acknowledges his postdoctoral grant (PTQ-16-08253) provided by the Spanish Ministry of Economy, Industry and Competitiveness.

Conflict of Interest

Eva Navascués is employee of Bodegas Pago de Carraovejas and Ignacio Belda developed part of this work as employee of Biome Makers (granted by Spanish Ministry of Economy, Industry and Competitiveness).

References

Ashworth, A.J., DeBruyn, J.M., Allen, F.L., Radosevich, M. and Owens, P.R. (2017) Microbial community structure is affected by cropping sequences and poultry litter under long-term no-tillage. *Soil Biol Biochem* **114**, 210–219.

Belda, I., Ruiz, J., Alastruey-Izquierdo, A., Navascués, E., Marquina, D. and Santos, A. (2016) Unraveling the enzymatic basis of wine "flavorome": a phylo-functional study of wine related yeast species. *Front Microbiol* **7**, 12. https://doi.org/10.3389/fmicb.2016.00012

Belda, I., Ruiz, J., Esteban-Fernández, A., Navascués, E., Marquina, D., Santos, A. and Moreno-Arribas, M.V.

(2017a) Microbial contribution to wine aroma and its intended use for wine quality improvement. *Molecules* **22**, 189. https://doi.org/10.3390/molecules22020189

Belda, I., Zarraonaindia, I., Perisin, M., Palacios, A. and Acedo, A. (2017b) From vineyard soil to wine fermentation: microbiome approximations to explain the "terroir" concept. *Front Microbiol* **8**, 821. https://doi.org/10.3389/fmicb.2017.00821

Beltrán, G., Torija, M.J., Novo, M., Ferrer, N., Poblet, M., Guillamón, J.M., Rozès, N. and Mas, A. (2002) Analysis of yeast populations during alcoholic fermentation: a six year follow-up study. *Syst Appl Microbiol* **25**, 287–293.

Bokulich, N.A., Ohta, M., Richardson, P.M. and Mills, D.A. (2013) Monitoring seasonal changes in winery-resident microbiota. *PLoS ONE* **8**, e66437. https://doi.org/10.1371/journal.pone.0066437

Capece, A., Romaniello, R., Siesto, G., Pietrafesa, R., Massari, C., Poeta, R. and Romano, P. (2010) Selection of indigenous *Saccharomyces cerevisiae* strains for Nero d'Avola wine and evaluation of selected starter implantation in pilot fermentation. *Int J Food Microbiol* **144**, 187–192.

Capece, A., Romaniello, R., Siesto, G. and Romano, P. (2012) Diversity of *Saccharomyces cerevisiae* yeasts associated to spontaneously fermenting grapes from an Italian "heroic vine-growing area". *Food Microbiol* **31**, 159–166.

Capozzi, V., Garofalo, C., Chiriatti, M.A., Grieco, F. and Spano, G. (2015) Microbial terroir and food innovation: the case of yeast biodiversity in wine. *Microbiol Res* **181**, 75–83.

Ciani, M., Comitini, F., Mannazzu, I. and Domizio, P. (2010) Controlled mixed culture fermentation: a new perspective on the use of non-*Saccharomyces* yeasts in winemaking. *FEMS Yeast Res* **10**, 123–133.

Cordero-Bueso, G., Arroyo, T., Serrano, A. and Valero, E. (2011a) Remanence and survival of commercial yeast in different ecological niches of the vineyard. *FEMS Microbiol Ecol* **77**, 429–437.

Cordero-Bueso, G., Arroyo, T., Serrano, A., Tello, J., Aporta, I., Vélez, M.D. and Valero, E. (2011b) Influence of the farming system and vine variety on yeast communities associated with grape-berries. *Int J Food Microbiol* **154**, 132–139.

Cubillos, F.A., Vásquez, C., Faugerón, S., Ganga, A. and Martínez, C. (2009) Self-fertilization is the main sexual reproduction mechanism in native wine yeast populations. *FEMS Microbiol Ecol* **67**, 162–170.

Fleet, G.H. and Heard, G.M. (1993) Yeast: growth during fermentation. In *Wine Microbiology and Biotechnology*, ed. Fleet, G.H. pp. 27–54. Chur, Switzerland: Harwood Academic Publishers.

Francesca, N., Chiurazzi, M., Romano, R., Aponte, M., Settani, L. and Moschetti, G. (2010) Indigenous yeast communities in the environment of 'Rovello bianco' grape variety and their use in commercial white wine fermentation. *World J Microbiol Biotechnol* **26**, 337–351.

Garofalo, C., El Khoury, M., Lucas, P., Bely, M., Russo, P., Spano, G. and Capozzi, V. (2015) Autochthonous starter cultures and indigenous grape variety for regional wine production. *J Appl Microbiol* **118**, 1395–1408.

Goddard, M.R., Anfang, N., Tang, R., Gardner, R.C. and Jun, C. (2010) A distinct population of *Saccharomyces cerevisiae* in New Zealand: evidence for local dispersal by insects and human-aided global dispersal in oak barrels. *Environ Microbiol* **12**, 63–73.

Grangeteau, C., Gerhards, D., Rousseaux, S., von Wallbrunn, C., Alexandre, H. and Guilloux-Benatier, M. (2015) Diversity of yeast strains of the genus *Hanseniaspora* in the winery environment: what is their involvement in grape must fermentation? *Food Microbiol* **50**, 70–77.

Grangeteau, C., Gerhards, D., von Wallbrunn, C., Alexandre, H. and Guilloux-Benatier, M. (2016) Persistence of two non-*Saccharomyces* yeasts (*Hanseniaspora* and *Starmerella*) in the cellar. *Front Microbiol* **7**, 268. https://doi.org/10.3389/fmicb.2016.00268

Grangeteau, C., David, V., Alexandre, H., Guilloux-Benatier, M. and Rousseaux, S. (2017a) The sensitivity of yeasts and yeasts-like fungi to copper and sulfur could explain lower yeast biodiversity in organic vineyards. *FEMS Yeast Res* **17**, fox092. https://doi.org/10.1093/femsyr/fox092

Grangeteau, C., Roullier-Gall, C., Rousseaux, S., Gougeon, R.D., Schmitt-Kopplin, P., Alexandre, H. and Guilloux-Benatier, M. (2017b) Wine microbiology is driven by vineyard and winery anthropogenic factors. *Microb Biotechnol* **10**, 354–370.

Guerra, E., Sordi, G., Mannazzu, I., Clementi, F. and Fatichenti, F. (1999) Occurrence of wine yeasts on grapes subjected to different pesticide treatments. *Ital J Food Sci* **11**, 221–230.

Knight, S.J. and Goddard, M.R. (2016) Sporulation in soil as an overwinter survival strategy in *Saccharomyces cerevisiae*. *FEMS Yeast Res* **16**, fov102. https://doi.org/10.1093/femsyr/fov102

Legrand, F., Picot, A., Cobo-Díaz, J.F., Carof, M., Chen, W. and Le Floch, G. (2018) Effect of tillage and static abiotic soil properties on microbial diversity. *Appl Soil Ecol* **132**, 135–145.

Legras, J.L. and Karst, F. (2003) Optimisation of interdelta analysis for *Saccharomyces cerevisiae* strain characterisation. *FEMS Microbiol Lett* **221**, 249–255.

Marqués, M.J., García-Muñoz, S., Muñoz-Organero, G. and Bienes, R. (2010) Soil conservation beneath grass cover in hillside vineyards under Mediterranean climatic conditions (Madrid, Spain). *Land Degrad Dev* **21**, 122–131.

Martini, A. (1993) Origin and domestication of the wine yeast *Saccharomyces cerevisiae*. *J Wine Res* **3**, 165–176.

Martini, A., Ciani, M. and Scorzzetti, G. (1996) Direct enumeration and isolation of wine yeasts from grape surfaces. *Am J Enol Vitic* **47**, 435–440.

Martiniuk, J.T., Pacheco, B., Russell, G., Tong, S., Backstrom, I. and Measday, V. (2016) Impact of

commercial strain use on *Saccharomyces cerevisiae* population structure and dynamics in Pinot Noir vineyards and spontaneous fermentations of a Canadian winery. *PLoS ONE* **11**, e0160259. https://doi.org/10.1371/journal.pone.0160259

Medina, K., Boido, E., Fariña, L., Gioia, O., Gomez, M.E., Barquet, M., Gaggero, C., Dellacassa, E. *et al.* (2013) Increased flavour diversity of Chardonnay wines by spontaneous fermentation and co-fermentation with *Hanseniaspora vineae*. *Food Chem* **141**, 2513–2521.

Morrison-Whittle, P. and Goddard, M.R. (2018) From vineyard to winery: a source map of microbial diversity driving wine fermentation. *Environ Microbiol* **20**, 75–84.

Mortimer, R. and Polsinelli, M. (1999) On the origins of wine yeast. *Res Microbiol* **150**, 199–204.

Palková, Z. and Váchová, L. (2006) Life within a community: benefit to yeast long-term survival. *FEMS Microbiol Rev* **30**, 806–824.

Pretorius, I.S. (2000) Tailoring wine yeast for the new millennium: novel approaches to the ancient art of winemaking. *Yeast* **16**, 675–729.

Querol, A., Barrio, E., Huerta, T. and Ramón, D. (1992) Molecular monitoring of wine fermentations conducted by active dry yeast strains. *Appl Environ Microbiol* **58**, 2948–2953.

Romano, P., Fiore, C., Paraggio, M., Caruso, M. and Capece, A. (2003) Function of yeast species and strains in wine flavor. *Int J Food Microbiol* **86**, 169–180.

Rosini, G., Federici, F. and Martini, A. (1982) Yeast flora of grape berries during ripening. *Microbiol Ecol* **8**, 83–89.

Salinas, F., Mandakovic, D., Urzua, U., Massera, A., Miras, S., Combina, M., Ganga, M.A. and Martínez, C. (2010) Genomic and phenotypic comparison between similar wine yeast strains of *Saccharomyces cerevisiae* from different geographic origins. *J Appl Microbiol* **108**, 1850–1858.

Schuller, D., Cardoso, F., Sousa, S., Gomes, P., Gomes, A.C., Santos, M.A.S. and Casal, M. (2012) Genetic diversity and population structure of *Saccharomyces cerevisiae* strains isolated from different grape varieties and winemaking regions. *PLoS ONE* **7**, e32507. https://doi.org/10.1371/journal.pone.0032507

Shannon, C.E. (2001) A mathematical theory of communication. *ACM SIGMOBILE M2R* **5**, 3–55.

Simpson, E.H. (1949) Measurement of diversity. *Nature* **163**, 688.

Tello, J., Cordero-Bueso, G., Aporta, I., Cabellos, J.M. and Arroyo, T. (2012) Genetic diversity in commercial wineries: effects of the farming system and vinification management on wine yeasts. *J Appl Microbiol* **112**, 302–315.

Tristezza, M., Gerardi, C., Logrieco, A. and Grieco, F. (2009) An optimized protocol for the production of interdelta markers in *Saccharomyces cerevisiae* by using capillary electrophoresis. *J Microbiol Methods* **78**, 286–291.

Valero, E., Schuller, D., Cambon, B., Casal, M. and Dequin, S. (2005) Dissemination and survival of commercial wine yeast in the vineyard: a large scale, three-year study. *FEMS Yeast Res* **5**, 959–969.

Valero, E., Cambon, B., Schuller, D., Casal, M. and Dequin, S. (2007) Biodiversity of *Saccharomyces* yeast strains from grape berries of wine-producing areas using starter commercial yeasts. *FEMS Yeast Res* **7**, 317–329.

Vaughan-Martini, A. and Martini, A. (1995) Facts, myths and legends on the prime industrial microorganism. *J Ind Microbiol* **14**, 514–522.

Vigentini, I., De Lorenzis, G., Fabrizio, V., Valdetara, F., Faccincani, M., Panont, C.A., Picozzi, C., Imazio, S. *et al.* (2015) The vintage effect overcomes the terroir effect: a three years survey on the wine yeast biodiversity in Franciacorta and Oltrepò Pavese, two Northern Italian vine-growing areas. *Microbiology* **161**, 362–373.

Wang, Y., Li, C., Tu, C., Hoyt, G.D., DeForest, J.L. and Hu, S. (2017) Long-term no-tillage and organic input management enhanced the diversity and stability of soil microbial community. *Sci Total Environ* **609**, 341–347.

Zarraonaindia, I., Owens, S.M., Weisenhorn, P., West, K., Hampton-Marcell, J., Lax, S., Bokulich, N.A., Mills, D.A. *et al.* (2015) The soil microbiome influences grapevine-associated microbiota. *MBio* **6**, e02527–14. https://doi.org/10.1128/mbio.02527-14

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article:

Figure S1 Wine appellations sampled in the study indicating geographical and climatic data.

Figure S2 Guide for identification of strains of yeasts of the species *Saccharomyces cerevisiae* using the developed computer tools.

Figure S3 Schematic representation of the soil distribution in vineyards pertaining to cellar B. Three vineyards (B1, B2, B3) closely located to the cellar were sampled to develop spontaneous fermentations. Vineyards B1 and B2 were SW-exposed and B3 was NE-exposed. Numbers from 1 to 7 indicate the different soil types present in vineyards: (1) Red mudstones, sandstones and conglomerate. (2) Dolostones and/or limestones with interbedded marls. (3) Limestones, dolostones and marls. (4) Red mudstones and sandstones and/or conglomerates and marls. (5) White mudstones and marls with pebbles and cobbles. Colluvium. (6) Alluvial fan. (7) Dark mudstones and siltstones with pebbles. Quaternary terraces and fluvial deposits.

Table S1 Isolates obtained from the four cellars of the study with the correspondent interdelta polymorphism patterns assigned through the utilization of the software developed in this work. The frequency refers to the percentage of each polymorphism in the total number of different polymorphisms detected.