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Indigenous *Saccharomyces cerevisiae* strains and their influence on the quality of Cataratto, Inzolia and Grillo white wines

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ABSTRACT

The present paper deals with three new strains of *Saccharomyces cerevisiae*, isolated in old wineries of Sicily, which were microbiologically and molecularly characterized and tested for their ability to produce white wines. Examined in terms of their growth pattern, fermentation vigour, sulphite tolerance, fermentative power, spore formation, and production of acetic acid, hydrogen sulphide and phenolic off-flavours, the strains were utilized as starters in experimental fermentations of musts obtained from the cultivars Inzolia, Grillo and Catarratto. Further, the three musts were also fermented using two commercial *S. cerevisiae* strains. The quality of the wines produced was confirmed by their principal oenochemical parameters, by sensory analysis and qualitative and quantitative determination of the volatile aroma constituents. All the data were statistically elaborated. Interestingly, the new selected yeasts were able to increase the pear notes (Z)-ethyl-4-decenoate, (E)-ethyl-3-decenoate, and (Z)-ethyl-3-decenoate which are fundamental for the aroma of these Sicilian wines. From our results, the new yeast strains were found to produce white wines of a quality which was not inferior to those obtainable with the best commercial strains selected in other geographical areas, but also with a distinctive aromatic profile.

1. Introduction

In winemaking, the use of a yeast starter culture ensures an adequate control of the alcoholic must fermentation. Today a wide variety of dried *Saccharomyces cerevisiae* yeast strains are commercially available and able to prevail over the native yeasts in the must and avoid the risks associated with the development of species potentially detrimental to the wine quality. However, in those regions which are well-known for typical wines, it would preferable to use a starter of indigenous yeasts of the same area (Moreno, Millàn, Ortega, & Medina, 1991); in fact, each strain of *S. cerevisiae* is able to produce different types and quantities of secondary compounds which are determinant on the desirable aromatic characteristics of a wine (Pretorius, 2000). Further, the selected yeast strains should produce very low quantities of unpleasant compounds which could compromise the quality of the bouquet. In fact, in the case of white wines, both *Saccharomyces* and non-*Saccharomyces* yeasts have been found to produce undesired phenolic compounds (Chatonnet, Dubourdieu, Boidron, & Pons, 1992). The volatile phenolic compounds, such as 4-vinyl guaiacol or 4-vinyl phenol, are produced through the decarboxylation of ferulic acid and p-cumaric acid, respectively, and the subsequent reduction of these compounds leads to the formation of 4-ethyl guaiacol and 4-ethyl phenol. These volatile phenols have a distinctive aroma judged to be "smoky", "pharmaceutical" or "leathery" even if present in small quantities in the grapes. When present at high levels in wine, they result in a defect called *phenolic off-flavour* (POF) (Thurston & Tubb, 1981).

The Regional Institute of Vine and Wine (IRVV) (Palermo, Italy) has isolated more than 900 *S. cerevisiae* yeasts from spontaneous fermenting musts, in order to study and preserve the biodiversity of these indigenous populations and also to identify promising yeast strains for use in winemaking. This collection of yeasts is characterized by high genetic variability and the identification of some characteristic phenotypes,

Abbreviations: POF, phenolic off flavour; IRVV, Istituto Regionale della Vite e del Vino; PCR, polymerase chain reaction; DIPROVAL, Dipartimento di Protezione e Valorizzazione Agroalimentare; mtDNA-RFLP, mitochondrial DNA-restriction fragments length polymorphisms; YPD, yeast peptone dextrose; TBE, tris borate EDTA; EDTA, ethylene diamine tetraacetic acid; EEC, Economic European Community; WL, Wallerstein Laboratory nutrient agar; HS-SPME–GC–MS, head space-solid phase micro extraction-gas chromatography–mass spectrometry; DVB/CAR/PDMS, DiVinylBenzene/Carboxen/PolyDiMethylSiloxane; CP-Wax 52 CB, Chrompack Wax 52 Chemically Bonded; PCA, principal component analysis; ANOVA, analysis of variance; Bp, base pairs.

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fundamental in the oenological selection of yeasts, allowed the selection of 35 strains as being potentially excellent grape must ferments (Di Maio, Polizzotto, Di Gangi, Foresta, & Oliva, 2009). One of these strains has been marketed as active dried yeast since 2006 and has given excellent results in the production of wines from Nero d'Avola and other black grape varieties (Di Maio et al., 2006; Oliva et al., 2006).

The present paper describes the results of experimental white wine production using three strains of yeast from the IRVV collection, identified by codes A2-40, A3-2 and A4-9, distinguished by the positive values of some important oenological properties. The quality of the wines produced was confirmed by their principal oenochemical parameters, by sensory analysis and qualitative and quantitative determination of the volatile aroma constituents. The aim of the research was to evaluate the contribution of the selected yeast for enhancing the quality of white wines from autochthonous grapes: Grillo, Cataratto and Inzolia.

2. Materials and methods

2.1. Yeast strains

S. cerevisiae yeast strains A2-40, A3-2 and A4-9 belong to the oenological yeasts collection of the Regional Institute of Vine and Wine (IRVV) and were isolated in Sicily (Italy). Commercial *S. cerevisiae* strains VL1 (POF-) and EC1118, used as controls in fermentation trials, are produced by Laffort (France) and Lallemand (Canada), respectively. Commercial *S. cerevisiae* strains BA11, ICV-K1, ICV-D254 and RC212 are produced by Lallemand (Canada); *S. cerevisiae* strain L404 belongs to the DIPROVAL collection-University of Bologna (Italy) and is commercialized by Oliver-Ogar (Italy). *Hanseniaspora uvarum* 1-03 strain belongs to the oenological yeasts collection of the Regional Institute of Vine and Wine (IRVV). All the yeasts were maintained at 4 °C on Sabouraud Dextrose Agar (Oxoid, Hampshire, UK) medium slants enriched with 1% Yeast Extract (Oxoid, Hampshire, UK). The DNA of the three strains A2-40, A3-2 and A4-9 was extracted following the method described by Querol, Barrio, Huerta, and Ramon (1992) and then used for subsequent experiments as described by Granchi, Bosco, Messini, and Vincenzini (1999). PCR products were then digested with 3 units of the restriction endonuclease HaeIII (New England Biolabs, Hertfordshire, England). For all three strains, fragments of 320, 225, 180 and 145 base pairs were obtained, typical of the species *S. cerevisiae* and *Saccharomyces paradoxus.* To further distinguish between these two species, a *S. cerevisiae*-specific PCR was performed according to Sabaté, Guillamon, and Cano (2000). In both analyses the *S. cerevisiae* 6167 and *Saccharomyces bayanus* 11719 DIPROVAL (Bologna University) yeasts were used as control strains.

2.2. Determination of oenological characteristics of yeast strains

Fermentation vigour and sulphite tolerance were assayed following Caridi, Cufari, and Ramondino (2002) in white must, made from concentrated must. The DIPROVAL *S. cerevisiae* L404 strain was used as positive control, while non-inoculated models acted as negative control. Fermentation vigour and sulphite tolerance were determined as the weight loss caused by the liberation of CO_2 (g CO_2/100 mL) after 2 and 7 days incubation at 25 °C. The mean values of fermentation vigour shown by the L404 strain were 4.66 g/100 mL (after 2 days) and 10.92 g/100 mL (after 7 days), while sulphite tolerance was 4.58 g/100 mL (after 2 days) and 10.48 g/100 mL (after 7 days). The growth pattern of each strain was evaluated observing samples in fermentation of the different strains using a Zeiss Axioskope2 Plus Microscope (Carl Zeiss, Oberkochen, Germany). Following Regodón, Peréz, Valdés, De Miguel, and Ramìrez (1997), killer factor production was evaluated on medium 4.7 MB on a layer of the sensitive strain BA11 (Lallemand). The commercial strains ICV-K1 and EC1118 (Lallemand) were used as positive controls, and the strains ICV-D254 and RC212 (Lallemand) as negative controls. In order to evaluate spore-producing capacity, the different strains were cultivated at 30 °C for 7 days on acetate agar as described in Caridi et al. (2002). Cellular films for microscope examination were coloured according to Schaeffer and Fulton (1933). The spores, coloured blue, and vegetative cells, coloured red, were then examined using a Zeiss Axioskope2 Plus Microscope (Carl Zeiss, Oberkochen, Germany). Acetic acid production was evaluated on calcium carbonate agar (Caridi et al., 2002). The DIPROVAL *S. cerevisiae* L404 strain was used as negative control, while the strain *H. uvarum* 1-03 (from the IRVV collection) (Romancino, Di Maio, Muriella, & Oliva, 2008) was used as positive control. Hydrogen sulphide production was evaluated on BiGGY agar as described in Nickerson (1953). The β-glucosidase production was assayed following Strauss, Jolly, Lambrechts, and van Rensburg (2001). Production of phenolic off-flavour (POF), was assayed according to Shinohara, Kubodera, and Yanagida (2000) in white grape must (20 Brix, pH 3.2). A control yeast strain (Zymaflore VL1, Laffort) and reference samples, consisting of must without acids, were used in each experiment.

2.3. Fermentation

The experimental winemaking was performed during the 2006 vintage. Inzolia and Catarratto grapes came from a vineyard situated in Biesina county (Marsala, Italy), Grillo grapes from the island of Mothia (Marsala, Italy). Transported to the IRVV Experimental Winery in Marsala (Italy), the grapes were pressed and the musts obtained were sulphited (0.05 g/L), dosed with ascorbic acid (0.05 g/L) and pectolytic enzymes (0.02 g/L), static cold clarified at 8 °C for 24 h and then subjected to the oenochemical analysis listed in Table 1. Microbiological monitoring before and after clarification showed a lowering of the indigenous bioburden before inoculation with the cultures of the selected yeast strains. The single lot of clarified must was subdivided into 5 aliquots of 100 L, each of which was then inoculated at a ratio of 5% (v/v) (Zambonelli, Tini, & Castellari, 2000) with the liquid culture of one of the 5 yeast strains, the IRVV strains A2-40, A3-2 and A4-9 and the commercial strains Zymaflore VL1 (Laffort, Bordeaux Cedex, France) and EC1118 (Lallemand, Montréal, Canada). The pure cultures of the 5 *S. cerevisiae* strains were obtained by reproduction in must (20 Brix, pH 3.20) obtained by diluting concentrated must. Fermentation was performed at 17 to 19 °C. During fermentation, the quantity of sugars present was monitored through densitometric measurement of Babo degrees every day, together with temperature and microbiological controls. The end of fermentation was determined on the basis of the exhaustion of reducing sugars (<3 g/L). Fermentation lasted 14 days for Catarratto musts and between 16 and 22 days for those of Inzolia and Grillo. The musts were then racked and sulphur dioxide (0.04 g/L) was added: samples of the dregs at the bottom of the fermentation vessels were immediately cryo-preserved for subsequent molecular analyses to identify the yeasts present at the end of the fermentation process. Wine samples were collected from each vessel for subsequent oenochemical analyses. In December 2006, after a further racking and a final addition of sulphur dioxide (0.04 g/L), the wines were bottled.

Table 1
Oenological parameters for Grillo, Inzolia and Catarratto musts before yeast inoculation.

	Grillo	Inzolia	Catarratto
Brix	22.2	23.2	22.2
pH	3.30	3.48	3.29
Total acidity (g/L)	6.8	6.3	6.0
Yeast available nitrogen (mg/L)	133a[a]	144b	232c

[a] Different letters in the same row represent significant differences at $P<0.05$ by Duncan's multiple range test.