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(Article begins on next page)

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Conflict of interest

Authors declare no conflict of interest.

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Clonal selection of wine yeasts with differential adsorption activities towards phenolics and ochratoxin A

ABSTRACT

To design a fast, easy, and cheap procedure for yeast selection with differential adsorption activities towards phenolics and ochratoxin A, 284 yeast strains were screened evaluating the type of growth during grape must fermentation, the acetic acid production on Chalk agar, the H_2S production on BiGGY agar, and the spore-formation on acetate agar. After that initial step, 84 strains were pre-selected and further studied by Petri plate tests and to determine their wine-making trials for their differential adsorption activities towards phenolics and ochratoxin A. Three yeast strains were selected based on the above tests. After confirming their belonging to the *Saccharomyces cerevisiae* species and their diploidy, a spore clone selection was performed. The strain Sc1741A_1D was selected and used in wine-making at six Calabrian wineries and found to be suitable as wine starter to improve quality and safety of red wines.

KEYWORDS: adsorption; clonal selection; ochratoxin A; phenolics; wine making; yeasts

1. Introduction

Though many types of yeast species are associated with wine fermentation, *Saccharomyces cerevisiae* is the main species involved (Mercado et al. 2010). *Saccharomyces* yeasts were used for millennia to produce wine and other fermented products (Eldarov et al. 2016). Wine results from a complex interaction between yeasts and grape musts, whose effects endure during wine aging (Ivanova et al. 2012). The impact of yeasts on wine quality is one of the most important factors (Bezerra-Bussoli et al. 2013). The microbial action allows controlling the alcoholic fermentation process, valorizing the produced wines in different ways such as increasing the flavor compounds (Belda et al. 2017). Over the last decade, oenological practices were renewed and improved both in vineyard and in winery, with a comparable evolution of yeast features required for producing valuable wines (Bonciani et al. 2016).

Mycotoxin-producing molds may be present at the end of grape ripening and produce ochratoxin A (OTA) in grape must and, consequently, in wine (Dachery et al. 2015). The European legal limit for OTA concentration in wine is of 2 μg/kg (Benito 2019). In Europe, the highest reported incidences and/or measured levels of OTA were found among wines from several climatically warmer Southern areas (De Jesus et al. 2018).

Different approaches have been conceived to remove OTA from grape and wine (Fiori et al. 2014; Piotrowska et al. 2013). Microbiological methods may represent a good strategy for wines detoxification, since it is well known that OTA can be adsorbed by some yeast and bacteria strains (Quintela et al. 2013). OTA adsorption by yeast during wine-making of both red and white wines has been proved to be strain dependent and can range from 37 to 80% (Meca et al. 2010). As already described (Petruzzi et al. 2014; Aponte and Blaiotta 2016), OTA was mainly adsorbed during yeast exponential grow phase and, in some cases, depending on strain, again released in wine. This phenomenon could be due to the premature autolysis of some yeast strains.

Recently interesting results were obtained selecting wine yeasts based on their different parietal adsorption activity, in order to obtain starter yeasts able to respect grape phenolic compounds, remove OTA eventually present in grape must and protect color of red wines (Caridi et al. 2020; Petruzzi et al. 2017; Pulvirenti et al. 2020). The correct approach to solve these problems is the application of breeding programs, primarily focused on oenological and technological properties, to select wine yeast strains as candidates for inbreeding program (Fernández-González et al. 2015).

The aim of this work was to perform the oenological characterization and the clonal selection of autochthonous strains of the genus *Saccharomyces* to improve and valorize red wines as regards their microbiological quality and safety. The strains were tested for

conventional oenological characteristics; the best strains were improved by spore clone selection for the following innovative characteristics: (a) wine color protection, (b) preservation of phenolics, and (c) removal of the OTA from grape must. Lastly, the best strain was used to control red wine-making at six Calabrian wineries.

2. Materials and methods

2.1. ***Pre-selection trials***

The starting point was the check of yeast strains from the microbial collection of the Department Agraria of Reggio Calabria. Two hundred and eighty-four strains were chosen, based on these characteristics: (a) autochthonous (South Italy) wild types, (b) oenological origin (grape musts or wines), (c) elliptic cells, (d) if deriving from the same origin matrix, exhibiting different morphology to avoid the presence of duplicates. The 284 yeast strains were pre-selected by evaluating: (a) type of growth: to exclude flocculent strains during grape must fermentation in test tubes containing 10 mL of pasteurized (110°C for 10 min) and filtered (through sterile gauze) must from white grapes of the Calabrian cultivar *Greco Bianco*; (b) acetic acid production: to exclude high acetic acid producer strains on Chalk agar at 30°C for 3 days according to Lemaresquier et al. (1995); (c) H_2S production: to exclude high H_2S producer strains on BiGGY agar at 25°C for 48 h according to Nickerson (1953); (d) production of spores of the genus *Saccharomyces*: to exclude the non-spore producer strains and the strains producing spores different from the genus *Saccharomyces* on acetate agar (anhydrous sodium acetate 10 g/L, agar 20 g/L) at 25°C for 10 days according to Fowell (1952). For each parameter the mean value was used as a reference criterion for selecting or excluding strains. This procedure allowed to select, among a high number of strains, a few strains surely able to act as wine starter.

97 *2.2.* ***Selection trials***

98 Based on the results obtained with the pre-selection trials, the yeast strains that had passed all
99 the pre-selection tests were selected for their low adsorption activity of phenolics and high
100 adsorption activity of OTA. The yeast strains were grown in the chromogenic grape-skin agar
101 medium (Caridi 2013) and, after 10 days of anaerobic incubation at 28°C, yeast biomass was
102 subjected to the computer-assisted evaluation of the red, green, and blue components using
103 Photoshop CS for Windows XP from Adobe. Moreover, the strains were tested in micro-
104 winemaking trials to confirm their low aptitude to adsorb grape pigments and phenolics during
105 fermentation. Black grapes of *Gaglioppo* cultivar were given pre-fermentative maceration to
106 extract pigments from skins and seeds. They were destemmed, crushed and cold soaked at 0°C
107 for 3 days, performing a punch down twice per day. The grape must (pH 3.50, °Brix 23) was
108 divided in aliquots of 20 mL using test tubes closed with cotton caps, immediately inoculated
109 at 5% in triplicate with 24-h precultures of each wine yeast in the same grape must, and
110 incubated at 20°C. The fermentation vigor after 3 days was referred to 100 mL of grape must
111 multiplying by 5 the weight loss caused by CO_2 production. At the end of fermentation, wines
112 were diluted 1:5 (v/v) with a pH 3.5 buffer (citric acid monohydrate 0.1 M, Na_2HPO_4 0.2 M)
113 after centrifugation. The absorbance at 420 nm (yellow component), 520 nm (red component),
114 and 620 nm (blue component) was read using an Anadeo1 spectrophotometer (Bibby Sterilin
115 Ltd); the color intensity was calculated with the following formula: $I = A_{420} + A_{520} + A_{620}$
116 (Glories 1984). The total phenolic content was determined using the FC index (Folin-
117 Ciocalteu's index) according to Singleton and Rossi (1965). Based on the results on strain
118 aptitude to adsorb grape pigments and phenolics, several strains were excluded. The remaining
119 strains were further studied for their aptitude to remove OTA from synthetic must (Yeast
120 Nitrogen Base 6.7 g/L, tartaric acid 5.0 g/L, malic acid 5.0 g/L, citric acid 0.2 g/L, dextrose 110

g/L, fructose 100 g/L, and saccharose 7 g/L, pH 3.3) contaminated with the addition of 5 ppb of OTA. Yeast precultures were prepared in YPD broth (yeast extract 10 g/L, peptone 10 g/L, dextrose 20 g/L) at 28°C for 48 h. Tests were performed inoculating in triplicate 10 mL of the synthetic must with 0.2 mL of the precultures. The fermentations were carried out at 25°C and after 28 days the OTA content of the wines was determined by HPLC (Meca et al. 2010). In brief OTA present in the musts/wines was analyzed by direct injection of 20 µL of sample in the high-performance liquid chromatography with fluorescence detection (HPLC-FLD) apparatus, previously it was filtered using a 0.45 µm filter. The method used acetonitrile, water, and acetic acid (49:49:2, vol./vol./vol., respectively) as the isocratic mobile phase (1 mL/min), a monolithic C18 column (Onyx Monolithic C18 100 x 3.0 mm, Phenomenex) and a guard cartridge kit (Onyx Monolithic C18 5 x 4.6 mm, Phenomenex).

2.3. ***Cytofluorimetric analysis and molecular identification of the selected yeasts***

The finally chosen strains of the genus *Saccharomyces* were studied by cytofluorimetric analysis in order to confirm their ploidy. The basic procedure followed was that of Paulovich and Hartwell (1993) modified according to Naumova et al. (2005). After that, the yeast DNA was extracted by UltraClean™ Microbial DNA Isolation Kit (MO BIO Laboratories, Inc., USA) according to company instructions. The yeast strains were identified by RFLP of the internal transcribed spacers (ITS1 and ITS2) and the 5.8S (Sidari et al. 2014). In details, the extracted DNA (50 ng/µL) was amplified using two specific primers of the ITS region, ITS1 (5’ TCCGTAGGTGAACCTGCGG 3’) and ITS4 (5’ TCCTCCGCTTATTGATATGC 3’), in 25 µL amplification mix: buffer A + $MgCl_2$ 1x, dNTPs 0.2 mM, ITS1 0.5 µM, ITS4 0.5 µM, Taq 2G Robust (KAPA Biosystems) 0.5 U, sterile MilliQ water, and 0.5 µl of DNA. The PCR program was: 95°C for 6 min; 35 cycles at 94°C for 30 secs, 55°C for 30 secs, 72°C for 60 secs;

72°C for 10 min. The amplified products were analyzed by electrophoresis on 1.5% agarose gel (Agarose Molecular Biology Grade, Scientifix™, Victoria, Australia) at 90 V for 1 h, stained with 1% ethidium bromide solution for 15 min, and visualized under UV light. Sizes were estimated by comparison against a DNA length standard (100 bp DNA ladder Mid, GeneWorks, Australia). Five µL of the amplified products were digested with *Hae*III, *Cfo*I, and *Hinf*I restriction enzymes (Promega). The restriction mixture contained 1 µL of buffer specific for each enzyme, 0.4 µL of restriction enzyme, and 3.6 µL of sterile water. After incubating at 37°C for 3 h, digested samples were visualized by electrophoresis on 2% MetaPhor® Agarose gel (Lonza, Rockland, ME, USA) at 90 V for 3 h, stained with 1% of ethidium bromide solution for 15 min, and visualized under UV light. Sizes were estimated by comparison against a DNA length standard (100 bp DNA ladder Mid, GeneWorks, Australia).

2.4. ***Sporulation, spore clone selection and wine-making trials***

The finally chosen strains of the genus *Saccharomyces* were grown at 28°C for 2 days on YPD broth, solidified with 2% agar when required. Sporulation was induced at 28°C for 7 days on acetate agar. Ascospores were isolated on YPD agar by a micromanipulator Singer MSM System series 300 manual. Ascus wall was digested at 25°C for 20 min using zymolyase 20T, 10 mg/mL (Seikagaku, Kogyo/Tokyo, Japan) diluted 1:9 with sterile distilled water. Parents and progenies were studied using the chromogenic grape-skin agar medium and performing micro-winemaking trials to determine their aptitude to adsorb grape pigments and phenolics during fermentation. Based on the results, several strains were excluded. The remaining strains were further studied for their aptitude to remove OTA from synthetic must. Based on the results of the spore clone selection trials, the best strain was used in wine-making at six Calabrian wineries: 1) Azienda Agrituristica Contessa, Lattarico (CS); 2) Azienda Vinicola Malaspina,

Melito Porto Salvo (RC); 3) Azienda Agricola Cosimo Murace, Bivongi (RC); 4) Azienda Vitivinicola Realarico Wines s.a.r.l., Carolei (CS); 5) Azienda Agricola Fratelli Zagarella, Arghillà (RC); 6) Verbicaro Viti e Vini S.r.l., Verbicaro (CS). Therefore, grape musts of the following cultivars were inoculated: *Gaglioppo*, *Greco Nero*, *Magliocco*, *Merlot*, and *Nerello*. Each winery received a 48-h preculture of the best strain in 5 L of pasteurized grape must. The preculture was promptly inoculated in 100 L of grape must; a control was prepared in accordance with the practices of each winery. At the end of wine-making, the experimental wines were analyzed for ethanol content by ebulliometer, absorbance at 420, 520, and 620 nm, color intensity, and OTA content (ppb).

2.5. ***Statistical analysis***

Three replicates of data obtained were expressed as mean value ± standard deviation

3. Results

3.1. ***Pre-selection trials***

The 284 yeast strains were reduced to 84 excluding all the strains unable to act as a starter. The 92.96% of the strains exhibited dispersed growth in grape must, while 7.04% were flocculent and this last category (20 strains) was excluded. The 92.25% of the strains did not produce acetic acid on Chalk agar or showed low acetic acid production, while 7.75% showed medium-high acetic acid production and this last category (22 strains) was excluded. The 14.09%, 45.77%, and 40.14% of the strains showed low, medium, and high H_2S production on BiGGY agar, respectively; the last category (114 strains) was excluded. The 26.05% of the strains showed either medium or high production of spores of genus *Saccharomyces* on acetate agar, 21.13% showed low spore production while 52.82% did not produce spores of genus

193 *Saccharomyces* or not at all; this category (150 strains) was equally excluded. So, 200 yeast
194 strains were excluded for one or more parameters pre-selecting 84 strains. This confirms the
195 usefulness of the experimental protocol to quickly reduce the number of strains: (a) excluding
196 those undoubtedly unable to be candidate as wine starter and (b) maintaining the strains with
197 dispersed growth, low production of acetic acid and H_2S, and able to produce spores of the
198 genus *Saccharomyces*.
199
200 *3.2.* ***Selection trials***
201 Table 1 reports the list of the 84 yeast strains studied for: red, green, and blue components of
202 the biomass color on grape-skin agar medium; fermentation vigor after 3 days; color intensity
203 and FC index. The red component of the biomass color on grape-skin agar ranged from a
204 minimum value of 56 (strain TT201) to a maximum value of 154 (strain Sc2306) with a mean
205 value of 106. Low values match high adsorption ability; 39 strains highlighted in grey exhibited
206 a value ≤ 106 were then excluded based on high adsorption activity. The green component of
207 the biomass color on grape-skin agar ranged from a minimum value of 27 (strain TT201) to a
208 maximum value of 124 (strain Sc2306) with a mean value of 64. Forty-four strains highlighted
209 in grey exhibited a value ≤ 64 were then excluded based on high adsorption activity. The blue
210 component of the biomass color on grape-skin agar ranged from a minimum value of 32 (strains
211 Sc1321 and TT201) to a maximum value of 112 (strain Sc2306) with a mean value of 59. Forty-
212 two strains highlighted in grey exhibited a value ≤ 59 were then excluded based on high
213 adsorption activity. The fermentation vigor after 3 days, referred to 100 mL of grape must,
214 ranged from a minimum value of 5.58 (strain Sc1583) to a maximum value of 9.57 (strain
215 TT141) with a mean value of 8.09; the 33 strains highlighted in grey exhibited a fermentation
216 vigor ≤ 8.09 and were excluded. The color intensity ranged from a minimum value of 1.168

(strain Sc1687) to a maximum value of 6.355 (strain Sc2774) with a mean value of 3.968; the 35 strains highlighted in grey exhibited a value ≤ 3.968 and were excluded. The FC index ranged from a minimum value of 7.88 (strain Sc1321) to a maximum value of 24.83 (strain B35-Sc128) with a mean value of 14.27. This parameter is proportional to the total quantity of phenolics present in wines; 44 strains highlighted in grey exhibited a value ≤ 14.27 were then excluded based on high adsorption activity. Data highlighted in grey in Table 1 are those that have caused the exclusion of the strains as they indicate lower fermentation vigor or higher adsorption activity of pigments and phenolics. The eight strains in bold in Table 1 are those that have passed all the tests and, consequently, were further studied for their aptitude to remove OTA from synthetic must with the following results: Sc1055 - 40.07%, Sc1674 - 49.92%, Sc1741 - 55.04%, Sc1905 - 54.74%, Sc2731 - 31.89%, Sc2774 - 43.90%, Sc2823 - 35.02%, TP5 - 39.47%. The percentage of OTA removal ranged from a minimum value of 31.89% to a maximum value of 55.04% with a mean value of 43.76%. Therefore, the five strains that exhibited a value ≤ 43.76% were excluded. So, based on the differential adsorption activities towards OTA and phenolics, three yeasts, Sc1674, Sc1741, Sc1905 were selected for their low aptitude to adsorb phenolics and wine color and for their high OTA removing activity.

3.3. ***Cytofluorimetric analysis and molecular identification of the selected yeasts***

Flow cytometry analysis of strain ploidy confirmed that the three strains are diploids. Using the haploid control strain BY 474i (M1), strain Sc1674 exhibited M2/M1=2.39, strain Sc1741 exhibited M2/M1=2.36, strain Sc1905 exhibited M2/M1=2.21 (Fig. 1). The three strains selected according to their adsorption characteristics were studied for their genetic profile according to the method reported by Esteve-Zarzoso et al. (1999); they indicated the use of 5.8S-ITS region and its PCR-RFLP as a method reproducible and useful to rapidly identify and

classify yeast species. As recommended by the same authors, the strains were studied in comparison with the type strain, in this case the *S. cerevisiae* CBS 1171^{T}, to confirm that they effectively belong to *S. cerevisiae* species. For all the tested strains, the ITS amplicon was 880 bp. All the strains showed the same restriction pattern with the three restriction enzymes used. In particular, *Hae*III gave a restriction profile of four bands with size 320, 220, 180, and 150, *Hinf*I gave a restriction profile of two bands with size 365 and 140 while *Cfo*I gave a restriction profile of three bands with size 380, 360, and 150 (Fig. 2).

3.4. ***Sporulation, spore clone selection and wine-making trials***

The three yeasts were induced to sporulate; parents and progenies were studied using the chromogenic grape-skin agar medium and performing micro-winemaking trials to determine their aptitude to adsorb grape pigments and phenolics during fermentation. It is interesting to note that the range of variability of each tested parameter allows the strain improvement but is different from strain to strain and for each parameter. Table 2 reports the list of the 27 yeast strains, three wine strains and their progeny (8 descendants for each parent) studied for: red, green, and blue components of the biomass color on grape-skin agar medium; color intensity and FC index. Obviously, the difference in results (Table 1 compared to Table 2) for the same parental strain depends on the different substrata, grape skin and grape must, used. The red component of the biomass color on grape-skin agar ranged from a minimum value of 94 (strains Sc1674B_1C and Sc1674C_1A) to a maximum value of 137 (strain Sc1741B_1C) with a mean value of 115. The 13 strains highlighted in grey exhibited a value ≤ 115 so they were excluded. The green component of the biomass color on grape-skin agar ranged from a minimum value of 47 (strain Sc1674B_1C) to a maximum value of 96 (strain Sc1741B_1C) with a mean value of 68. The 14 strains highlighted in grey exhibited a value ≤ 68 so they were excluded. The blue

component of the biomass color on grape-skin agar ranged from a minimum value of 47 (strain Sc1674B_1C) to a maximum value of 82 (strain Sc1741B_1C) with a mean value of 63. The 13 strains highlighted in grey exhibited a value ≤ 63 so they were excluded. The color intensity ranged from a minimum value of 2.264 (strain Sc1905) to a maximum value of 3.103 (strain Sc1674C_1C) with a mean value of 2.713; the 12 strains highlighted in grey exhibited a value ≤ 2.713 and were excluded. The FC index ranged from a minimum value of 14.10 (strain Sc1905C_1B) to a maximum value of 18.77 (strain Sc1741A_1D) with a mean value of 15.93. The 13 strains highlighted in grey exhibited a value ≤ 15.93 so they were excluded. Data highlighted in grey in Table 2 are those that have caused the exclusion of the strains as they indicate higher adsorption activity of pigments and phenolics. The four strains in bold in Table 2 are those that have passed all the tests and, consequently, were further studied for their aptitude to remove OTA from synthetic must with the following results: Sc1674B_1A - 19.48%, Sc1741A_1C - 13.00%, Sc1741A_1D - 19.82%, Sc1741B_1C - 11.58%. The percentage of OTA removal ranged from a minimum value of 11.58% to a maximum value of 19.82% with a mean value of 15.97%. Based on the tested parameters, the strain preferred to perform wine-making at the six wineries was *S. cerevisiae* Sc1741A_1D. Table 3 reports the analytical parameters of the wines produced. The ethanol content, which indicates the fermentation degree of the sugars present in the grape musts, is higher for the wines produced using the selected strain Sc1741A_1D compared to the respective controls. At Realarico winery the two values are similar. The absorbance at 520 nm and the color intensity are strongly higher for the wines produced using the selected strain Sc1741A_1D at Malaspina, Contessa, and Zagarella wineries; similarly, the wine produced at Murace winery has very high values. Last but not least, in all the six wineries the wines produced using the selected strain Sc1741A_1D exhibited an OTA content always lower (30-60%) than the controls or not detectable.

4. Discussion

The natural variability of yeasts for the studied traits may be exploited to obtain a further enhancement of the adsorption/non-adsorption activity of wine yeasts. Due to phosphodiester bridges in both N- and O-linked mannosyl side chains the cell surface of yeast contains numerous negative charges (Jigami and Odani 1999). In fact, the sensitivity of yeast cells to the mycotoxin OTA depends on the presence of mannosyl phosphate groups at the cell surface (Ibeas et al. 2000). The mannoprotein content of cell wall and its phosphorylation grade is very variable among different yeast strains and depends on grow phase also, so these variations could explain both the difference in detoxification ability and its release into wine following autolysis (Gil-Serna et al. 2018). Recently it was demonstrated that anthocyanins enhance yeast's adsorption of OTA during the alcoholic fermentation (Cecchini et al. 2019). However, the trend of the yeast adsorption performance is that strains that remove more OTA are the same that adsorb more color and phenolics from wine. Consequently, the best strains for red wine-making obtained with the present selection possess intermediary adsorption characteristics. Better results may be obtained by a yeast breeding program with the goal to obtain new strains through sexual recombination of the best strains obtained by clonal selection. The inheritable nature of the adsorption of wine color (Caridi et al., 2007) and OTA (Caridi et al., 2012) were analyzed on descendants derived from wine strains of *S. cerevisiae*. Investigation on the progeny demonstrated that adsorption of wine color and adsorption of OTA are polygenic inheritable quantitative traits loci, partially and interdependently correlated to color and phenolic content of wines. This may justify the employment of genomic strategies for genetic improvement of the strains. The employed experimental protocol has allowed: (a) reduction in the high number of considered strains, excluding those undoubtedly unable to be candidate as wine starter; (b)

selection of strains for innovative characteristics connected to their different adsorption activity; (c) improving of the strains by spore clone selection.

In conclusion, this research has shown how, using a fast, easy, and cheap procedure, it is possible to select, among a high number of strains, a few strains that can be improved by sporification to obtain a strain of *S. cerevisiae* suitable as wine starter to improve and valorize red wines as regards their microbiological quality and safety. Lastly, it is interesting to remember that the most common corrective techniques to reduce OTA content in wines are agents like active carbon or amicrobial filtration, which are efficient but have important undesirable side effects, such losses of color and aroma.

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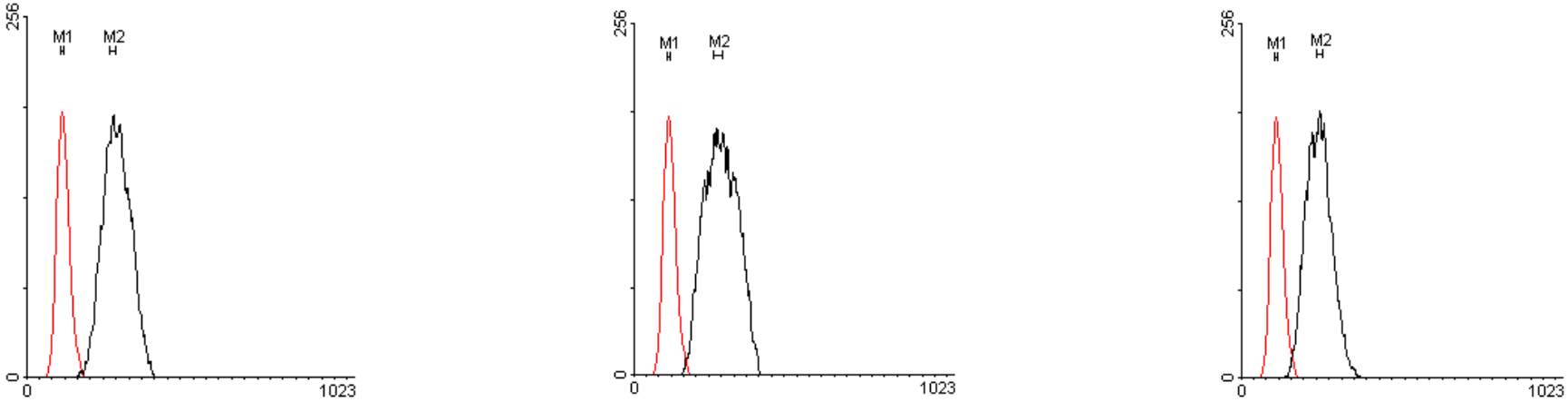
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(a) (b) (c)

Fig. 1 Flow cytometry analysis of the three selected strains using the haploid control strain BY 474i (in red): a) strain Sc1674, b) strain Sc1741, c) strain Sc1905

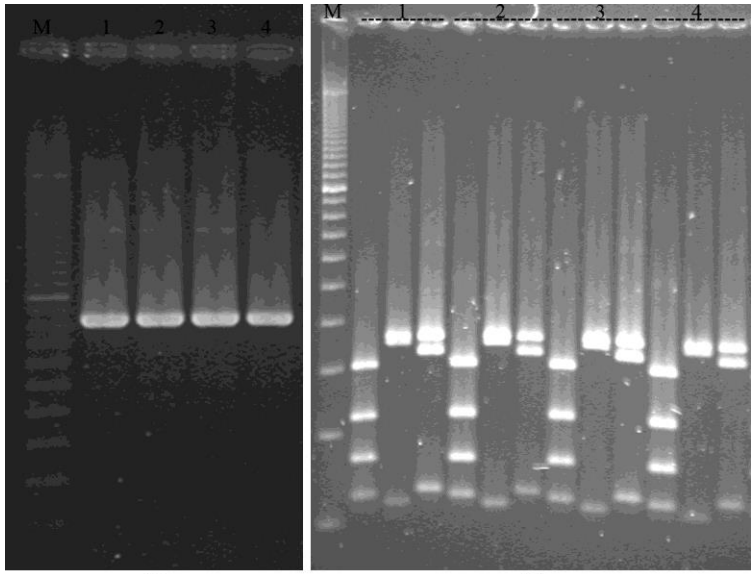


Fig. 2 Electrophoretic pattern of rITS–PCR products (a) and restriction profiles of the 5.8S-ITS region using *Hae*III, *Hinf*I, and *Cfo*I restriction enzymes (b) of the three yeast strains selected compared with a type strain. M: 100 bp molecular marker. (a) 1: *Saccharomyces cerevisiae* CBS 1171^{T}, 2: Sc1674, 3: Sc1741, 4: Sc1905; (b) the order of the restriction enzymes is *Hae*III, *Hinf*I, and *Cfo*I. 1: *Saccharomyces cerevisiae* CBS 1171^{T}, 2: Sc1674, 3: Sc1741, 4: Sc1905

Table 1 List of the 84 yeast strains. Data highlighted in grey have caused the exclusion of the strains as they indicate lower fermentation vigor or higher adsorption activity of pigments and phenolics. The eight strains in bold are those that have passed all the tests. The data are expressed as mean value ± standard deviation

	Biomass color on grape-skin agar					
Strain	Red component	Green component	Blue component	Fermentation vigor after 3 days	Color intensity	FC index
B35-Sc8	97±2	53±1	53±2	6.97±0.68	5.502±0.558	14.50±7.48
B35-Sc90	113±2	69±2	63±3	7.03±0.55	5.783±0.645	16.78±0.75
B35-Sc128	87±4	49±1	48±2	7.13±0.23	5.958±0.528	24.83±7.40
B35-Sc147	88±5	47±2	46±1	8.98±0.33	4.295±0.100	17.30±6.86
MCR5	93±3	50±2	48±2	8.12±0.19	1.793±0.277	8.83±1.88
MCR6	109±4	61±3	58±3	7.72±0.32	4.052±0.254	14.27±0.25
Sc251	78±2	41±2	41±2	9.20±0.20	4.917±0.092	12.20±4.99
Sc321	122±1	75±2	68±2	6.33±0.84	4.825±0.596	13.65±1.98
Sc329	99±2	53±2	50±2	8.15±0.30	3.112±1.177	10.75±5.30
Sc384	100±4	61±6	60±5	8.60±0.18	3.090±0.241	14.43±0.42
Sc396	112±4	61±4	56±5	9.30±0.15	3.987±0.062	15.65±1.39
Sc428	106±1	63±1	55±2	8.30±0.31	4.802±0.043	14.25±0.67
Sc468	83±1	42±2	44±1	8.75±0.39	2.073±0.933	9.32±5.45
Sc632	125±4	80±4	72±4	6.98±0.36	5.150±0.368	17.67±2.65
Sc786	110±2	65±3	59±3	7.75±0.36	5.642±0.224	20.70±0.52
Sc1055	116±5	72±5	64±4	8.17±0.46	5.212±0.469	16.97±1.21
Sc1128	99±4	53±2	49±2	8.52±0.33	2.915±0.725	12.92±4.65
Sc1228	101±3	57±2	52±3	8.98±0.25	5.157±0.609	12.42±3.60
Sc1240	95±1	53±2	50±2	8.65±0.30	4.143±0.101	16.38±3.43
Sc1321	61±1	28±2	32±3	8.68±0.10	1.517±0.540	7.88±4.51
Sc1326	115±5	72±5	69±4	7.78±0.12	4.512±0.241	17.90±1.31
Sc1340	95±4	51±4	49±6	8.32±0.51	3.303±0.749	12.50±4.18
Sc1349	144±3	109±4	94±4	8.60±0.48	4.050±0.150	13.70±0.61
Sc1526	101±5	59±5	55±5	8.85±0.25	3.608±0.455	14.20±0.46

Sc1541	124±3	85±2	75±3	8.00±0.35	3.653±0.432	17.67±2.83
Sc1572	122±3	81±3	71±3	6.17±0.36	4.355±0.332	17.27±2.03
Sc1583	81±4	45±5	45±5	5.58±0.35	5.315±0.116	15.05±2.17
Sc1591	97±4	54±5	52±6	8.68±0.28	4.033±0.508	9.93±3.12
Sc1674	119±5	76±5	69±5	8.38±0.13	4.103±0.494	18.67±1.51
Sc1687	109±6	65±6	60±8	9.12±0.12	1.168±0.086	11.33±0.76
Sc1721	120±2	70±2	68±2	9.40±0.15	1.452±0.210	11.02±1.01
Sc1741	118±5	70±4	63±5	8.68±0.10	4.650±0.097	18.38±0.08
Sc1751	138±5	89±6	83±4	6.88±0.50	5.080±0.414	19.08±1.33
Sc1771	121±1	76±1	70±1	8.68±0.10	3.882±0.241	14.87±0.38
Sc1798	142±3	96±4	84±5	8.78±0.13	2.880±0.094	14.73±0.33
Sc1819	135±1	87±1	78±2	6.82±0.06	1.252±0.039	13.60±0.30
Sc1864	77±3	37±2	36±2	9.48±0.13	1.240±0.266	8.73±2.63
Sc1871	124±5	81±5	69±5	8.52±0.19	3.097±0.737	17.38±2.08
Sc1905	120±4	71±4	61±4	8.88±0.06	4.567±0.155	17.48±1.72
Sc1936	99±5	53±3	51±2	8.80±0.48	4.730±0.217	12.73±2.63
Sc1951	118±3	74±2	67±2	8.62±0.12	2.265±0.605	14.97±0.68
Sc2306	154±6	124±9	112±11	6.25±0.30	5.965±0.169	15.90±3.34
Sc2319	89±4	50±3	48±3	7.98±0.23	2.743±1.011	16.10±8.37
Sc2361	67±3	32±2	36±2	7.95±0.20	4.942±0.826	21.08±6.08
Sc2363	128±2	85±2	76±2	9.05±0.23	3.775±0.465	14.05±0.82
Sc2366	130±3	88±4	77±2	8.20±0.26	3.515±1.252	17.02±10.29
Sc2485	87±3	44±2	44±3	8.72±0.40	4.562±0.133	13.28±2.95
Sc2616	101±3	60±3	55±2	8.83±0.38	2.422±1.043	11.80±0.83
Sc2621	81±1	42±1	42±1	9.17±0.15	1.293±0.091	8.68±1.62
Sc2705	75±3	38±1	40±2	8.55±0.26	4.492±0.149	11.62±2.38
Sc2708	99±5	58±4	54±3	8.37±0.28	5.052±0.417	18.43±8.65
Sc2710	115±4	72±3	66±4	7.68±0.35	5.120±0.136	13.80±1.13
Sc2712	107±4	64±5	60±5	8.05±0.13	4.328±0.286	12.95±0.53
Sc2713	126±1	82±1	76±1	8.43±0.33	4.538±0.079	14.12±0.03

Sc2715	118±4	78±3	70±2	8.37±0.29	4.683±0.353	14.27±0.19
Sc2723	89±3	48±1	46±3	6.88±0.86	3.227±1.056	10.40±4.89
Sc2724	92±3	52±2	51±2	8.08±0.60	2.698±1.215	15.83±6.50
Sc2731	117±1	72±1	66±1	8.17±0.37	5.535±0.039	15.63±0.91
Sc2734	121±3	79±1	68±2	7.08±0.13	3.968±0.209	14.48±0.79
Sc2744	73±3	36±2	38±2	8.67±0.20	2.483±0.508	10.52±6.31
Sc2750	120±5	76±4	69±3	7.33±0.53	4.728±0.453	15.32±1.00
Sc2751	108±5	66±3	62±3	8.50±0.56	5.412±0.339	13.72±0.61
Sc2753	80±3	42±1	42±1	7.48±0.06	3.385±0.444	10.93±6.11
Sc2754	101±1	59±2	59±2	6.63±1.25	2.372±0.387	11.32±0.70
Sc2760	114±2	69±2	64±2	8.73±0.33	3.437±0.718	14.40±0.73
Sc2762	107±2	61±1	56±2	7.75±0.22	2.065±0.088	13.78±0.71
Sc2770	90±1	48±1	50±1	7.95±0.70	2.173±0.435	11.18±1.95
Sc2773	101±3	58±2	56±1	6.92±0.15	5.718±0.253	15.87±1.07
Sc2774	130±1	86±1	77±3	8.50±0.17	6.355±0.744	15.67±0.83
Sc2776	104±2	62±1	57±2	6.68±0.34	5.767±0.378	13.30±0.61
Sc2786	92±2	48±2	47±2	8.75±0.58	1.480±0.740	12.23±8.22
Sc2803	128±2	84±2	76±3	9.07±0.24	4.235±0.511	13.37±3.70
Sc2814	117±2	76±1	66±3	6.52±1.17	5.180±0.437	13.05±2.18
Sc2823	141±2	105±3	91±2	8.13±0.35	4.967±0.274	15.57±0.95
TP1	109±1	65±2	60±2	6.35±0.84	2.858±0.150	11.15±1.33
TP4	124±2	83±1	73±1	7.40±0.48	3.693±0.640	11.02±0.99
TP5	129±4	88±4	77±3	8.12±0.42	5.167±0.074	14.90±1.78
TT51	112±4	64±2	61±1	8.50±0.30	4.422±0.211	15.28±1.00
TT104	126±3	83±1	75±1	8.60±0.13	1.635±0.126	11.85±0.78
TT141	75±3	34±1	36±2	9.57±1.94	5.468±0.207	12.08±2.62
TT193	60±2	28±2	34±1	7.47±0.20	5.253±0.363	16.77±6.58
TT201	56±3	27±2	32±4	8.67±0.65	4.915±0.271	10.13±2.08
TT241	101±2	61±2	58±3	8.62±0.31	5.087±0.284	17.00±0.26
TT254	120±1	75±3	68±2	7.88±0.15	5.052±0.015	16.00±0.20

Min	*56*	*27*	*32*	*5.58*	*1.168*	*7.88*
Max	*154*	*124*	*112*	*9.57*	*6.355*	*24.83*
Mean	*106*	*64*	*59*	*8.09*	*3.968*	*14.27*

Table 2 List of the 27 yeast strains - three wine strains and their progeny (8 descendants for each parent) - studied for: red, green, and blue components of the biomass color on grape-skin agar medium; color intensity and FC index. Data highlighted in grey have caused the exclusion of the strains as they indicate higher adsorption activity of pigments and phenolics. The four strains in bold are those that have passed all the tests. The data are expressed as mean value ± standard deviation

Strain	Biomass color on grape-skin agar			Color intensity	FC index
	Red component	Green component	Blue component		
Sc1674	116±3	67±2	64±2	2.780±0.047	16.18±0.03
Sc1674B_1A	118±6	70±3	68±5	2.867±0.033	17.80±0.20
Sc1674B_1B	100±4	53±3	52±3	2.994±0.068	16.08±0.25
Sc1674B_1C	94±5	47±2	47±3	2.835±0.020	16.95±0.13
Sc1674B_1D	99±5	53±5	54±3	3.050±0.022	14.70±0.09
Sc1674C_1A	94±3	49±2	49±2	2.754±0.036	16.35±0.30
Sc1674C_1B	106±3	60±4	58±4	2.869±0.036	15.85±0.15
Sc1674C_1C	117±2	68±4	67±3	3.103±0.020	14.90±0.13
Sc1674C_1D	99±7	54±6	54±4	3.024±0.054	15.12±0.28
Sc1741	127±5	83±3	77±4	2.375±0.044	14.20±0.17
Sc1741A_1A	112±6	67±7	67±8	2.910±0.132	16.23±0.03
Sc1741A_1B	114±4	65±2	64±2	2.460±0.048	15.62±0.26
Sc1741A_1C	117±1	69±2	67±3	2.964±0.061	17.08±0.06
Sc1741A_1D	129±3	81±2	69±2	2.765±0.026	18.77±0.06
Sc1741B_1A	128±3	84±2	70±3	2.560±0.028	16.45±0.10
Sc1741B_1B	123±3	77±3	70±2	2.570±0.021	17.70±0.20
Sc1741B_1C	137±1	96±2	82±3	2.958±0.035	16.90±0.05
Sc1741B_1D	120±4	74±3	60±2	2.750±0.024	17.10±0.09
Sc1905	115±7	69±4	63±6	2.264±0.019	14.17±0.18
Sc1905B_1A	114±6	66±4	62±4	2.498±0.025	15.97±0.06
Sc1905B_1B	129±5	80±4	68±3	2.595±0.025	15.02±0.25
Sc1905B_1C	135±4	85±3	71±3	2.552±0.051	14.17±0.03
Sc1905B_1D	124±6	79±5	63±6	2.770±0.065	16.12±0.31

Sc1905C_1A	121±2	74±3	66±2	2.586±0.056	15.67±0.19
Sc1905C_1B	95±4	52±3	51±3	2.555±0.040	14.10±0.13
Sc1905C_1C	114±7	68±6	58±6	2.409±0.025	14.97±0.13
Sc1905C_1D	106±4	60±3	51±4	2.428±0.041	15.92±0.38
Min	*94*	*47*	*47*	*2.264*	*14.10*
Max	*137*	*96*	*82*	*3.103*	*18.77*
Mean	*115*	*68*	*63*	*2.713*	*15.93*

Table 3 Analytical parameters of the wines produced at the six wineries using the selected strain of *Saccharomyces cerevisiae* Sc1741A_1D (nd = not detectable)

Cultivar	Winery	Wine-making	Ethanol (vol.%)	Absorbance			Color intensity	OTA content (ppb)
				420 nm	520 nm	620 nm		
Gaglioppo	Azienda Vinicola Malaspina, Melito Porto Salvo (RC)	Sc1741A_1D	14.50	2.420	2.357	0.496	5.273	0.56
		control	13.00	1.227	0.922	0.225	2.374	0.79
Greco Nero	Verbicaro Viti e Vini S.r.l., Verbicaro (CS)	Sc1741A_1D	13.30	1.311	1.621	0.288	3.220	0.10
		control	12.80	2.270	3.565	0.641	6.476	0.20
Magliocco	Azienda Agrituristica Contessa, Lattarico (CS)	Sc1741A_1D	14.55	3.340	5.015	1.157	9.512	0.12
		control	13.85	2.745	4.270	0.963	7.978	0.30
	Azienda Vitivinicola Realarico Wines s.a.r.l., Carolei (CS)	Sc1741A_1D	14.70	3.346	4.995	1.156	9.497	nd
		control	14.90	4.923	5.767	2.026	12.716	nd
Merlot	Azienda Agricola Fratelli Zagarella, Arghillà (RC)	Sc1741A_1D	13.60	1.533	1.928	0.403	3.864	nd
		control	13.10	1.233	1.277	0.295	2.805	nd
Nerello	Azienda Agricola Cosimo Murace, Bivongi (RC)	Sc1741A_1D	14.30	3.740	5.767	1.496	11.003	nd