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# Selection of 80 newly isolated autochthonous yeast strains from the Tikveš region of Macedonia and their impact on the quality of red wines produced from Vranec and Cabernet Sauvignon grape varieties

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ABSTRACT

The main objectives of this study were to (i) isolate newly autochthonous yeast strains from the Tikveš region of Macedonia and (ii) test their impact on the quality of red wines from Vranec and Cabernet Sauvignon grape varieties. The newly isolated yeast strains were obtained by spontaneous fermentation of grape must from Vranec and Cabernet Sauvignon varieties collected from ten different micro-regions in Macedonia. The grapevines from both varieties grown in "Barovo" micro-region were the richest sources of yeast strains. In addition, the molecular identification and typing of strains were also carried out. The monomeric anthocyanins, polyphenolic content and other oenochemical characteristics of the wines were also compared with the wines from commercial yeast strain "SiHa". The Vranec wine from yeast strain F-8 and Cabernet Sauvignon wine from yeast strain F-20 had significantly ($p < 0.05$) higher concentrations of monomeric anthocyanins and total phenolic compounds than other wines.



## 1. Introduction

Domestication of wine yeast, while inadvertent until recent decades, has generated strains that differ considerably from "wild" *S. cerevisiae* strains. Inoculations of "wild" *S. cerevisiae* yeasts can influence the process of fermentation and greatly affect the quality of the wine. Isolating strains from successful fermentations for inoculation in subsequent vintages was being practiced during winemaking in order to avoid unwanted malolactic or acetic fermentation. Largely, the specifics and the most important quality characteristics of the wine are due to the natural microflora of the grape of the viticulture region. Bokulich, Thorngate, Richardson, and Mills (2014) proposed several promising strategies for improving grape and wine quality of individual varieties regarding the region, climate, and microbial patterns. The yeast ecology of sweet, botrytized wine fermentations from two individual vintages was investigated by new TRFLP approach for identification and discrimination of 121 yeast strains from 24 genera and 72 species (Bokulich, Hwang, Liu, Boundy-Mills, & Mills, 2012).

As reported by Capece et al. (2010), two Nero d'Avola indigenous strains expressed completely different strain behavior after inoculation in the same must divided into two different tanks because of different ability to dominate the natural microflora present in the grape must. The low strain implantation can be due to the strong competition between wild yeasts and starter cultures and during alcoholic fermentation selected yeast strains cannot predominate the natural microflora during the whole process (Capece et al., 2010). The effect of different aging techniques on the polysaccharides, phenolic composition and sensory characteristics of Syrah red wines fermented using two different HPS and FERM yeast strains was studied (Del Barrio-Galán, Medel-Marabolí, & Peña-Neira, 2015). HPS yeast strain released a higher amount of low-molecular-weight polysaccharides during alcoholic fermentation than the FERM yeast strain. The group of Del Barrio-Galán indicated the possible interaction between polysaccharides released from the HPS yeast strain and phenolic compounds in the wine. Consequently, wines produced by HPS strain had lower phenolic compounds than those produced by the FERM yeast strain. Furthermore, the study of the effect of different *Saccharomyces cerevisiae* yeasts on the level of stilbenes in Vranec wines proved that the wines produced by French yeast "Levuline CHP" had higher level of resveratrol than the wines from same

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grape variety produced by Macedonian yeast "Vinalco" (Kostadinović et al., 2012).

The effect of three different *Saccharomyces cerevisiae* yeasts (1EV, 2EV and 7EV) on the level of anthocyanins, pyranoanthocyanins and non-anthocyanins phenolic compounds in red wines produced by two grape varieties Tempranillo and Cabernet Sauvignon showed that anthocyanins and especially diglycosides were compounds the most affected by the type of yeast strain (Monagas, Goméz-Cordovéz, & Bartolomé, 2007). Furthermore, the absorption of anthocyanins on the cell walls of the yeasts was influenced by their polarity and structural conformation (Morata et al., 2016). The effect of quantity of commercial strains during spontaneous fermentation of wines produced from grapevines from Mendoza region in Argentina reported by Mercado, Dalcero, Masuelli, and Combina (2007). Isolation and identification of 240 yeast Saccharomyces strain from the spontaneous fermentation of must from the region of La Mancha, Spain resulted in 21 different molecular profiles and micro vinification of the adequate yeasts for implantation and production of high-quality wines (Ortiz, Barrajón, Baffi, Arévalo-Villena, & Briones, 2013). Furthermore, *S. cerevisiae* strains selected from the indigenous population of domestic winemaking enable the alcoholic fermentation to proceed more effectively in comparison with commercial yeast strains (Settanni, Sannino, Francesca, Guarcello, & Moschetti, 2012). Among 26 Saccharomyces strains isolated from "Moscato di Saracena" wine from Calabria region in Italy, the autochthonous yeast strain M3-5 was more appropriate for fermentation than commercial strains (Aponte & Blaiotta, 2016).

In the current study, the wider range of micro-regions from which grape samples were selected, will increase the probability of isolating wine yeasts with different properties, and that will, in turn, increase the opportunities for selection of specific and varietal local strains. Therefore, the present work represents the first approach to spontaneous fermentation Saccharomyces population dynamics in Tikveš region, the most important vinicultural region in Macedonia that has never been characterized before. Our previous report (Ilieva, Kostadinović Veličkovska, Dimovska, & Spasov, 2016) indicated third-stage selected yeast strain F-78 as the best strain for alcoholic fermentation of Vranec wines. In the present study, the isolation of 80 yeast strains from spontaneously fermented grape must from 10 different micro-regions of Tikveš vinicultural region was applied and the fermentation activity of the yeast was studied. After the first and second stage selection, the molecular identification and typing of the most appropriate 10 yeasts were carried out. Finally, the effect of the 10-second stage selected strains on the oenochemical parameters, monomeric anthocyanins and total phenolic compounds of wines produced from two grape varieties Vranec and Cabernet Sauvignon was investigated. To the best of our knowledge, no similar report has been published regarding the isolation of a wide range of autochthonous yeast strains from the Tikveš region and their application in red wines from Vranec and Cabernet Sauvignon grape varieties.

## 2. Materials and methods

### 2.1. Grapes from Vranec and Cabernet Sauvignon grape variety for spontaneous fermentation

A spontaneous fermentation of seven different lots of Vranec grapes and three different lots of Cabernet Sauvignon grapes from different micro-regions from Tikveš region was held in "Popova Kula" vinery, Demir Kapija (Table 1). From each grape variety, 150 kg samples were collected. The grapes from Cabernet Sauvignon grape variety were harvested at optimal maturity. The sugar content was in the range of 21–24%, acidity 5.3–6.4 g/L and pH ranged from 3.32 to 3.45. The data for Vranec grape variety were reported by Ilieva et al. (2016).

### 2.2. Spontaneous fermentation of Vranec and Cabernet Sauvignon grape varieties from "Tikveš" region

A spontaneous fermentation of the must from three different lots of Cabernet Sauvignon grapes from "Barovo", "Kavadarci" and "Ljubash" micro-regions was held from the harvest 2010. Regarding the Vranec grape variety, the lots from seven micro regions are presented in Table 1. The wines obtained by spontaneous fermentation of additional three lots from this grape variety were not used for isolation of yeast strains. Table 1 shows the alcohol content, residual sugars, pH, titratable and volatile acids, monomeric anthocyanins and color intensity (IC) of the wines from differently fermented grape must. The procedure of isolation of pure culture of yeast strains and three stage selection were established by Ilieva et al. (2016).

### 2.3. Determination of oenochemical parameters in trial wines

Determination of the amount of alcohol was performed ebuliometrically with Dujardin – Salleron ebuliometer and for determination of reducing sugars the Schoorle method was used. Determination of titratable and volatile acidity of trial wines was performed by the previous method (Boulton, 1980). The color intensity (IC) was measured spectrophotometrically at 420 nm (yellow color), 520 nm (red color), 620 nm (blue color) by UV spectrophotometer Shimadzu 1800, Shimadzu corporation, Kyoto, Japan. Determination of monomeric anthocyanins and total phenolic method was performed by the colorimetric method (Singleton & Rossi, 1965).

### 2.4. Molecular identification and typing of newly isolated yeast strains

For molecular identification and typing of seven strains of the species *Saccharomyces cerevisiae*, PCR-Δ multiplication of the DNA fragments was applied. In this study, the visualization of the result was made by gel-electrophorese (Legras & Karst, 2003; Ness, Kowenz, Casini, Graf, & Leutz, 1993). The reaction mixture used for PCR with reaction buffer contained: Tris-HCl (10 mM), KCl (50 mM) and Triton® X-100 (0.1% v/v), $MgCl_2$ (1.25 mM), primers (0.83 μmol), deoxyribonucleotides: dATP, dTTP, dCTP et dGTP (160 μM each) and DNA polymerase (0.042 U.μL-1). For the analyses of the inter-δ region, the primer pairs δ12 (TCAACAATGGAATCCCAAC) and δ21 (CATCTTAACACCGTATATGA) by sequence (5′ → 3′) was used for PCR amplification. The amplification of δ region was performed directly from the colony, without previous DNA extraction, by increasing the time and the temperature of initial denaturation. After initial denaturation at 95 °C for 10 min, the reaction mixture was cycled 35 times using by following program: 30 s. denaturation at 95 °C, 30 s. primer annealing at 46 °C and 90 s. primer extension at 72 °C following by 10 min. The final extension at 72 °C. A microfermentation trials were performed in order to detect the weight loss stemming from $CO_2$ releasing from the system. The glass bottles of 330 mL volume were poured by 150 mL must with the sugar concentration of 222 g/L, titratable acidity 4.1 g/L, the pH value of 3.52% and 3% inoculation culture. Fermentation was considered when no weight loss was any longer recorded within 24 h. Fermentation rate was expressed as grams of $CO_2$ produced by 100 g of must during the first 72 h of fermentation (Rinaldi, Blaiotta, Aponte, & Luigi Moio, 2016). Each trial was performed in duplicate.