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This is the author's manuscript

Original Citation:

Availability:

This version is available http://hdl.handle.net/2318/1647294 since 2020-04-03T17:36:41Z

Published version:

DOI:10.14674/IJFS-767

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(Article begins on next page)

09 August 2024

Saccharomyces cerevisiae biodiversity in Monferrato, North West Italy and selection of indigenous starter cultures for Barbera wine production

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ABSTRACT

The aim of this study was to examine the biodiversity of *Saccharomyces cerevisiae* isolates from Barbera grapes and musts, from the Monferrato area, in the Piedmont region – North West Italy. An interdelta element PCR analysis was used to identify and discriminate 636 *S. cerevisiae* isolates at a strain level. Ninety-six *S. cerevisiae* that showed different molecular fingerprints were characterized through physiological tests and laboratory scale fermentations. A chemical analysis of experimental wines obtained from inoculated fermentations showed significant differences between the wines. The main variables considered in the strain differentiation were the residual sugars and the production of acetic acid, which ranged from 148.64 to 3.44 g/l and from 0.20 to 0.60 g/l, respectively. As a consequence, strain variability should be considered as a relevant resource to select suitable starter cultures in order to improve or characterize wines with a close bond to the geographic region.

Keywords: *Saccharomyces cerevisiae*, Yeast biodiversity, indigenous starter, Interdelta PCR, Selection.

INTRODUCTION

Wine production is an ancient tradition that has been carried out for centuries through the spontaneous fermentation of grape juice, which takes place due to the presence of indigenous yeasts from different genera and species (FLEET, 2003; PRETORIUS, 2000; ROMANO *et al.*, 2003). The number of species and their presence during fermentation depends on several factors (PRETORIUS *et al.*, 1999), which lead to subsequent wine quality variations from region to region, but also from one year to another, and all this makes the outcome of spontaneous fermentation difficult to predict (PRETORIUS, 2000). In an attempt to address this issue, many winemakers have used pure commercial *Saccharomyces cerevisiae* cultures inoculated into the must (PRETORIUS, 2000). However, it has been suggested that native *S. cerevisiae* strains are better suited to the micro-area climatic conditions of the wine production region (LOPES *et al.*, 2002) and can therefore more easily dominate the natural biota (LA JEUNE *et al.*, 2006).

S. cerevisiae, the most relevant species in winemaking, is usually chosen as the wine yeast, and the particular strain is chosen according to a set of physiological features that are indicative of their potential usefulness for industrial wine production. In addition to the primary end products of the glycolytic fermentation of glucose and fructose, certain oenological criteria must be considered in order to select yeast strains that show desirable characteristics, including: tolerance and high ethanol production, exhaustion of the sugar in must and high fermentation activity, growth at high sugar concentrations, high glycerol production, resistance and low sulphur dioxide production, good enzymatic profile (high β-glucosidase and proteolytic activities) and low acetic acid formation (ESTEVE-ZARZOSO *et al.*, 2000).

At present, there is increasing interest, in the wine community, in the use of indigenous *S. cerevisiae* strains that may contribute to the overall sensorial quality of wine and reflect the characteristics of a given region, even in guided fermentations using selected *S. cerevisiae* starter cultures (CAPECE *et al.*, 2010; SUZZI *et al.*, 2012). Recently, in an attempt to respond to these aspects coupled with the current emphasis on the preservation of all forms of genetic biodiversity, some research groups have focused on the selection of yeasts from restricted areas (SETTANNI *et al.*, 2012; FRANCESCA *et al.*, 2009; ORLIC *et al.*, 2007; LOPES *et al.*, 2007).

We have previously extensively studied the indigenous mycobiota originating from the Barbera grapes from the Monferrato area, Piedmont region, North-West Italy (ALESSANDRIA *et al.*, 2015). Barbera grapes produce a ruby-red coloured wine with berry, cherry, plum and spicy flavours, depending on the clone, as well as the location and the age of the plant (BOSSO *et al.*, 2011). In this study, *S. cerevisiae* isolates were characterized to establish their genetic and technological variability in order to contribute to the preservation of the *S. cerevisiae* genetic resources of the Barbera of Monferrato *terroir*. Interdelta-PCR was used to help establish the genetic diversity of the isolates. Physiological tests, which focused on the production of extracellular hydrolytic enzymes and on their growth in different ethanol and total SO_2 concentrations, were then conducted. Finally, selected genotypes were used to ferment Barbera must, during micro-fermentation trials, in order to evaluate their fermentation potential.

MATERIAL AND METHODS

Fermentation set up and yeast isolation

Spontaneous fermentations were conducted using *Vitis Vinifera* L. Barbera grapes obtained from fifteen different vineyards (Fig. 1), located in five areas in the Asti and Alessandria districts of the Piedmont region (ALESSANDRIA *et al.*, 2015). The vineyards, which were identified on the basis of their geographical locations, were: 1 (Murisengo), 2 (San Martino Alfieri), 3 (Costigliole d'Asti), 4 (Isola d'Asti), 5 (Montegrosso d'Asti), 6 (Agliano Terme), 7 (Vinchio), 8 (Nizza Monferrato), 9

(Incisa Scapaccino), 10 (Loazzolo), 11 (Ricaldone), 12 (Alice bel colle), 13 (Acqui -Terme Crocera south west zone), 14 (Acqui Terme - Crocera south est zone) and 15 (Acqui Terme – Dannona zone).

Approximately 25 kg of healthy grapes, without signs of bird damage or *Botryotinia fuckeliana* infection, were harvested. The grapes were crushed in the laboratory and the obtained juice (about 15 L volume) was transferred to sterile jugs where it underwent spontaneous fermentation at room temperature (between 22 and 25°C). Yeasts were isolated from each container at the beginning of the fermentation (after 1 day and 3 days), in the middle (after 7 days) and when alcoholic fermentation was completed. The alcoholic fermentation was monitored with a densitometer. Aliquots (0.1 mL each) of several decimal dilutions, in a 0.1% ringer solution (Oxoid, Milan, Italy), were plated on Wallerstein Laboratory Nutrient (WLN) medium (Oxoid) (Pallmann et al., 2001). The plates were incubated at 28°C for 5 days. WLN allows the presumptive identification of the yeast species according to the colony morphology and colour (URSO *et al.*, 2008). At least 10 colonies were selected from each sample and at each fermentation stage and were isolated on WLN; priority was given to putative colonies of *Saccharomyces* spp. The isolates were stored at −80 °C in YPD broth (1% (w/v) yeast extract, 2% (w/v) peptone and 2% (w/v) glucose, all obtained from Oxoid) after the addition of glycerol (30%, v/v) (Sigma-Aldrich, Milan, Italy).

Yeast identification

The putative *Saccharomyces* spp. isolates were subsequently identified and simultaneously differentiated at a strain level on the basis of a PCR interdelta element analysis (δ-PCR). In order to conduct the δ-PCR analysis, the total DNA was extracted from 1 millilitre of an overnight culture in YPD broth, according to COCOLIN *et al.* (2000), quantified using a Nanodrop ND-1000 spectrophotometer (Celbio, Milan, Italy) and standardized at 100 ng/µl. The delta12 (5′-TCAACAATGGAATCCCAAC-3′) and delta21 (5′-CATCTTAACACCGTATATGA-3′) oligonucleotide primers were used to amplify regions between the repeated interspersed delta sequences (Legras and Karst, 2003). Amplification reactions were performed with a PTC-200 DNA Engine MJ Research thermal cycler (Biorad, Milan, Italy) using the following programme: initial denaturation at 95°C (5 min), 35 cycles of denaturing at 94°C (1 min), annealing at 50°C (1 min), extension at 72°C (1 min) and a final extension at 72°C (10 min). The PCR products were separated in 1.5% agarose gels and stained with ethidium bromide. The resulting fingerprints were analyzed by means of the BioNumerics V4.0 software package (Applied-Maths, Sint-Martens-Latem, Belgium). Similarity among the digitized profiles was calculated using the Pearson correlation, and an average linkage (UPGMA) dendrogram was derived from the profiles. A coefficient of correlation of 85% was arbitrarily selected to distinguish the clusters. The yeasts that were not amplified with δ-PCR, were subsequently identified by using the RFLP of the ribosomal region method as described in ALESSANDRIA *et al.* (2015).

Physiological characterization

Hydrogen sulphite production

The ability to produce hydrogen sulphite was determined by streaking single colonies onto Biggy agar (Oxoid) and incubating them at 25°C for 48– 72 h. Colony colour was observed and scored as being white, pale hazel, hazel, dark hazel or black.

Enzymatic activities

The esterase, protease and β-glucosidase activities of the isolates were screened as described by ENGLEZOS *et al.* (2015).

Ethanol and SO_2 tolerance assays

Ethanol tolerance and SO_2 tolerance were determined in microplates, according to the method proposed by ARROYO-LOPEZ *et al.* (2010) and TOFALO *et al.* (2012), with some modifications. Yeast Nitrogen Base with amino acids (YNB, 6.7 g/L, [Remel, Lenexa, KS, USA]) and pH 5.5 was supplemented with 20 g/L of glucose and sterilized by filtration using a 0.2 μm membrane filter (VWR, Milan, Italy). The medium was supplemented with different concentrations of ethanol (Sigma) (final concentrations of 0, 12, 14 and 16% v/v) in order to test for ethanol tolerance, while, in order to establish SO_2 resistance, different amounts of total SO_2 were added (after adjustment to pH 3.0) until final concentrations of 0, 50, 100 and 150 mg/L. Cells for the inoculation were prepared from an overnight culture in 1 mL of YPD medium, centrifuged at 9000 rpm for 10 min. The obtained pellet was washed twice in a sterile salt solution (8 g/L NaCl) and then re-suspended in the same solution to obtain a concentration of about 10_6 cells/mL. The diluted cells (20 μL) were mixed with 180 μL of YNB, prepared as above. The microplates were incubated at 25 °C and the optical density (OD) was measured at 600 nm using a microtiter plate reader (Savatec Instruments, Torino, Italy) at 24 and 48 hours after an orbital shaking of 30 s, in order to re-suspend the cells in the medium before the measurement. YNB without ethanol or SO_2 was used as the control. Cell growth was determined on the basis of the ratio (%) of OD values obtained in medium with and without ethanol or SO_2 for the specific incubation times. Tests were carried out in triplicate. Isolates with a percentage of growth < 10% were considered sensitive.

Microfermentations

The fermentation potential of the different genotypes was evaluated in microfermentation trials. These were carried out in duplicate for 14 days, in 50 mL tubes containing 25 mL of Barbera grape must (120 g/L glucose, 124 g/L fructose, 5.25 titratable acidity as g/L of tartaric acid, pH 3.20 and 184 mg/L yeast available nitrogen (YAN)). Before the inoculation, the must was thermally treated at 60 °C for 50 min, and the absence of viable populations was evaluated by plating 100 μL of the must after the treatment on the WLN medium, followed by incubation at 28 °C for 5 days. Pre-cultures were prepared in must at 25 °C for 24 h, and then used to inoculate each fermentation with a cell concentration of 10_6 per mL, which was determined through a microscopical cell count. The fermentations were carried out under static conditions at 25 °C.

Chemical analysis

After 14 days of fermentation, the sugar consumption (glucose and fructose) and the ethanol, glycerol and acetic acid production were evaluated directly by means of HPLC, according to the method proposed by ROLLE *et al.* (2012). YAN was measured using the protocols reported in ENGLEZOS *et al.*, 2016).

Data analysis

The results of the chemical composition of the wines obtained from the micro-fermentation trials were subjected to a Principal Component Analysis (PCA), in order to evaluate the intraspecific biodiversity of *S. cerevisiae* isolates. Statistical analyses were performed using the IBM SPSS Statistics software package (version 19.0, IBM Corp., Armonk, NY, USA).

RESULTS AND DISCUSSION

The possible association between territory and yeasts is being actively investigated in recent years and is believed to have a positive impact and influence purchase decision-making by the consumer. The use of indigenous selected yeasts could represent a useful alternative to spontaneous fermentation in order to optimize the typical attributes of the grape variety (CLEMENTE JIMENEZ *et al.*, 2004; REMENTERIA *et al.*, 2003; ROMANO *et al.*, 2008). The goal of this study was to isolate and characterize indigenous *S. cerevisiae* yeasts present on Barbera grapes in the vineyards of the Monferrato area.

Fifteen vineyards, located in the Piedmont region (Fig. 1) and cultivated with the Barbera grape variety, were studied during the 2012 harvest season and the collected grapes were crushed to obtain 15 spontaneous alcoholic fermentations. Overall, 943 yeast colonies were isolated during the fermentations, and after molecular identification, most of them (636 isolates) were identified, through the use of δ-PCR, as *S. cerevisiae*. Other species, namely *Hanseniaspora uvarum* (248 isolates), *Starmerella bacillaris* (synonym *Candida zemplinina*) (11 isolates), *Pichia anomala* (7 isolates) and *Torulaspora delbruecki* (7 isolates) were also isolated, mainly at the beginning and middle of the fermentations.

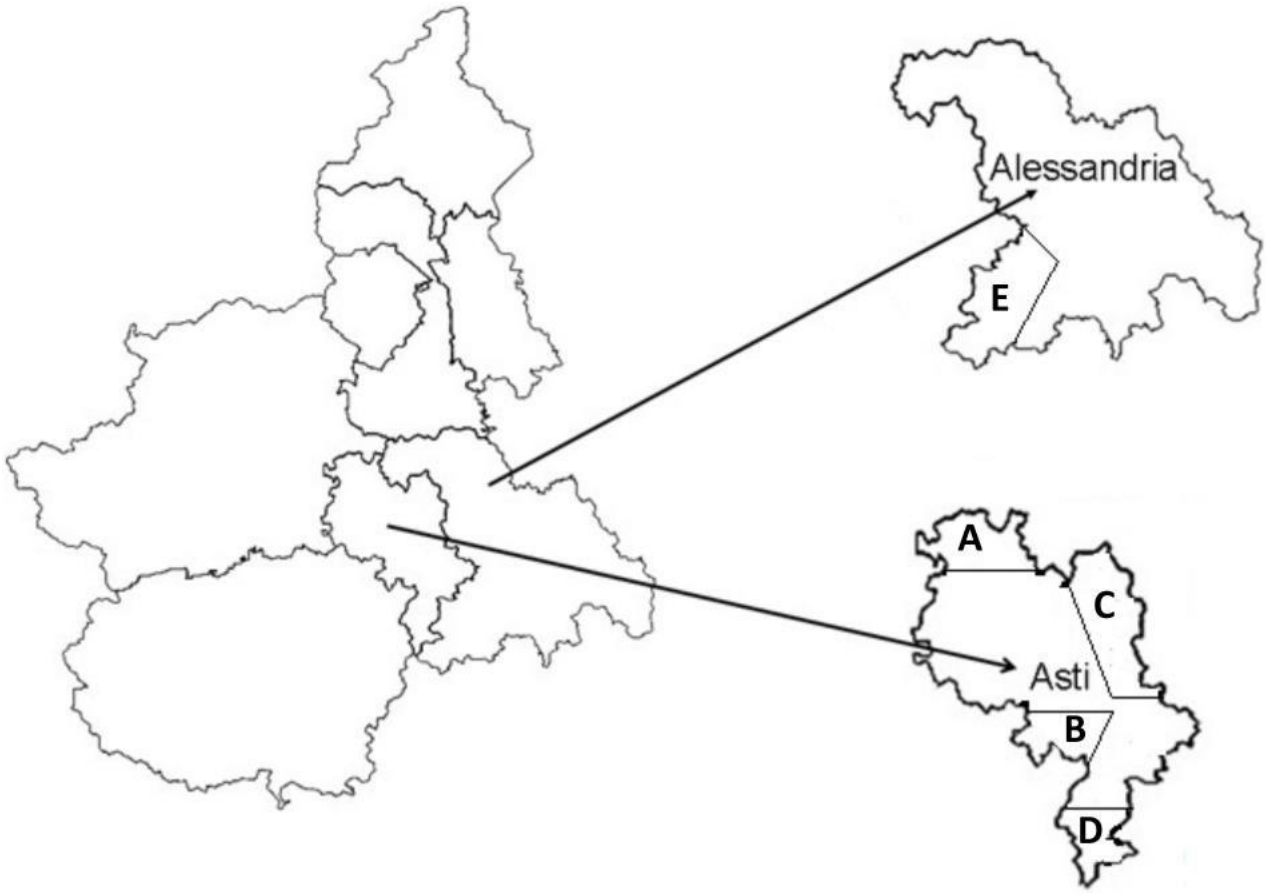


Figure 1. Geographic location of the fifteen vineyards in the Monferrato region (Asti and Alessandria) with indication of the 5 areas considered for the population genetic analysis reported in Table 2. The distribution of the vineyards in the five areas was as follows: A (Murisengo), B (Montegrosso d'Asti, Costigliole d'Asti, San Martino Alfieri, Agliano Terme, Isola d'Asti), C (Vinchio, Nizza Monferrato, Incisa Scapaccino), D (Loazzolo) and E (Ricaldone, Aqui Terme, Alice bel colle).

The PCR amplification of the δ interspersed sequences was also used to identify the genetic differences among the *S. cerevisiae* isolated during the fermentations. The molecular fingerprinting analysis, using a coefficient of similarity of 85%, allowed numerous strains among the isolates to be distinguished. The dendrogram resulting from the analysis of 636 *S. cerevisiae* isolates highlighted the presence of 62 clusters and 37 strains, which were unique and did not cluster with any other isolate. The most numerous clusters were: XVII and XLI with 60 and 44 isolates, respectively (Tab. 1). It is interesting to notice the different level of heterogeneity of *S. cerevisiae* isolated from the different vineyards. For example, most of the isolates from vineyard 1 grouped in one single cluster (XLI), while in other cases (vineyards 12, 13 and 14) a high level of diversity was observed. Only 37 *S. cerevisiae* δ-PCR patterns were unique, demonstrating a feeble biodiversity of indigenous *S. cerevisiae* strains in Monferrato area. Considering the ratio between the number of *Saccharomyces* isolated and the number of observed patterns, as an approximate biodiversity estimation, our results showed similar values to those found in Portugal (Schuller et al., 2005) and in France (VALERO *et al.*, 2007).

Table 1. Clusters obtained from a comparison of the different fingerprinting profiles of the *S. cerevisiae* isolates examined in this study with/by means of the molecular technique. The arbitrarily selected coefficient of similarity was 85%. The table shows their composition according to the geographical locations (vineyard) from which the isolates were obtained.

Cluster	Number of strains in the cluster	Monferrato's vineyards														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
I	5	/	/	/	/	/	/	/	/	/	/	5	/	/	/	/
II	15	/	/	/	/	11	/	4	/	/	/	/	/	/	/	/
III	14	/	/	6	/	/	/	4	/	/	/	2	/	2	/	/
IV	6	/	/	6	/	/	/	/	/	/	/	/	/	/	/	/
V	3	/	3	/	/	/	/	/	/	/	/	/	/	/	/	/
VI	6	/	/	/	/	/	/	/	/	/	2	/	/	/	3	1
VII	2	/	/	/	/	/	/	/	/	/	/	/	/	/	/	2
VIII	24	/	14	5	/	/	/	/	2	/	/	/	/	1	/	2
IX	4	/	4	/	/	/	/	/	/	/	/	/	/	/	/	/
X	7	/	/	/	/	3	/	/	/	/	/	4	/	/	/	/
XI	10	/	/	/	/	/	/	/	/	3	/	/	/	7	/	/
XII	8	/	/	/	/	/	/	/	/	4	/	/	/	4	/	/
XIII	7	/	7	/	/	/	/	/	/	/	/	/	/	/	/	/
XIV	18	/	4	/	/	10	/	/	/	/	/	1	/	2	1	/
XV	4	/	/	/	/	/	2	/	/	/	/	/	/	2	/	/
XVI	2	/	/	/	/	/	/	/	/	/	1	/	/	/	1	/
XVII	60	6	4	3	2	1	/	3	8	/	2	/	2	5	9	15
XVIII	6	/	/	/	/	/	/	2	2	/	/	/	/		2	/
XIX	10	/	/	4	/	/	/	/	/	/	/	/	/	4	/	2
XX	15	1	/	/	9	2	/	/	/	/	/	/	/	/	2	1
XXI	13	/	/	/	2	2	/	/	2	/	2	/	/	2	2	1
XXII	28	2	/	1	/	/	/	/	1	/	/	/	1	1	4	18
XXIII	2	/	/	1	/	/	/	/	/	/	/	/	/	1	/	/
XXIV	3	/	/	/	/	/	/	/	/	/	3	/	/	/	/	/
XXV	3	/	/	/	/	/	/	/	/	/	3	/	/	/	/	/
XXVI	4	/	/	/	/	/	/	/	/	/	/	/	/	/	/	4
XXVII	5	/	/	3	/	/	/	/	/	/	/	/	/	/	1	1
XVIII	2	/	/	/	/	/	/	/	/	/	/	/	/	2	/	/
XXIX	10	/	/	/	/	/	/	/	/	/	/	/	/	10	/	/
XXX	6	/	/	/	/	/	/	/	/	/	/	/	/	3	3	/
XXXI	7	/	/	/	/	/	/	/	/	/	3	/	/	3	/	1
XXXII	3	1	/	/	/	/	/	/	/	/	2	/	/	/	/	/
XXXIII	6	/	/	/	/	/	/	/	/	/	/	/	5	1	/	/
XXXIV	5	/	/	/	/	/	/	/	/	/	/	/	5	/	/	/
XXXV	3	/	/	/	1	/	/	/	/	/	/	/	/	2	/	/
XXXVI	5	/	/	/	/	/	5	/	/	/	/	/	/	/	/	/
XXXVII	9	/	/	/	2	/	/	/	2	/	4	/	/	/	/	1
XXXVIII	4	/	/	/	/	/	/	/	/	/	/	/	/	/	/	4

Table 1. Continued

Cluster	Number of strains in the cluster	Monferrato's vineyards														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
XXXIX	2	/	/	/	/	/	/	/	2	/	/	/	/	/	/	/
XL	7	/	/	/	/	/	/	/	/	/	/	/	/	7	/	/
XLI	44	38	/	/	/	/	/	/	/	/	/	/	3	3	/	/
XLII	7	/	/	/	/	/	2	/	1	4	/	/	/	/	/	/
XLIII	9	/	/	5	/	/	/	/	/	1	/	/	2	/	1	/
XLIV	7	/	/	/	/	/	/	/	3	3	1	/	/	/	/	/
XLV	6	/	/	/	/	/	/	/	/	/	/	/	/	6	/	/
XLVI	5	/	/	/	/	/	/	5	/	/	/	/	/	/	/	/
XLVII	10	/	/	/	9	/	/	/	/	/	/	/	/	1	/	/
XLVIII	15	/	/	/	/	/	/	/	/	/	/	13	2	/	/	/
XLIX	6	/	/	/	/	2	4	/	/	/	/	/	/	/	/	/
L	10	/	3	/	/	/	/	/	6	/	1	/	/	/	/	/
LI	4	/	/	/	/	/	/	/	/	3	/	1	/	/	/	/
LII	30	/	/	/	/	2	16	10	/	/	/	/	/	2	/	/
LIII	12	/	/	/	/	2	/	7	/	/	/	/	1	/	2	/
LIV	2	/	/	/	/	/	/	/	/	/	/	/	/	/	2	/
LV	10	/	/	/	/	/	/	/	/	/	/	/	/	/	/	10
LVI	5	/	/	/	/	3	2	/	/	/	/	/	/	/	/	/
LVII	19	2	/	/	/	2	/	/	1	/	/	/	/	6	8	/
LVIII	15	/	/	/	/	3	/	/	/	/	2	/	10	/	/	/
LIX	25	/	/	/	/	/	5	7	/	/	/	/	1	/	12	/
LX	22	/	/	/	/	/	/	/	1	/	7	/	/	12	1	1
LXI	3	/	/	/	/	/	/	/	/	/	1	/	/	2	/	/
LXII	17	/	3	/	/	/	/	1	/	/	/	4	8	/	1	/

In order to investigate further the *S. cerevisiae* diversity, 96 strains were selected on the basis of the cluster analysis, and screened for desirable oenological characteristics (ESTEVE-ZARZOSO *et al.*, 2000) such as: a low production of hydrogen sulphide and tolerance to a final concentration of 150 mg/L total sulphur dioxide and 16% (v/v) of ethanol (Supplementary Table 1). All the strains exhibited a medium hydrogen sulphide production level; 5% of them appeared to be pale hazel on Biggy agar, while the others were hazel. Concerning the results of the tolerance to SO_2, the selected strains were able to grow in the presence of 50 and 100 mg/L of SO_2 (83% and 60% of the isolates, respectively), while only a few isolates (32%) grew at 150 mg/L of SO_2 within 24 h. Extending the incubation time to 48 h, the number of the isolates that were able to grow at 150 mg/L of SO_2 increased to 63%. Only one strain (ScBa20) was totally inhibited by SO_2. As far as ethanol tolerance is concerned, 60% of the strains grew at 14% v/v within 24 h. Ethanol mainly affected yeast growth by increasing the lag phase, and this evidence explains why after increasing the incubation time to 48 h, 95% of the strains were able to grow in all the ethanol concentrations (Suppl. Tab. 1). β-glucosidase activity was found in only 2.7% of the strains, thus indicating possible production and activity during the fermentation. Protease activity was detected in 37.8% of the tested *S. cerevisiae*, while 21.6% were able to hydrolyse esters (data not shown).

In order to extend the information on the 96 *S. cerevisiae* strains of the Monferrato area, alcoholic fermentations were carried out in Barbera grape must. The experimental wines obtained were analyzed to establish the content of some by-products correlated to the organoleptic quality of the

wine and the obtained results are reported in Table 2. The values of the residual sugars ranged from 3.44 to 148.64 g/L. Only seven isolates (ScBa4, ScBa5, ScBa13, ScBa26, ScBa44, ScBa60 and ScBa63) were able to leave less than 5 g/L of residual sugars after 14 days of fermentation. All the strains, except ScBa12, ScBa47, ScBa48 and ScBa57, were capable of consuming almost all the glucose of the must, confirming the glucophylic character of this species. All the yeast strains, that completed the fermentation, formed low amounts of acetic acid in the wines (less than 0.6 g/L). Glycerol production was relatively low, ranging from 5.20 to 7.86 g/L. The fermentation purity was high; most strains had a low ratio between the produced acetic acid and ethanol (range 0.01-0.09) and only three strains showed values above 0.04. As regards ethanol production, 52% of the strains produced more than 13%, and 6% of them managed to develop more than 14%.

Table 2. Chemical analysis of the wines obtained from the fermentation of the pure indigenous *S. cerevisiae* cultures. The data are means ± standard deviations. With * were reported strains which were unique and did not cluster with any other isolate.

Strains	Vineyard	Cluster	Residual glucose (g/L)	Residual fructose (g/L)	Glycerol (g/L)	Acetic acid (g/L)	Ethanol (%v/v)	Fermentation purity[a]	Ethanol yield[b]	Glycerol yield[c]	Acetic acid yield[d]
ScBa1	11	I	2.18 ± 0.54	24.23 ± 2.14	7.00 ± 0.15	0.45 ± 0.00	12.57 ± 0.19	0.036 ± 0.001	0.058 ± 0.001	0.032 ± 0.001	0.0359 ± 0.0008
ScBa2	13	*	0.88 ± 0.07	8.68 ± 0.80	6.86 ± 0.05	0.51 ± 0.02	13.77 ± 0.05	0.037 ± 0.001	0.059 ± 0.001	0.029 ± 0.001	0.0367 ± 0.0012
ScBa3	5	II	0.65 ± 0.08	4.43 ± 1.04	7.01 ± 0.01	0.56 ± 0.01	14.11 ± 0.11	0.039 ± 0.001	0.059 ± 0.001	0.029 ± 0.001	0.0395 ± 0.0005
ScBa4	7	*	0.68 ± 0.01	2.77 ± 1.47	7.42 ± 0.82	0.29 ± 0.03	14.14 ± 0.11	0.021 ± 0.002	0.059 ± 0.001	0.031 ± 0.003	0.0208 ± 0.0023
ScBa5	7	III	0.38 ± 0.27	3.08 ± 1.84	6.89 ± 0.10	0.30 ± 0.05	14.27 ± 0.01	0.021 ± 0.004	0.059 ± 0.001	0.029 ± 0.001	0.0207 ± 0.0037
ScBa6	7	*	1.14 ± 0.37	6.57 ± 2.71	7.08 ± 0.03	0.31 ± 0.07	13.89 ± 0.35	0.022 ± 0.006	0.059 ± 0.001	0.026 ± 0.001	0.0224 ± 0.0056
ScBa7	3	IV	1.58 ± 0.73	12.15 ± 1.93	6.92 ± 0.16	0.33 ± 0.00	13.82 ± 0.03	0.024 ± 0.001	0.060 ± 0.001	0.030 ± 0.001	0.0240 ± 0.001
ScBa8	2	V	2.98 ± 3.16	16.78 ± 18.98	6.54 ± 0.43	0.47 ± 0.34	12.60 ± 0.90	0.037 ± 0.025	0.056 ± 0.002	0.029 ± 0.005	0.0367 ± 0.0247
ScBa9	5	*	0.69 ± 0.25	5.68 ± 4.83	7.07 ± 0.01	0.29 ± 0.02	13.73 ± 0.20	0.021 ± 0.001	0.058 ± 0.002	0.030 ± 0.001	0.0215 ± 0.0014
ScBa10	10	VI	1.65 ± 0.49	15.33 ± 2.45	6.81 ± 0.14	0.41 ± 0.04	13.46 ± 0.13	0.031 ± 0.002	0.059 ± 0.001	0.030 ± 0.001	0.0307 ± 0.0025
ScBa11	15	VII	1.11 ± 0.96	11.44 ± 10.36	7.44 ± 0.08	0.35 ± 0.01	13.46 ± 0.58	0.026 ± 0.001	0.058 ± 0.001	0.032 ± 0.001	0.026 ± 0.0007
ScBa12	2	VIII	78.13 ± 0.19	70.52 ± 0.15	5.20 ± 0.10	0.43 ± 0.01	4.90 ± 0.09	0.088 ± 0.001	0.051 ± 0.001	0.054 ± 0.001	0.0879 ± 0.0001
ScBa13	2	IX	0.82 ± 0.25	2.80 ± 0.68	6.63 ± 0.08	0.20 ± 0.02	13.87 ± 0.28	0.014 ± 0.002	0.058 ± 0.001	0.028 ± 0.001	0.0144 ± 0.0015
ScBa14	11	X	1.14 ± 0.72	13.37 ± 5.12	6.99 ± 0.07	0.29 ± 0.03	13.59 ± 0.45	0.021 ± 0.003	0.059 ± 0.001	0.030 ± 0.001	0.0213 ± 0.0033
ScBa15	14	XI	2.88 ± 0.61	28.44 ± 1.79	7.10 ± 0.03	0.44 ± 0.01	12.38 ± 0.05	0.035 ± 0.001	0.058 ± 0.001	0.033 ± 0.001	0.0352 ± 0.0008
ScBa16	14	XII	3.71 ± 0.58	28.71 ± 1.54	6.82 ± 0.22	0.38 ± 0.04	12.04 ± 0.14	0.032 ± 0.003	0.057 ± 0.001	0.032 ± 0.001	0.0319 ± 0.0028
ScBa17	2	XIII	1.09 ± 0.07	6.93 ± 0.34	7.37 ± 0.01	0.39 ± 0.01	14.03 ± 0.03	0.028 ± 0.001	0.059 ± 0.001	0.031 ± 0.001	0.0275 ± 0.0004
ScBa18	2	*	0.89 ± 0.37	11.64 ± 5.35	6.46 ± 0.65	0.41 ± 0.18	13.00 ± 0.32	0.031 ± 0.013	0.056 ± 0.001	0.028 ± 0.004	0.0314 ± 0.0129
ScBa19	5	XIV	0.83 ± 0.23	8.09 ± 5.27	6.85 ± 0.09	0.36 ± 0.05	13.34 ± 0.71	0.028 ± 0.006	0.056 ± 0.004	0.029 ± 0.001	0.0275 ± 0.0055
ScBa20	14	XV	0.63 ± 0.24	7.80 ± 2.79	7.26 ± 0.02	0.41 ± 0.00	13.89 ± 0.07	0.029 ± 0.001	0.060 ± 0.001	0.031 ± 0.001	0.0292 ± 0.0004
ScBa21	10	XVI	1.58 ± 0.21	10.27 ± 2.32	6.72 ± 0.04	0.44 ± 0.06	13.40 ± 0.15	0.030 ± 0.001	0.0059 ±0.001	0.030 ±0.001	0.0234 ± 0.003
ScBa22	15	XVII	1.54 ± 0.62	17.88 ± 4.13	7.88 ± 0.12	0.38 ± 0.04	12.88 ± 0.14	0.030 ± 0.004	0.057 ± 0.001	0.035 ± 0.001	0.0297 ± 0.0037
ScBa23	13	*	1.04 ± 0.65	13.68 ± 5.92	7.35 ± 0.04	0.34 ± 0.01	13.21 ± 0.26	0.026 ± 0.001	0.058 ± 0.001	0.032 ± 0.001	0.026 ± 0.0012
ScBa24	14	*	1.18 ±	15.75 ±	7.13 ±	0.34 ±	13.14 ±	0.026 ±	0.058 ±	0.031 ±	0.0261 ±

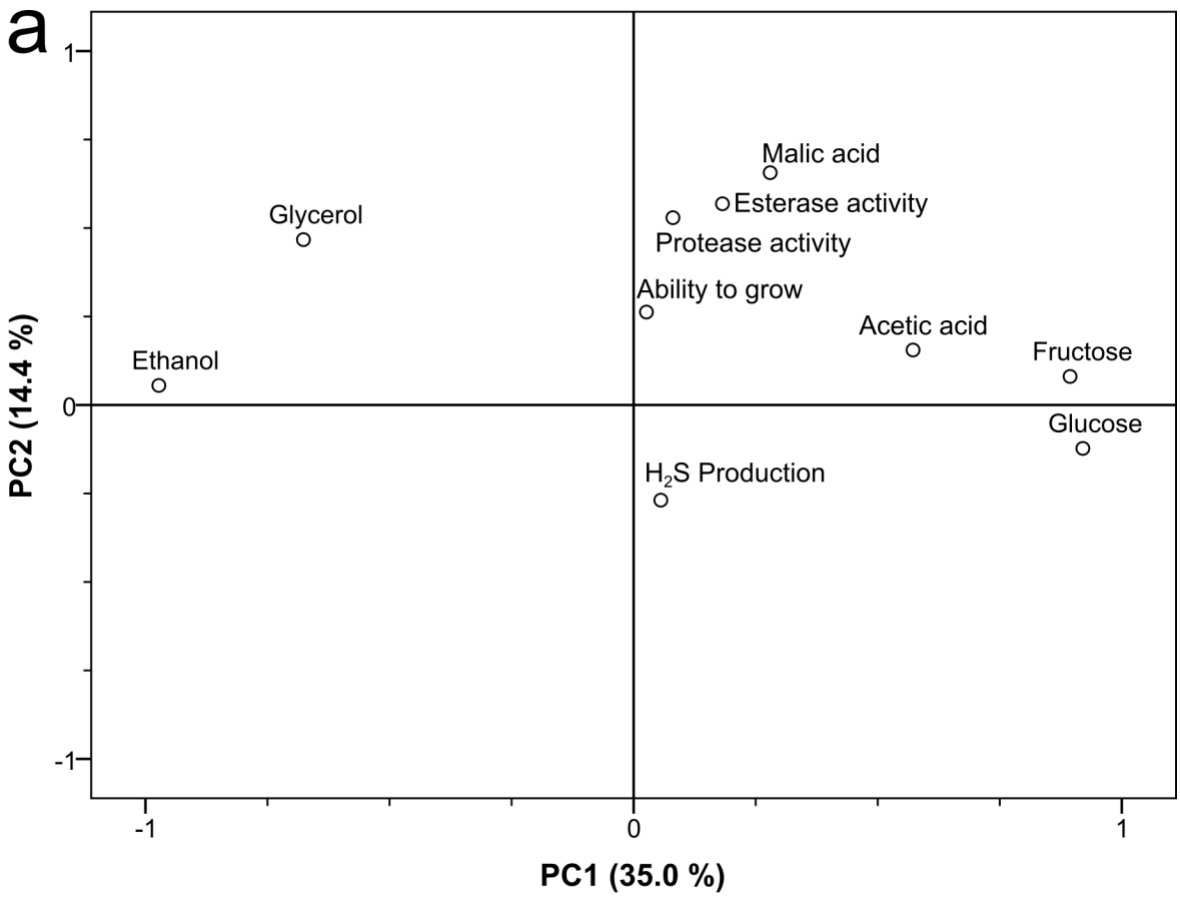
4			0.04	0.21	0.20	0.01	0.19	0.001	0.001	0.001	0.0007
ScBa25	7	*	3.86 ± 1.76	22.9 ± 5.06	6.81 ± 0.17	0.26 ± 0.01	12.82 ± 0.71	0.021 ± 0.001	0.059 ± 0.001	0.031 ± 0.001	0.0206 ± 0.0006
ScBa26	7	XVIII	0.69 ± 0.08	4.20 ± 1.12	7.13 ± 0.21	0.25 ± 0.04	13.96 ± 0.22	0.018 ± 0.003	0.058 ± 0.001	0.030 ± 0.001	0.0181 ± 0.0035
ScBa27	14	XIX	0.73 ± 0.48	9.18 ± 6.55	6.88 ± 0.13	0.24 ± 0.04	13.72 ± 0.64	0.017 ± 0.004	0.059 ± 0.001	0.029 ± 0.001	0.0174 ± 0.0037
ScBa28	4	XX	0.75 ± 0.10	7.31 ± 2.50	6.65 ± 0.12	0.32 ± 0.07	13.68 ± 0.30	0.024 ± 0.006	0.058 ± 0.001	0.028 ± 0.001	0.0237 ± 0.0058
ScBa29	5	XXI	1.84 ± 0.13	16.38 ± 0.98	6.80 ± 0.11	0.41 ± 0.05	13.32 ± 0.20	0.031 ± 0.004	0.059 ± 0.001	0.030 ± 0.001	0.0307 ± 0.0043
ScBa30	15	XXII	5.12 ± 2.03	31.28 ± 5.30	6.65 ± 0.03	0.44 ± 0.01	11.8 ± 0.54	0.037 ± 0.001	0.057 ± 0.001	0.032 ± 0.001	0.037 ± 0.0008
ScBa31	2	XXIII	1.15 ± 0.44	11.07 ± 4.09	6.59 ± 0.01	0.40 ± 0.06	13.56 ± 0.41	0.029 ± 0.005	0.058 ± 0.001	0.028 ± 0.001	0.0293 ± 0.0051
ScBa32	10	XXIV	0.96 ± 0.47	6.09 ± 0.38	7.03 ± 0.84	0.37 ± 0.02	13.67 ± 0.17	0.027 ± 0.002	0.058 ± 0.001	0.03 ± 0.004	0.0269 ± 0.0015
ScBa33	10	XXV	0.65 ± 0.17	5.69 ± 1.43	6.42 ± 0.17	0.44 ± 0.03	13.97 ± 0.27	0.031 ± 0.003	0.059 ± 0.002	0.027 ± 0.001	0.0313 ± 0.003
ScBa34	13	*	1.13 ± 0.54	18.34 ± 1.89	7.78 ± 0.05	0.32 ± 0.00	12.82 ± 0.01	0.025 ± 0.001	0.057 ± 0.001	0.035 ± 0.001	0.0249 ± 0.0001
ScBa35	7	*	0.62 ± 0.20	5.13 ± 1.56	7.44 ± 0.03	0.52 ± 0.02	13.93 ± 0.20	0.037 ± 0.002	0.058 ± 0.001	0.031 ± 0.001	0.0372 ± 0.0019
ScBa36	15	XXVI	1.14 ± 0.42	15.81 ± 2.76	6.69 ± 0.08	0.40 ± 0.06	12.99 ± 0.12	0.031 ± 0.004	0.057 ± 0.001	0.029 ± 0.001	0.0306 ± 0.004
ScBa37	3	XXVII	0.57 ± 0.13	5.55 ± 2.26	6.59 ± 0.07	0.33 ± 0.01	13.87 ± 0.12	0.024 ± 0.001	0.058 ± 0.001	0.028 ± 0.001	0.0239 ± 0.0012
ScBa38	10	*	0.79 ± 0.02	7.81 ± 1.69	6.99 ± 0.32	0.41 ± 0.02	13.23 ± 0.50	0.031 ± 0.002	0.056 ± 0.003	0.030 ± 0.001	0.0310 ± 0.0002
ScBa39	14	XXVIII	1.58 ± 0.35	17.36 ± 1.76	7.86 ± 0.10	0.31 ± 0.01	12.99 ± 0.23	0.024 ± 0.001	0.058 ± 0.001	0.035 ± 0.001	0.0240 ± 0.0010
ScBa40	14	XXIX	2.68 ± 0.47	24.44 ± 0.38	7.49 ± 0.68	0.42 ± 0.05	12.24 ± 0.90	0.036 ± 0.007	0.054 ± 0.004	0.034 ± 0.003	0.0356 ± 0.0068
ScBa41	12	*	1.40 ± 0.86	19.33 ± 4.79	7.12 ± 0.16	0.39 ± 0.02	13.01 ± 0.28	0.03 ± 0.002	0.058 ± 0.001	0.032 ± 0.001	0.0297 ± 0.0025
ScBa42	13	XXX	2.52 ± 0.62	25.2 ± 1.99	6.98 ± 0.23	0.36 ± 0.00	12.6 ± 0.17	0.029 ± 0.001	0.058 ± 0.001	0.032 ± 0.001	0.0287 ± 0.0003
ScBa43	15	XXXI	3.07 ± 1.42	26.09 ± 4.50	6.91 ± 0.20	0.36 ± 0.02	12.50 ± 0.68	0.029 ± 0.001	0.058 ± 0.002	0.032 ± 0.001	0.0289 ± 0.0001
ScBa44	1	XXXII	0.62 ± 0.01	3.63 ± 0.59	6.99 ± 0.08	0.27 ± 0.01	14.05 ± 0.04	0.019 ± 0.001	0.058 ± 0.001	0.029 ± 0.001	0.0189 ± 0.0003
ScBa45	12	XXXIII	3.88 ± 0.02	30.01 ± 0.48	6.86 ± 0.11	0.41 ± 0.03	12.11 ± 0.02	0.034 ± 0.002	0.058 ± 0.001	0.033 ± 0.001	0.0338 ± 0.0020
ScBa46	12	XXXIV	3.32 ± 0.14	24.06 ± 0.06	7.69 ± 0.27	0.39 ± 0.00	12.43 ± 0.22	0.031 ± 0.001	0.057 ± 0.001	0.035 ± 0.001	0.0313 ± 0.0003
ScBa47	4	XXXV	61.26 ± 8.62	48.21 ± 7.22	5.65 ± 0.44	0.52 ± 0.02	7.42 ± 0.89	0.071 ± 0.006	0.055 ± 0.001	0.042 ± 0.002	0.0708 ± 0.0058
ScBa48	6	XXXVI	68.47 ± 0.49	53.25 ± 1.42	5.51 ± 0.29	0.54 ± 0.02	6.86 ± 0.28	0.079 ± 0.001	0.056 ± 0.002	0.045 ± 0.002	0.0786 ± 0.0003
ScBa49	15	XXXVII	1.39 ± 0.90	17.01 ± 3.35	6.94 ± 0.05	0.37 ± 0.00	12.88 ± 0.40	0.029 ± 0.001	0.057 ± 0.001	0.031 ± 0.001	0.0288 ± 0.0012
ScBa50	9	*	2.78 ± 0.19	25.25 ± 0.23	6.75 ± 0.15	0.39 ± 0.01	12.6 ± 0.08	0.031 ± 0.001	0.058 ± 0.001	0.031 ± 0.001	0.0311 ± 0.0003
ScBa51	15	XXXVIII	2.48 ± 0.03	24.83 ± 0.26	6.81 ± 0.18	0.36 ± 0.01	12.49 ± 0.22	0.029 ± 0.001	0.058 ± 0.001	0.031 ± 0.001	0.0291 ± 0.0001
ScBa52	8	XXXIX	0.71 ± 0.09	8.32 ± 0.62	6.67 ± 0.11	0.43 ± 0.02	13.73 ± 0.08	0.031 ± 0.001	0.058 ± 0.001	0.028 ± 0.001	0.0312 ± 0.0010
ScBa53	11	*	1.78 ± 1.01	21.99 ± 5.78	6.80 ± 0.04	0.42 ± 0.03	12.60 ± 0.14	0.033 ± 0.002	0.057 ± 0.001	0.031 ± 0.001	0.0333 ± 0.0019
ScBa54	14	XL	1.72 ± 1.39	15.94 ± 7.53	7.06 ± 0.01	0.31 ± 0.00	13.24 ± 0.42	0.023 ± 0.001	0.058 ± 0.001	0.031 ± 0.001	0.0233 ± 0.0011
ScBa55	1	XLI	2.11 ± 1.08	17.06 ± 5.14	6.70 ± 0.06	0.24 ± 0.01	13.18 ± 0.48	0.018 ± 0.001	0.059 ± 0.001	0.030 ± 0.001	0.0181 ± 0.0015
ScBa56	6	XLII	1.85 ± 0.39	15.86 ± 1.64	6.78 ± 0.01	0.32 ± 0.01	13.35 ± 0.16	0.024 ± 0.001	0.059 ± 0.001	0.030 ± 0.001	0.0238 ± 0.0007
ScBa57	3	XLIII	68.19 ± 0.5	45.76 ± 2.71	5.21 ± 0.13	0.60 ± 0.00	7.39 ± 0.17	0.081 ± 0.002	0.057 ± 0.001	0.040 ± 0.002	0.0811 ± 0.0024
ScBa58	8	XLIV	4.84 ±	30.43 ±	6.53 ±	0.43 ±	11.73 ±	0.038 ±	0.055 ±	0.031 ±	0.0375 ±

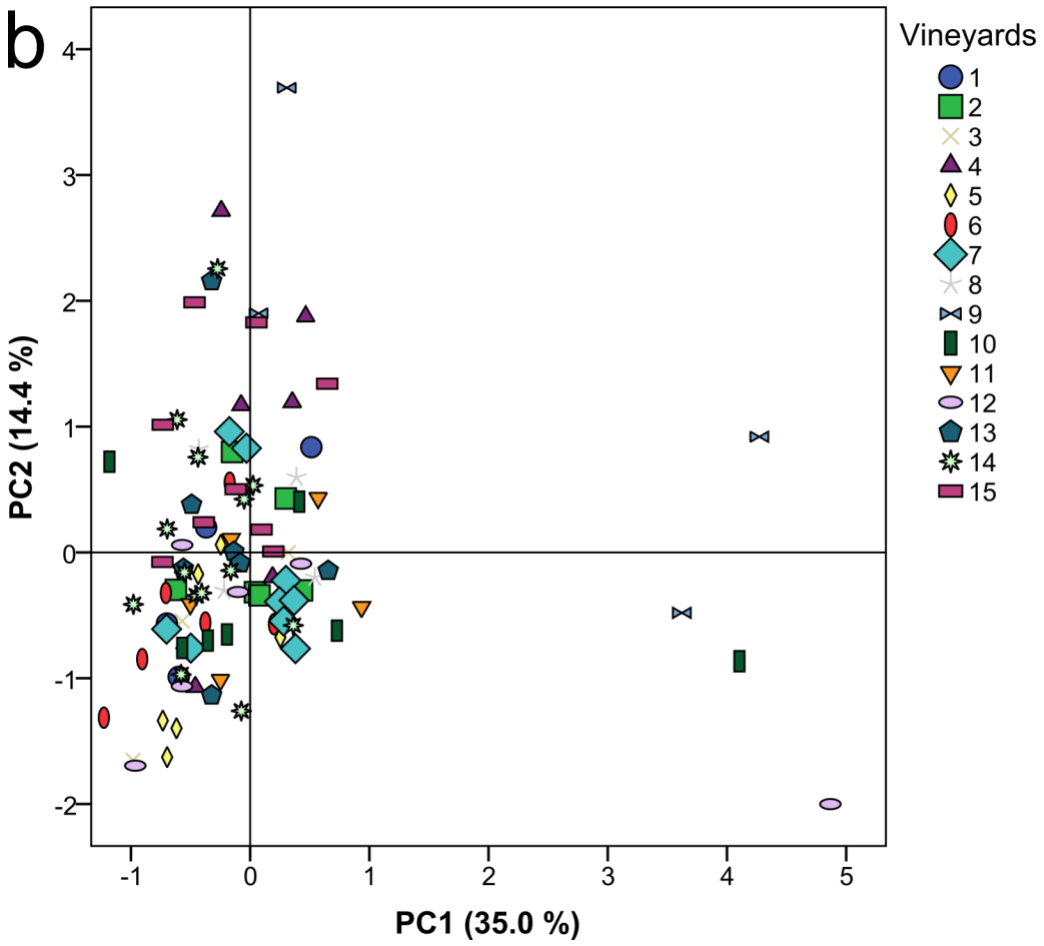
8			3.73	10.18	0.20	0.01	1.22	0.004	0.002	0.003	0.0045
ScBa59	1	*	3.60 ± 1.45	28.12 ± 4.22	6.84 ± 0.01	0.42 ± 0.00	12.20 ± 0.46	0.034 ± 0.001	0.057 ± 0.001	0.032 ± 0.001	0.0341 ± 0.0011
ScBa60	10	XLIV	0.69 ± 0.20	4.12 ± 2.80	7.81 ± 0.20	0.30 ± 0.02	14.08 ± 0.03	0.022 ± 0.001	0.059 ± 0.001	0.033 ± 0.001	0.0216 ± 0.0013
ScBa61	14	XLV	0.56 ± 0.05	7.10 ± 0.59	6.96 ± 0.19	0.42 ± 0.00	13.79 ± 0.18	0.030 ± 0.001	0.058 ± 0.001	0.029 ± 0.001	0.0305 ± 0.0007
ScBa62	13	*	0.98 ± 0.08	13.73 ± 0.37	6.43 ± 0.17	0.32 ± 0.01	13.21 ± 0.28	0.024 ± 0.001	0.058 ± 0.001	0.028 ± 0.001	0.0239 ± 0.0001
ScBa63	14	*	0.39 ± 0.15	3.85 ± 1.71	7.20 ± 0.07	0.50 ± 0.14	13.58 ± 0.48	0.038 ± 0.012	0.056 ± 0.002	0.030 ± 0.001	0.0377 ± 0.0116
ScBa64	14	*	0.98 ± 0.54	7.81 ± 1.64	6.87 ± 0.14	0.32 ± 0.09	13.33 ± 0.61	0.025 ± 0.008	0.056 ± 0.002	0.029 ± 0.001	0.0248 ± 0.0079
ScBa65	7	XLVI	1.07 ± 0.51	9.91 ± 4.72	6.46 ± 0.09	0.23 ± 0.01	13.42 ± 0.50	0.017 ± 0.001	0.057 ± 0.001	0.028 ± 0.001	0.0173 ± 0.0013
ScBa66	4	XLVII	3.00 ± 0.12	25.73 ± 0.44	6.76 ± 0.01	0.39 ± 0.01	12.15 ± 0.07	0.032 ± 0.001	0.056 ± 0.001	0.031 ± 0.001	0.0319 ± 0.0005
ScBa67	4	*	3.17 ± 1.05	25.26 ± 2.74	7.72 ± 1.37	0.37 ± 0.01	12.71 ± 0.56	0.029 ± 0.001	0.059 ± 0.004	0.036 ± 0.007	0.0292 ± 0.0002
ScBa68	4	*	0.89 ± 0.22	9.31 ± 2.35	6.60 ± 0.22	0.22 ± 0.03	13.46 ± 0.31	0.016 ± 0.002	0.057 ± 0.001	0.028 ± 0.001	0.0162 ± 0.0017
ScBa69	4	*	0.85 ± 0.42	8.44 ± 4.20	6.54 ± 0.03	0.24 ± 0.02	13.41 ± 0.27	0.018 ± 0.002	0.057 ± 0.001	0.028 ± 0.001	0.0182 ± 0.0021
ScBa70	12	XLVIII	4.27 ± 1.33	31.23 ± 3.89	6.88 ± 0.02	0.40 ± 0.03	12.27 ± 0.27	0.033 ± 0.001	0.059 ± 0.003	0.033 ± 0.001	0.0330 ± 0.0014
ScBa71	11	*	0.72 ± 0.33	4.54 ± 2.92	6.72 ± 0.09	0.30 ± 0.01	13.88 ± 0.39	0.021 ± 0.001	0.058 ± 0.001	0.028 ± 0.001	0.0214 ± 0.0001
ScBa72	6	XLIX	0.66 ± 0.09	7.05 ± 0.76	6.84 ± 0.01	0.40 ± 0.01	13.78 ± 0.08	0.029 ± 0.001	0.058 ± 0.001	0.029 ± 0.001	0.0291 ± 0.0009
ScBa73	14	*	0.78 ± 0.04	7.81 ± 0.64	7.10 ± 0.04	0.42 ± 0.00	13.82 ± 0.02	0.031 ± 0.001	0.059 ± 0.001	0.030 ± 0.001	0.0307 ± 0.0002
ScBa74	8	L	0.93 ± 0.28	9.96 ± 2.72	6.64 ± 0.01	0.40 ± 0.01	13.49 ± 0.11	0.030 ± 0.001	0.058 ± 0.001	0.028 ± 0.001	0.0300 ± 0.0007
ScBa75	9	LI	3.57 ± 2.41	27.49 ± 8.01	6.79 ± 0.13	0.38 ± 0.01	12.35 ± 0.66	0.031 ± 0.002	0.058 ± 0.001	0.032 ± 0.001	0.0309 ± 0.0022
ScBa76	9	*	3.63 ± 1.97	30.74 ± 4.86	6.58 ± 0.05	0.37 ± 0.00	12.01 ± 0.29	0.031 ± 0.001	0.057 ± 0.001	0.031 ± 0.001	0.0308 ± 0.0008
ScBa77	9	*	3.96 ± 1.15	30.12 ± 3.88	6.61 ± 0.04	0.37 ± 0.03	11.93 ± 0.41	0.031 ± 0.001	0.057 ± 0.001	0.031 ± 0.001	0.0314 ± 0.0011
ScBa78	7	*	1.02 ± 0.22	9.71 ± 2.42	6.15 ± 0.11	0.31 ± 0.04	13.42 ± 0.11	0.023 ± 0.003	0.057 ± 0.001	0.026 ± 0.001	0.0228 ± 0.0033
ScBa79	11	*	1.23 ± 0.37	12.73 ± 2.91	6.52 ± 0.13	0.23 ± 0.01	13.37 ± 0.06	0.017 ± 0.001	0.058 ± 0.001	0.028 ± 0.001	0.0169 ± 0.0007
ScBa80	8	*	6.79 ± 2.11	36.88 ± 3.98	6.57 ± 0.00	0.41 ± 0.02	11.22 ± 0.36	0.037 ± 0.003	0.056 ± 0.001	0.033 ± 0.001	0.0366 ± 0.0032
ScBa81	7	LII	1.96 ± 1.25	15.41 ± 6.55	6.96 ± 0.03	0.40 ± 0.01	13.30 ± 0.38	0.030 ± 0.002	0.059 ± 0.001	0.031 ± 0.001	0.0299 ± 0.0017
ScBa82	6	LIII	1.00 ± 0.06	10.89 ± 0.92	6.65 ± 0.04	0.21 ± 0.00	13.36 ± 0.07	0.016 ± 0.001	0.057 ± 0.001	0.029 ± 0.001	0.0157 ± 0.0001
ScBa83	5	LIV	2.43 ± 1.29	17.35 ± 5.92	6.66 ± 0.15	0.38 ± 0.01	12.82 ± 0.45	0.030 ± 0.002	0.057 ± 0.001	0.030 ± 0.001	0.0298 ± 0.0017
ScBa84	15	LV	2.14 ± 0.86	23.56 ± 3.33	6.68 ± 0.05	0.40 ± 0.02	12.47 ± 0.29	0.032 ± 0.002	0.057 ± 0.001	0.031 ± 0.001	0.0318 ± 0.0022
ScBa85	14	*	3.92 ± 1.20	29.25 ± 3.40	6.70 ± 0.02	0.38 ± 0.00	12.10 ± 0.16	0.031 ± 0.001	0.057 ± 0.001	0.032 ± 0.001	0.0314 ± 0.0008
ScBa86	6	LVI	1.06 ± 0.15	10.25 ± 1.61	6.73 ± 0.13	0.42 ± 0.01	13.60 ± 0.20	0.031 ± 0.001	0.058 ± 0.002	0.029 ± 0.001	0.0307 ± 0.0011
ScBa87	1	LVII	1.74 ± 0.96	21.21 ± 4.94	6.85 ± 0.07	0.39 ± 0.02	12.54 ± 0.20	0.031 ± 0.002	0.057 ± 0.001	0.031 ± 0.001	0.0311 ± 0.0021
ScBa88	12	LVIII	2.85 ± 2.21	25.68 ± 9.50	6.64 ± 0.24	0.37 ± 0.02	12.39 ± 0.02	0.029 ± 0.002	0.058 ± 0.003	0.031 ± 0.003	0.0295 ± 0.0016
ScBa89	13	*	2.24 ± 0.24	19.4 ± 0.70	7.12 ± 0.07	0.33 ± 0.02	13.07 ± 0.18	0.025 ± 0.001	0.059 ± 0.001	0.032 ± 0.001	0.0253 ± 0.0009
ScBa90	13	LIX	2.55 ± 0.28	24.87 ± 0.73	7.43 ± 0.20	0.32 ± 0.01	12.29 ± 0.13	0.026 ± 0.001	0.057 ± 0.002	0.034 ± 0.001	0.0258 ± 0.0011
ScBa91	10	LX	2.64 ± 0.41	19.53 ± 1.64	6.36 ± 0.17	0.37 ± 0.01	12.64 ± 0.11	0.029 ± 0.001	0.057 ± 0.001	0.029 ± 0.001	0.0292 ± 0.0007
ScBa9	14	LXI	3.43 ±	26.05 ±	6.49 ±	0.36 ±	12.06 ±	0.030 ±	0.056 ±	0.030 ±	0.0301 ±

2			2.51	8.16	0.02	0.01	0.33	0.001	0.001	0.002	0.0001
ScBa93	6	LXII	0.67 ± 0.31	6.69 ± 5.00	6.46 ± 0.18	0.21 ± 0.01	13.55 ± 0.34	0.016 ± 0.001	0.057 ± 0.002	0.027 ± 0.001	0.0156 ± 0.0001
ScBa94	5	XX	0.83 ± 0.05	10.16 ± 0.53	6.59 ± 0.05	0.28 ± 0.00	13.46 ± 0.05	0.021 ± 0.001	0.058 ± 0.001	0.028 ± 0.001	0.0205 ± 0.0003
ScBa95	15	LXII	2.42 ± 0.73	24.31 ± 2.48	6.55 ± 0.17	0.37 ± 0.01	12.31 ± 0.24	0.030 ± 0.002	0.057 ± 0.002	0.030 ± 0.001	0.0301 ± 0.0017
ScBa96	12	LIX	3.18 ± 1.87	25.13 ± 5.26	6.67 ± 0.06	0.40 ± 0.02	12.12 ± 0.28	0.033 ± 0.001	0.056 ± 0.001	0.031 ± 0.001	0.0329 ± 0.0007

[a] Fermentation purity: acetic acid (g/L)/ethanol % (v/v), [b] Ethanol yield: ethanol % (v/v)/sugar consumption (g/L), [c] Glycerol yield: glycerol (g/L)/sugar consumption (g/L), [d] Acetic acid: acetic acid (g/L)/)/sugar consumption(g/L).

The physiological data from the growth tests at 50 mg/L of SO_2 after 24 h (enological conditions, variable: "Ability to grow"), the presence or absence of enzymatic activities (esterase and protease activity), H_2S production and the chemical composition (glucose, fructose, malic and acetic acids, glycerol, and ethanol) of the wines obtained after 14 days of fermentation were used to evaluate the technological diversity of the strains. A Principal Component Analysis (PCA) was carried out and the outcome is presented in Figure 2, including the loadings plot (Figure 2a) and the scatter plot (Figure 2b). PC1 (35.0 % of variance explained) was better correlated with the strains that left high level of residual sugars present in the wine and that produced high amount of acetic acid, while PC2 (14.4 % of variance explained) was mainly correlated to the high production of glycerol and low degradation of malic acid (Figure 2a). Two groups may be differentiated according to the scatter plot (PC1 or PC2 with values close or higher than 2, respectively). Some strains from vineyards 4, 13, 14, and 15 produced high glycerol amounts and preserved malic acid contents, and two isolates from vineyards 10 and 12 were unsatisfactory due to their sugars degradation. In addition, all the four isolates from vineyard 9 were present either in the first or in the second group (Figure 2b).


a
Malic acid
Esterase activity
Glycerol
Protease activity
Ability to grow
Acetic acid
Fructose
Ethanol
Glucose
H2S Production
PC2 (14.4 %)
PC1 (35.0 %)
1
0
-1


b
Vineyards
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
PC2 (14.4 %)
PC1 (35.0 %)
-2
-1
0
1
2
3
4
5

308

Figure 2. Principal component analysis of the *S. cerevisiae* strains from the fifteen vineyards (as reported Figure 1), according to the wine chemical composition: loadings plot (a) and scatter plot (b). The fifteen vineyard considered were: 1 (Murisengo), 2 (San Martino Alfieri), 3 (Costigliole d'Asti), 4 (Isola d'Asti), 5 (Montegrosso d'Asti), 6 (Agliano Terme), 7 (Vinchio), 8 (Nizza Monferrato),9 (Incisa Scapaccino), 10 (Loazzolo), 11 (Ricaldone), 12 (Alice bel colle), 13 (Acqui Terme Crocera south west), 14 (Acqui Terme Crocera south est) and 15 (Acqui Terme Dannona).

CONCLUSIONS

The present study investigated the genetic and technological diversity of autochthonous *S. cerevisiae* in the North-West of Italy, in the Monferrato area. It was possible to genetically and phenotypically differentiate the strains. In order to investigate the ability of the isolated strains to properly ferment Barbera must, at pilot scale level first and in industrial settings after, relevant technological characteristics, such as sugar consumption and acetic acid production, should be taken into consideration. All the data presented here were obtained from pasteurized natural must, and the ability of the selected strains to dominate the natural grape and must mycobiota should therefore be determined throughout the fermentation process. In parallel, the ability to complete the fermentation in the competitive environment of a natural grape must should also be confirmed. Finally, the production of compounds, such as alcohols, esters, carbonyl compounds and fatty acids that have an impact on the sensory characteristics of wine should also be evaluated.

Acknowledgments

This work has been funded by EU FP7 under Grant Agreement 315065-WILWINE (http:// www.wildwine.eu/). The information in this document only reflects the authors' views and the Community is not liable for any use that may be made of the information contained herein.

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Supplementary table 1. Results of the resistance to ethanol and SO_2 of the tested strains. The values presented are the ratio between the OD of the isolates in broth with and without ethanol or SO_2 times 100 at the specific incubation times. The values are the means of triplicate experiments.

Strains	SO_2 growth (mg/L)						Ethanol growth (% vol.)					
	24 hours of incubation			48 hours of incubation			24 hours of incubation			48 hours of incubation		
	50	100	150	50	100	150	12	14	16	12	14	16
ScBa1	36	6	14	81	74	81	0	0	0	64	0	0
ScBa2	82	87	71	93	99	89	100	93	100	100	100	100
ScBa3	81	46	36	100	91	100	36	6	7	97	47	0
ScBa4	34	3	4	100	72	36	2	1	1	17	0	0
ScBa5	81	46	36	98	99	96	36	6	7	98	73	4
ScBa6	44	46	16	87	67	52	11	1	1	13	1	1
ScBa7	58	48	42	95	88	61	9	4	4	90	6	5
ScBa8	76	49	42	98	94	93	8	4	5	85	7	5
ScBa9	70	42	43	96	92	94	5	4	5	72	4	5
ScBa10	26	15	9	70	69	60	19	1	0	70	0	0
ScBa11	22	6	6	87	83	10	2	1	1	5	0	0
ScBa12	9	6	2	44	7	7	4	2	2	3	1	1
ScBa13	60	32	32	99	93	83	9	3	3	97	3	3
ScBa14	38	2	1	85	55	16	51	1	0	61	0	0
ScBa15	26	3	3	72	30	14	49	0	0	58	0	0
ScBa16	2	4	23	6	40	77	51	0	0	61	0	0
ScBa17	12	0	0	91	54	17	37	0	0	76	0	1
ScBa18	47	0	0	55	9	0	1	1	0	38	0	0
ScBa19	87	58	52	85	84	75	40	0	0	77	0	0
ScBa20	63	0	0	64	2	0	0	0	0	13	0	0
ScBa21	52	31	46	60	59	50	55	0	0	83	0	0
ScBa22	96	91	83	100	97	96	100	95	97	98	93	99
ScBa23	67	63	54	96	66	40	96	70	71	100	92	100

474

Strains	SO_2 growth (mg/L)						Ethanol growth (% vol.)					
	24 hours of incubation			48 hours of incubation			24 hours of incubation			48 hours of incubation		
	50	100	150	50	100	150	12	14	16	12	14	16
ScBa1	36	6	14	81	74	81	0	0	0	64	0	0
ScBa2	82	87	71	93	99	89	100	93	100	100	100	100
ScBa3	81	46	36	100	91	100	36	6	7	97	47	0
ScBa4	34	3	4	100	72	36	2	1	1	17	0	0
ScBa5	81	46	36	98	99	96	36	6	7	98	73	4
ScBa6	44	46	16	87	67	52	11	1	1	13	1	1
ScBa7	58	48	42	95	88	61	9	4	4	90	6	5
ScBa8	76	49	42	98	94	93	8	4	5	85	7	5
ScBa9	70	42	43	96	92	94	5	4	5	72	4	5
ScBa10	26	15	9	70	69	60	19	1	0	70	0	0
ScBa11	22	6	6	87	83	10	2	1	1	5	0	0
ScBa12	9	6	2	44	7	7	4	2	2	3	1	1
ScBa13	60	32	32	99	93	83	9	3	3	97	3	3
ScBa14	38	2	1	85	55	16	51	1	0	61	0	0
ScBa15	26	3	3	72	30	14	49	0	0	58	0	0
ScBa16	2	4	23	6	40	77	51	0	0	61	0	0
ScBa17	12	0	0	91	54	17	37	0	0	76	0	1
ScBa18	47	0	0	55	9	0	1	1	0	38	0	0
ScBa19	87	58	52	85	84	75	40	0	0	77	0	0
ScBa20	63	0	0	64	2	0	0	0	0	13	0	0
ScBa21	52	31	46	60	59	50	55	0	0	83	0	0
ScBa22	96	91	83	100	97	96	100	95	97	98	93	99
ScBa23	67	63	54	96	66	40	96	70	71	100	92	100

475