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Selection of an autochthonous yeast starter culture for industrial production of Primitivo "Gioia del Colle" PDO/DOC in Apulia (Southern Italy)

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ABSTRACT

The aim of the present study was to isolate and characterize yeast strains as good candidates for driving the industrial fermentation process, from natural must fermentations of "Primitivo" grape cultivar, grown in the PDO/DOC "Gioia del Colle" (Apulia, Southern Italy). The selection protocol was based on parameters such as low production of acetic acid and hydrogen sulphide, complete sugar consumption during fermentation, significant production of some classes of volatile molecules responsible for wine aroma. Three *Saccharomyces cerevisiae* strains, named ITEM14088, ITEM14090 and ITEM14093, successfully dominated the fermentation process and contributed to increase organoleptic quality of the produced wines. The best performing strain, namely ITEM14093, was used as fermentation starter for three different industrial vinifications. The wines obtained were characterized by high levels of esters, associated to fruity nuances, as well as of alcohols responsible for vinous, sweet and floral notes. Furthermore, from a sensory point of view, all wines were positively judged, being characterized by frankness, gustatory persistence and intensity, good balance and body wine.

1. Introduction

Apulia (Southern Italy) is the second Italian area for wine production (ISMEA, 2017). The Apulian wines detain several peculiarities because of pedologic features of the production area, climatic conditions of this region and the specific adopted technologies, all contributing to the definition of a unique "terroir". The International Organization of Vine and Wine established in 2010 that "terroir" pertains to "an area in which collective knowledge of the interactions between the identifiable physical and biological environment and applied viticulture and oenological practices develops, providing distinctive characteristics for the products originating from this area" (Capozzi, Russo, & Spano, 2012; Capozzi & Spano, 2011). Several investigations have underlined the pivotal role of the microbiota associated with the "terroir" in which a particular grape cultivar is grown, able to give unique organoleptic properties to the produced wine (Di Maio et al., 2012). The "microbial terroir" associated to the grape/wine background has been recently studied and the obtained findings highlighted the close connection among microbial consortium, climate and production area (Bokulich et al., 2016; Bokulich, Thorngated, Richardsone, & Mills, 2014). A rising number of scientific surveys strongly focused on microbial biodiversity associated with spontaneous grape must fermentation, with the aim to identify autochthonous strains, characterized by optimal physiological and technological properties, to be used as fermentation starters in industrial production (Tristezza et al., 2013, 2014, 2012; Capozzi et al., 2010; Capozzi, Garofalo, Chiriatti, Grieco, & Spano, 2015; Cappello, Stefani, Grieco, Logrieco, & Zapparoli, 2008; Garofalo et al., 2015; Grieco et al., 2011).

As already reported, the diversity of indigenous yeast strains allows the production of wines denoted by high quality and peculiar flavour (Capozzi et al., 2015; Pérez-Coello, Briones Pérez, Ubeda Iranzo, & Martin Alvarez, 1999; Romano, Fiore, Paraggio, Caruso, & Capece, 2003; Tristezza et al., 2014). In contrast, the massive employment of commercial starters could affect the unique properties that differentiate typical regional wines (Cappello, Bleve, Grieco, Dellaglio, & Zacheo, 2004).

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Primitivo is one of the most important vines grown in Southern Italy and, particularly, in the Apulia Region. Primitivo grapes produce wines with high alcohol levels and a ruby-purple colour denoted by the Protected Designation of Origin (PDO/DOC) in two different areas in Apulia, Manduria and Gioia del Colle (Southern Italy; Antonacci, 2004). Even though, the Gioia del Colle - Primitivo PDO/DOC wine consumer's appreciation has been recently increasing worldwide, scarce knowledge is available on the chemical and sensory characteristics of Primitivo wines and none studies give information on the yeast population associated to this area. (Baiano, Terracone, Gambacorta, & La Notte, 2009; Trani, Verrastro, Punzi, Faccia, & Gambacorta, 2016).

During a previous study, a population consisting of one thousand different isolates of *S. cerevisiae* was isolated, during the last step of the spontaneous alcoholic fermentation of Primitivo grape (collected in district of Gioia del Colle; Grieco et al., 2011) and subjected to oenological selection procedure (Tristezza et al., 2012). The genetic analysis of the rDNA region of 104 low H_2S-producers isolates confirmed that they all belonged to the species *S. cerevisiae* and it allowed the identification of 15 different strains, that were deposited in the International ISPA Collection (http://server.ispa.cnr.it/ITEM/Collection/).

The present investigation describes the genetic diversity of wild *Saccharomyces cerevisiae* strains in spontaneous fermentations of a Primitivo wine produced with grapes collected in the Gioia del Colle - Primitivo PDO/DOC area. A selection approach able to identify autochthonous yeast strains and providing significant oenological properties was performed and the selected strains tested in pilot- and industrial-scale vinification. To our knowledge, this study is the first investigation on the *S. cerevisiae* populations associated to the above PDO/DOC area grapes and of the employment of autochthonous starter cultures for the industrial production of this typical wine.

2. Materials and methods

2.1. Yeast strains genetic analysis

Yeast populations were sampled at the end of alcoholic fermentation. Yeast total genomic DNA was extracted according to De Benedictis et al. (2011) and isolates were genetically distinguished at strain level by inter-delta typing (Tristezza, Gerardi, Logrieco, & Grieco, 2009).

2.2. Lab-scale fermentations

Selected yeasts fermentation performances were evaluated by micro-fermentation trials. The must (sugars 215 g/L, pH 3.25, assimilable nitrogen 142.6 g/L) was centrifuged and sterilized by filtration (through 0.22 μm Ø membrane), then potassium metabisulphite (100 mg/L) was added. One liter of must was inoculated with a yeast culture (up to a concentration of 10^6 CFU/mL) grown in the same must. The lab-scale fermentations were carried in triplicate out at 20 °C. Samples were daily subjected to gravimetric analysis in order to record CO_2 production until the weight remained constant. A sample of fermented must (100 mL) was stored at −20 °C, the remaining was used for instrumental analysis. During fermentation, the hydrogen sulphide production was evaluated as described by Tufariello et al. (2014).

2.3. Pilot-scale fermentations

Pilot-scale fermentations were carried out in 100 L stainless steel vats. Primitivo must (3 L) was inoculated with 1.5×10^6 CFU/mL of yeast and left for 6 h at room temperature. After this period, the yeast-must mixture was added to 90 kg of Primitivo must (sugars 202 g/L, pH 3.2, assimilable nitrogen 167.2 g/L). The fermentation process was carried out at 25 °C and its kinetics was followed daily by measuring the sugars consumption. At the end of alcoholic fermentation (0–1 °Babo), wine and residual lees were collected and yeast population was isolated for further molecular analyses.

2.4. Industrial-scale fermentations

Yeast biomass productions were carried out by employing a Biostat C fermenter (Sartorius, Germany) as previously described (Tristezza et al., 2012). The initial yeast inoculum (1.5×10^6 CFU/mL) was mixed with 300 L of Primitivo must and left for 6 h at room temperature. Then, the yeast-must mix was added to 15 tons of Primitivo must. The alcoholic fermentations were carried out at 25 °C and their kinetics were monitored daily by measuring the concentration of reducing sugars. At the end of alcoholic fermentation (0 °Babo), samples of wine and residual lees were collected for further analyses. The industrial test was conducted on Primitivo wines from three wineries located in the “Gioia del Colle” DOC area in Apulia Region (Southern Italy) specifically located in Cassano delle Murge (denoted as GT and LZ) and Locorotondo (denoted as LR).

2.5. Chemical analysis

Wines and musts were centrifuged at 8000 rpm for 10 min and then were analyzed by Fourier Transform Infrared Spectroscopy (FTIR), using the WineScan Flex (FOSS Analytical, DK). Acetaldehyde, ethyl acetate, 2-methyl-1-propanol, higher alcohols (3-methyl- and 2-methyl-1-butanol) and acetoin were determined by GC-FID system according to De Benedictis et al. (2011). Separation of wines from solids was performed, and then wines were bottled and stored at 16–19 °C. Volatile aroma compounds were extracted in triplicate by solid phase extraction (SPE) technique according to Tufariello, Capone, and Siciliano (2012).

2.6. Sensory analysis

The sensory analysis was performed by a panel composed of 15 professional experts, chosen among oenologists and producers involved in Primitivo wine production. The judges were asked to assign a score for different parameters of the wines, such as frankness, gustatory-intensity, balance, acidity, body, gustatory-persistence and aftertaste attributes, using a sensory analysis-tasting sheet with a scale ranging from 0 (absence of perception) to 10 (maximum perception). The mean scores of attributes were submitted to Quantitative Descriptive Analysis (QDA) according to Trani and Coworkers (2016).

2.7. Statistical analysis

The results were expressed as mean values ± standard deviations. Analysis of variance (ANOVA) of the mean values obtained for the volatiles concentrations was performed, followed by Tukey's post-hoc test when $P < 0.05$. In order to reveal any grouping of the wines based on the composition of volatile compounds, as well as to identify the main components contained within each group, the data were subjected to principal component analysis (PCA).

3. Results and discussion

3.1. Oenological characterization of selected strains

The oenological selection of indigenous wine yeast strains is fundamental for wine producers in order to have starter cultures able either to control wine fermentations or to link wines to their productive area. Even tough, the employment of autochthonous yeast starters for industrial-scale wine production is, to date, scarcely adopted by local winemakers (Berbegal, Spano, Tristezza, Grieco, & Capozzi, 2017; Petruzzi et al., 2017). Yeasts play a substantial role in the transformation of grape must in wine (Howell, Cozzolino, Bartowsky, Fleet, & Henschke, 2006; Romano, Fiore, Paraggio, Carusi & Capece, 2003) and the use of selected autochthonous strains was employed to produce wines with peculiar aroma (Alves et al., 2015) or to enhance the aromatic properties of a specific grape cultivar (Garofalo et al., 2015;