Identification and technological characteristics of yeast strains from vineyards in Slovakia

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Summary
Slovakia, a central European country, has a long lasting tradition of vine growing and producing wine, where yeasts significance in winemaking process and their region- and climate-depending specificity lies in the centre of research interest. In this study, yeast consortium profiles of several vineyards and vine varieties in four following years were identified and confirmed by biochemical tests. Pure yeast cultures were obtained from grapes, must, fermenting must and young wines. Isolated genera such as *Saccharomyces*, *Kloeckera*, *Candida*, *Rhodotorula* or *Lachancea* were placed in the wine yeast collection. Significant technological properties of identified yeast strains (ethanoltolerance, osmotolerance and thermotolerance) were studied. All strains isolated from variety Sauvignon Blanc harvested in all four investigated years showed high ethanol- and osmotolerance. Two *Kloeckera apiculata* strains from variety Blaufränkisch (year 2017) were osmotolerant, identically with *K. apiculata* isolated from the Green Veltliner in the same year. Four *Saccharomyces cerevisiae* strains isolated in 2017 from White Riesling showed high ethanoltolerance and osmotolerance.

Keywords
wine; yeast; osmotolerance; thermotolerance; ethanoltolerance

The composition of yeast consortia during wine fermentation is known to have a strong influence on the quality of wine, involving visual aspect, taste and aroma. There exist two potential natural sources of yeasts. One of them is the natural occurrence of yeasts, on berries and leaves of the grape, another source is the plant itself, with which wine comes into contact. Yeasts could be also found in the soil, or transmitted by insects from other plants by sucking out the flowing berry juice [1, 2].

Yeasts are significant in winemaking, because they carry out the alcoholic fermentation but, simultaneously, they can cause spoilage of wine. Their autolytic products may affect sensory quality and influence the growth of malolactic and spoilage bacteria [3]. Prominent in the winemaking process are *Saccharomyces* species (predominantly *S. cerevisiae*), which dominate the alcoholic fermentation, but their growth can be detrimental if it occurs in a finished and bottled wine. Therefore, efforts to determine the population diversity and potential impact of different microorganisms on the winemaking process is critical to production of flavourful product [4]. Occurrence of specific yeasts may be typical for individual wine grape cultivars and for individual wine-producing regions. Data on yeast consortium composition of various vine cultivars and at the different stages of wine production are essential for production of high quality wines and their subsequent successful performance on the market. Yeast representation differs between varieties and varies between regions, as well as within the vineyard, even the dominant strain may be different for each variety [5].

The traditional approach to wine yeast characterization is auxotrophy profiling, which is based on studying the ability of cultures to grow on various saccharides as a sole carbon source [6]. In this way, useful information in terms of distinguishing *S. cerevisiae* from non-*Saccharomyces* yeasts can be obtained. Several regional wine yeast profiles were published, mostly in countries

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with extended tradition of wine production such as France [7], Greece [8], Argentina [9], Italy [10] or Spain [11, 12]. Research on microbial diversity, however, has escalated trend also in other wine-producing countries, e.g. Brasil [13], Macedonia [14], China [15], Croatia [16], Czech Republic [17] or Turkey [18].

Certain differences in strain presentation, however, could be affected by climatic conditions [3, 19, 20]. Therefore, monitoring of yeast diversity, invovling both *Saccharomyces* and non-*Saccharomyces* yeasts, during several vegetation periods could be of great interest, as the profiles could be compared and provide a more comprehensive view at microbiological trends.

Technologically relevant yeast strains could be gained as pure commercially produced cultures or as the autochtonous microflora. Commercially produced yeasts are easily accessible and applicable, could be specifically or universally used and provide high quality of wine. However, wines produced using commercial yeast strains are uniform to a considerable extent and originality as well as typical regional character of wine is suppressed. Autochtonous yeast strains provide high quality wine, increasing wine autheticity and variabilty due to terroir, however, they are difficult to access and are applicable only in liquid form. The use of conventional microbiological methods, like microbial population enumeration on nutritive selective media for yeast strains, demonstrate significant microbial differences between different wine-producing areas and differences in technological characteristics such as ethanoltolerance, thermotolerance and osmotolerance. Specific yeast strains are, therefore, apparently responsible for unique character of wines.

The aim of this work was to identify the yeast species isolated from individual grapevine varietes, vintage years and wine regions of Slovakia, compare their diversity and evaluate their technologically significant characteristics.

MATERIALS AND METHODS

Isolation and identification of yeast strains

Yeast strains were isolated from wine grape samples of varietes ‘Rulandské sivé’ (Pinot Gris), ‘Veltlínske zelené’ (Green Veltliner), ‘Sauvignon’ (Sauvignon Blanc), ‘Rizling rýnsky’ (White Riesling) and ‘Frankovka modrá’ (Blaufränkisch) harvested at maturity stage in September–October as follows: Pinot Gris from Small Carpathian, Nitra and South Slovakian regions in the season 2015 and from Nitra region in the years 2016, 2017,

Tab. 1. Identified yeast species from harvest 2015.

Sample	Yeast species
Pinot Gris (Small Carpathian region)	
Berry	*Candida rugosa* *Rhodotorula glutinis* *Cryptococcus albidus*
Must	*Candida rugosa*
Fermenting must	*Kloeckera apis*
Young wine	*Kloeckera apis* *Saccharomyces cerevisiae*
Pinot Gris (Nitra region)	
Berry	*Candida pulcherrima* *Kloeckera apis*
Must	*Candida pulcherrima* *Kloeckera apis*
Fermenting must	*Candida pulcherrima* *Kloeckera apis/apiculata*
Young wine	*Saccharomyces cerevisiae* *Kloeckera apis/apiculata*
Pinot Gris (South Slovakian region)	
Berry	*Rhodotorula glutinis* *Candida pulcherrima* *Cryptococcus humicola* *Kloeckera apis*
Must	*Rhodotorula glutinis* *Candida rugosa* *Candida dattila* *Candida pulcherrima* *Cryptococcus laurenti* *Kloeckera apis*
Fermenting must	*Rhodotorula mucilaginosa* *Candida pulcherrima* *Cryptococcus humicola* *Kloeckera apis*
Young wine	*Cryptococcus humicola* *Saccharomyces cerevisiae*
Green Veltliner (South Slovakian region)	
Berry	*Rhodotorula glutinis* *Candida pulcherrima* *Cryptococcus humicola* *Kloeckera apis*
Must	*Rhodotorula glutinis* *Candida rugosa* *Candida dattila* *Cryptococcus laurenti* *Kloeckera apis* *Candida pulcherrima*
Fermenting must	*Kloeckera apis* *Cryptococcus humicola* *Candida pulcherrima* *Rhodotorula mucilaginosa*
Young wine	*Cryptococcus humicola* *Saccharomyces cerevisiae*

Tab. 1. *continued*

Sample	Yeast species
Sauvignon Blanc (South Slovakian region)	
Berry	*Rhodotorula glutinis* *Cryptocuccus laurenti* *Candida rugosa*
Must	*Rhodotorula glutinis* *Kloeckera apis* *Cryptocuccus laurenti*
Fermenting must	*Kloeckera apis*
Young wine	*Kloeckera apis* *Saccharomyces cerevisiae*
Blaufränkisch (South Slovakian region)	
Must	*Candida pulcherrima* *Rhodotorula mucilaginosa* *Kloeckera apis*
Fermenting must	*Kloeckera apis* *Cryptococcus humicola*
Young wine	*Saccharomyces cerevisiae* *Cryptococcus humicola*

Tab. 2. Identified yeast species from harvest 2016.

Sample	Yeast species
Pinot Gris (Nitra region)	
Berry	*Candida pulcherrima* *Kloeckera apis/apiculata*
Must	*Candida rugosa* *Candida pelliculosa* *Zygosaccharomyces* sp. *Kloeckera apis/apiculata*
Fermenting must	*Candida pelliculosa* *Candida pulcherrima* *Zygosaccharomyces* sp. *Kloeckera apis/apiculata*
Young wine	*Candida rugosa*
Sauvignon Blanc (South Slovakian region)	
Berry	*Candida pulcherrima* *Kloeckera apis/apiculata*
Must	*Candida pulcherrima* *Kloeckera apis/apiculata*
Fermenting must	*Candida pulcherrima* *Candida pulcherrima* *Kloeckera apis/apiculata*
Young wine	*Saccharomyces cerevisiae*
White Riesling (South Slovakian region)	
Berry	*Saprochaete capitata* *Kloeckera apis/apiculata*
Must	*Candida pulcherrima* *Kloeckera apis/apiculata*
Fermenting must	*Candida pulcherrima* *Kloeckera apis/apiculata*
Young wine	*Saccharomyces cerevisiae*

Tab. 3. Identified yeast strains from harvest 2017.

Sample	Yeast species
Pinot Gris (Nitra region)	
Berry	*Kloeckera apiculata*
Must	*Kloeckera apiculata*
Fermenting must	*Saccharomyces cerevisiae*
Young wine	*Kloeckera apiculata* *Saccharomyces cerevisiae*
Sauvignon Blanc (South Slovakian region)	
Berry	*Kloeckera apiculata*
Fermenting must	*Kloeckera apiculata* *Saccharomyces cerevisiae*
Young wine	*Saccharomyces cerevisiae*
Green Veltliner (South Slovakian region)	
Berry	*Kloeckera apiculata*
Must	*Kloeckera apiculata*
Fermenting must	*Kloeckera apiculata*
Young wine	*Kloeckera apis*
Blaufränkisch (South Slovakian region)	
Berry	*Kloeckera apiculata*
Must	*Kloeckera apiculata*
Fermenting must	*Kloeckera apiculata* *Candida pulcherima*
Young wine	*Saccharomyces cerevisiae* *Kloeckera apiculata*
White Riesling (South Slovakian region)	
Berry	*Kloeckera apiculata*
Must	*Kloeckera apiculata*
Fermenting must	*Kloeckera apiculata* *Saccharomyces cerevisiae*
Young wine	*Saccharomyces cerevisiae*

Tab. 4. Identified yeast strains from harvest 2018.

Sample	Yeast species
Pinot Gris (Nitra region)	
Berry	*Kloeckera apiculata* *Candida pulcherrima* *Rhodotorula glutinis* *Lachancea kluyverii* *Cryptococcus laurentii*
Must	*Kloeckera apiculata* *Candida pulcherrima* *Zygosaccharomyces* sp.
Fermenting must	*Saccharomyces cerevisiae* *Kloeckera apiculata*
Young wine	*Candida famata* *Saccharomyces cerevisiae*

2018; the other varieties were analysed only from South Slovakian region: Green Veltliner (2015, 2017), Sauvignon Blanc (2015, 2016, 2017), White Riesling (2016, 2017) and Blaufränkisch (2015, 2017). In only one growing season, yeasts from vine variety Pinot Gris, Small Carpathian region (2015) and from vine variety White Riesling, South Slovakian region (2017) were isolated. Data on numbers of isolated strains from individual varieties, at different stages of fermentation and in each cultivation season, are shown in Tab. 1–4.

For the analysis of grape surface microflora, grape berries were suspended in the physiological saline solution (0.9 % NaCl) and shaken on an orbital shaker PSU-10i (Biosan, Riga, Latvia) at laboratory temperature for 4 h at 3.3 Hz. Must was prepared from approximately 1 kg of grapes by hand-crushing in a sterile plastic bag. Must was fermented for 21 days at 15 °C in the dark in a cooled incubator (VELP Scientifica, Usmate, Italy). A volume of 100 μl of the homogenized grape-rinse saline solution, must, vigorously fermenting must and young wine were plated on Wallerstein Laboratories Nutrient (WLN) medium with chloramphenicol (Sigma-Aldrich, St. Louis, Missouri, USA).

Yeast strains were isolated from berries, must (after pressing), fermenting must (3–5 days of fermentation) and young wine (14–21 days of fermentation). Individual yeast species were distinguished by different colonies morphology and colour, subsequently separated by serial dilutions and isolated to pure cultures. The obtained isolates were identified using a biochemical test API ID 32C (BioMérieux, Marcy-l'Étoile, France). For short term, strains were stored on yeast peptone dextrose agar (YPD agar) plates at 4 °C for a maximum of 4 weeks. The oenologicaly significant strains were lyophilised and placed to the Wine Yeast Collection at the Food Research Institute of National Agricultural and Food Centre (Bratislava, Slovakia), which contains many significant strains and has been maintained since the fifties of the 20th century.

Technological properties testing

The isolated yeast strains were tested regarding technologically significant characteristics, namely, ethanoltolerance, osmotolerance and thermotolerance. Test were carried out in yeast extract-dextrose (YD) broth (yeast extract 10 g, D-glucose 20 g, distilled water to 1000 ml; final pH 6.0–6.5 at 25 °C; autoclaved at 121 °C for 20 min). For ethanoltolerance testing, ethanol was added to the broth in concentrations of 0, 5, 10, 12.5, 15, 20 % (v/v); for osmotolerance testing, glucose was added at levels of 0, 200, 400, 500 and 600 $g \cdot l^{-1}$. Thermotolerance was tested at temperatures of 4, 25, 37 and 54 °C. Broth portions of 5 ml were inoculated by 100 μl fresh yeast suspension (1-day culture resuspended in 1 ml of distilled water, diluted to 3 McFarland units (McF; DensiCHEK plus, BioMérieux) and biomass formation was observed after 6 days of incubation at 26 °C.

RESULTS AND DISCUSSION

Samples of berries, must, fermenting must and young wine originated from three Slovakian wine regions. They were collected in 2015, 2016, 2017 and 2018 with the aim of yeast consortium analysis. Comparing Small Carpathian, Nitra and South Slovakian Pinot Gris variety, *Kloeckera, Rhodotorula, Candida, Cryptococcus* and *Saccharomyces* species were identified (Tab. 1–4). The greatest diversity of strains was found in the wine from South Slovakian region from season 2015, where 4 strains from berries and fermenting must, 6 strains from must and 2 strains from young wine were isolated (Tab. 1). Pinot Gris was examined as the only variety in all four seasons (2015–2018). Interesting technological properties showed the osmotolerant *Candida rugosa* and *Rhodotorula glutinis* strains, as well as the ethanoltolerant *Saccharomyces cerevisiae* strain. One strain of *Candida pulcherrima* and two strains of *Kloeckera apis*/*apiculata* from Nitra region, year 2015, were osmotolerant and they grew in media containing more than 500 $g \cdot l^{-1}$ glucose (Tab. 5).

Comparing Green Veltliner yeast profiles in 2015 and 2017 from the South Slovakian vine region, the greater diversity was evident in the samples harvested in the first one (Tab. 1, 3). *K. apiculata* was the main and exclusively identified yeast species at all stages of the vinification process (berries, must, fermenting must) in 2017. This species demonstrated high ethanoltolerance, growing in 15 % (v/v) of ethanol. Isolates of 2015 season represented mostly *Candida, Rhodotorula* and *Cryptococcus* species, while one strain of *S. cerevisiae* and one *R. glutinis* were osmotolerant and showed good oenological properties (Tab. 5, *7*).

Sauvignon Blanc grape samples were harvested in 2015, 2016 and 2017 from South Slovakian wine region and were used in wine production with spontaneous fermentation process (Tab. 1–3). Remarkable predominance of *S. cerevisiae* was evident even at the stage of fermenting must (year 2017) and all isolated strains showed high ethanol- and osmotolerance (Tab. 5–7). Noticeably greater

Tab. 5. Technological properties of strains isolated in 2015.

Variety and region	Species	Ethanoltolerance						Thermotolerance				Osmotolerance				
		Ethanol [%]						Temperature [°C]				Glucose [g·l^{-1}]				
		0	5	10	12.5	15	20	4	25	37	54	0	200	400	500	600
***Saccharomyces* species**																
Pinot Gris (Small Carpathian region)		+++	+++	++	+	–	–	–	+	+	–	++	++	+	+/–	+/–
Pinot Gris (Nitra region)		+++	+++	–	–	–	–	+	+	+	–	++	++	+	+	–
Green Veltliner (Nitra region)		+++	++	–	–	–	–	+/–	+	–	–	++	++	+	+	+/–
Sauvignon Blanc (South Slovakian region)		+++	++	++	–	–	–	–	+/–	–	–	++	++	+	+/–	–
Blaufränkisch (South Slovakian region)		+++	++	–	–	–	–	–	+	–	–	+++	+++	++	+	+/–
Non-*Saccharomyces* species																
Pinot Gris (Small Carpathian region)	*Candida rugosa*	+++	–	–	–	–	–	+/–	+	–	–	++	++	++	+	+
Pinot Gris (Small Carpathian region)	*Rhodotorula glutinis*	+++	–	–	–	–	–	+/–	+	–	–	+++	+++	++	++	+
Pinot Gris (Nitra region)	*Candida pulcherrima*	+++	+++	–	–	–	–	+/–	+	+/–	–	+++	++	++	++	+/–
Pinot Gris (Nitra region)	*Kloeckera apis* 1	++	++	–	–	–	–	+/–	+	+/–	–	+++	+++	++	++	+/–
Pinot Gris (Nitra region)	*Kloeckera apis* 2	++	++	–	–	–	–	+/–	+	+/–	–	++	++	++	+	+/–
Green Veltliner (South Slovakian region)	*Rhodotorula glutinis*	++	–	–	–	–	–	+	+	+/–	–	++	+	+	+	–

Symbols of biomass formation: (–) – no biomass formation, (+/–) – very weak, (+) – weak, (++) – moderate, (+++) – strong.

Tab. 6. Technological properties of strains isolated in 2016.

Variety/ Region	Species	Ethanoltolerance						Thermotolerance				Osmotolerance				
		Ethanol [%]						Temperature [°C]				Glucose [g·l^{-1}]				
		0	5	10	12.5	15	20	4	25	37	54	0	200	400	500	600
***Saccharomyces* species**																
Sauvignon Blanc (South Slovakian region)		+++	+++	+++	+	–	–	–	+	+	–	+++	+++	+	–	–
Pinot Gris (Nitra region)		+++	+++	–	–	–	–	–	+	+	–	+++	+++	++	+/–	–
Non-*Saccharomyces* species																
Pinot Gris (Nitra region)	*Candida pulcherrima*	+++	++	–	–	–	–	+	+	+	–	+++	+++	++	+	–
Pinot Gris (Nitra region)	*Candida rugosa*	+++	+++	+	–	–	–	–	+	+/–	–	+++	+++	+/–	+/–	–
Sauvignon Blanc (South Slovakian region)	*Candida pulcherrima 1*	+++	++	–	–	–	–	+	+	–	–	+++	+++	++	+	–
Sauvignon Blanc (South Slovakian region)	*Candida pulcherrima 2*	+++	+++	–	–	–	–	+	+	–	–	+++	+++	+++	+	–

Symbols of biomass formation: (–) – no biomass formation, (+/–) – very weak, (+) – weak, (++) – moderate, (+++) – strong.

Tab. 7. Technological properties of strains isolated in 2017.

Variety and region	Species	Ethanoltolerance						Thermotolerance				Osmotolerance				
		Ethanol [%]						Temperature [°C]				Glucose [g·l^{-1}]				
		0	5	10	12.5	15	20	4	25	37	54	0	200	400	500	600
***Saccharomyces* species**																
Pinot Gris (Nitra region)		+++	++	++	++	+	+/–	+/–	+++	+++	–	+++	++	++	–	–
Pinot Gris (Nitra region)		+++	+++	+++	++	+	–	+/–	+++	+++	–	+++	+++	++	+	–
Sauvignon Blanc (South Slovakian region)		+++	++	++	++	+	–	+/–	+++	+++	–	+++	+++	++	+/–	–
Sauvignon Blanc (South Slovakian region)		+++	+++	++	++	+	–	+/–	+++	+++	–	+++	+++	++	+/–	–
Sauvignon Blanc (South Slovakian region)		+++	+++	++	++	+	–	+/–	++	++	–	++	++	++	+/–	–
Sauvignon Blanc (South Slovakian region)		+++	++	++	+	+/–	–	+/–	+++	+++	–	+++	++	++	+/–	–
Sauvignon Blanc (South Slovakian region)		+++	++	++	++	+	–	+/–	+++	+++	–	++	++	++	+/–	–
Sauvignon Blanc (South Slovakian region)		+++	++	++	++	+	–	+/–	+++	+++	–	++	++	++	+/–	–
White Riesling (South Slovakian region)		+++	+++	+++	+++	+/–	–	+/–	+++	+++	–	++	+++	++	+/–	–
White Riesling (South Slovakian region)		+++	+++	+++	++	+	–	+/–	+++	+++	–	++	+++	+++	+/–	–
White Riesling (South Slovakian region)		+++	+++	++	++	+	–	+	+++	++	–	+++	++	++	+/–	–
White Riesling (South Slovakian region)		+++	++	++	+	+/–	–	+/–	++	++	–	++	++	++	+/–	–
Non-*Saccharomyces* species																
Green Veltliner (South Slovakian region)	*Kloeckera apiculata* 1	+++	++	+/–	+/–	+/–	–	+/–	+	–	–	+++	++	+/–	+/–	–
Green Veltliner (South Slovakian region)	*Kloeckera apiculata* 2	+++	++	+/–	+/–	–	–	+/–	+	–	–	+++	++	+/–	–	–
Green Veltliner (South Slovakian region)	*Kloeckera apiculata* 3	+++	++	+/–	+/–	+/–	–	+/–	+	–	–	+++	++	+/–	–	–
Blaufränkisch (South Slovakian region)	*Kloeckera apiculata* 1	+++	++	+/–	+/–	+/–	–	+/–	+	–	–	+++	++	+/–	+/–	–
Blaufränkisch (South Slovakian region)	*Kloeckera apiculata* 2	+++	++	+/–	+/–	+/–	–	+/–	+	–	–	+++	++	+/–	+/–	–

Symbols of biomass formation: (–) – no biomass formation, (+/–) – very weak, (+) – weak, (++) – moderate, (+++) – strong.

Tab. 8. Technological properties of strains isolated in 2018.

Variety and region	Species	Ethanoltolerance						Thermotolerance				Osmotolerance				
		Ethanol [%]						Temperature [°C]				Glucose [g·l^{-1}]				
		0	5	10	12.5	15	20	4	25	37	54	0	200	400	500	600
Pinot Gris (Nitra region)	*Saccharomyces cerevisiae* 1	+++	+++	+++	+++	–	–	+/–	++	++	–	+++	+++	+++	+	–
Pinot Gris (Nitra region)	*Saccharomyces cerevisiae* 2	++	+++	+++	+	–	–	+/–	++	++	–	++	+++	++	+/–	–
Pinot Gris (Nitra region)	*Saccharomyces cerevisiae* 3	++	++	++	++	–	–	+/–	++	++	–	++	++	++	+/–	–
Pinot Gris (Nitra region)	*Zygosaccharomyces* sp.	+++	+++	+/–	–	–	–	–	+++	+++	+/–	++	+++	+	+/–	–
Pinot Gris (Nitra region)	*Kloeckera apiculata* 1	++	++	–	–	–	–	–	++	+/–	–	++	+	–	–	–

Tab. 8. *continued*

Variety and region	Species	Ethanoltolerance						Thermotolerance				Osmotolerance				
		Ethanol [%]						Temperature [°C]				Glucose [g·l⁻¹]				
		0	5	10	12.5	15	20	4	25	37	54	0	200	400	500	600
Pinot Gris (Nitra region)	*Kloeckera apiculata* 2	++	++	+/–	–	–	–	–	++	–	–	++	++	+	+/–	–
Pinot Gris (Nitra region)	*Kloeckera apiculata* 3	++	++	–	–	–	–	–	++	–	–	++	++	+	+/–	–
Pinot Gris (Nitra region)	*Candida pulcherrima* 1	+++	++	–	–	–	–	+/–	+++	+++	–	+++	+++	++	+/–	–
Pinot Gris (Nitra region)	*Candida pulcherrima* 2	++	++	–	–	–	–	+	+++	+/–	–	+++	+++	++	+	–
Pinot Gris (Nitra region)	*Candida pulcherrima* 3	+++	+++	–	–	–	–	+/–	+++	+++	–	+++	+++	+++	+	–
Pinot Gris (Nitra region)	*Candida pulcherrima* 4	++	++	–	–	–	–	+/–	+++	+++	–	+++	+++	++	+	–
Pinot Gris (Nitra region)	*Candida pulcherrima* 5	+++	+++	–	–	–	–	+/–	+++	+++	–	+++	++	++	+	
Pinot Gris (Nitra region)	*Candida pulcherrima* 6	+++	+++	–	–	–	–	+/–	+++	+++	–	+++	+++	++	+	–
Pinot Gris (Nitra region)	*Candida pulcherrima* 7	+++	+++	–	–	–	–	+/–	+++	+++	–	+++	++	++	+	–
Pinot Gris (Nitra region)	*Candida pulcherrima* 8	+++	+++	+/–	–	–	–	+/–	+++	+++	–	+++	++	++	+	–
Pinot Gris (Nitra region)	*Candida pulcherrima* 9	+++	++	–	–	–	–	+/–	+++	+++	–	+++	+++	++	+	–
Pinot Gris (Nitra region)	*Candida famata*	+++	+++	+++	+	–	–	+/–	+++	+++	–	+++	+++	++	+	–
Pinot Gris (Nitra region)	*Rhodotorula glutinis* 1	+++	–	–	–	–	–	+/–	+++	–	–	+++	+++	++	+	–
Pinot Gris (Nitra region)	*Rhodotorula glutinis* 2	++	+/–	–	–	–	–	+	++	+	–	++	++	++	++	–
Pinot Gris (Nitra region)	*Lachancea kluyverii*	++	–	–	–	–	–	+/–	+++	+	–	++	++	++	++	–
Pinot Gris (Nitra region)	*Cryptococcus laurentii*	++	–	–	–	–	–	–	++	–	–	++	++	++	+	–

Symbols of biomass formation: (–) – no biomass formation, (+/–) – very weak, (+) – weak, (++) – moderate, (+++) – strong.

yeast diversity was found in previous years depicting *Rhodotorula, Cryptococcus, Kloeckera* and *Candida* species.

Blaufränkisch samples of grapes harvested in South Slovakian wine region in 2015 and 2017 were analysed regarding yeasts (Tab. 1, 3). Comparing the yeast diversity with that of Green Veltliner from the same region, *K. apiculata* dominated also in Blaufränkisch from the 2017 harvest at the berries, must, fermenting must and young wine fermentation stages. Two *K. apiculata* strains were osmotolerant, identically with *K. apiculata* isolated from the Green Veltliner in the same year (Tab. 5, 7).

White Riesling samples were collected in 2016 and 2017 from the South Slovakian wine region. *K. apiculata* was frequently isolated from samples of berries, must and fermenting must from both years. Young wine was characteristic with *S. cerevisiae* growth. Regarding berries, *Saprochaete capitata* was isolated from samples from the 2016 harvest (Tab. 2, 3). Four *S. cerevisiae* strains isolated from White Riesling vine samples of 2017 showed high ethanoltolerance and osmotolerance (Tab. 7).

In Pinot Gris berry samples collected from the 2018 season from Nitra region, great diversity of yeasts was found (Tab. 4, 8). Seven different strains of *C. pulcherrima* were isolated and several of them were osmo- and thermotolerant. From the same berry sample, osmotolerant *Saccharomyces*-related *Lachancea kluyverii* was isolated. *Lachancea* spp. are known for their transmission by fruit flies (*Drosophila pinicola*) and resorting to fermentation only when oxygen is limiting.

It is known that only a limited population of yeasts is present on immature grapes (10^1–10^3 CFU·g^{-1}) but the population increases by 10- to 100-fold. Yeasts of the genera *Rhodotorula Cryptococcus* and *Candida* had been identified on immature grape berries [3]. *Kloeckera*

apiculata (teleomorph: *Hanseniaspora uvarum*) is the predominant species on the surface of grape berries, representing approximately 50–75 % of the total yeast populations [21, 22]. Less numerous are apiculate yeasts *Candida* spp. (e.g. *C. pulcherrima*), *Brettanomyces* spp. (e.g. *B. intermedius*, *B. custer*), *Cryptococcus* spp., *Kluyveromyces* spp., *Metschnikowia* spp. (e.g. *M. pulcherrima*), *Pichia* spp. (e.g. *P. membranaefaciens*), as well as yeast from genera previously categorized as *Hansenula* (e.g. *H. anomala*) and *Rhodotorula* (e.g. *R. minuta*). Unlike the low prevalence of natural habitats in the vineyard population *Saccharomyces* sp. grows in grape juice, during the middle fermentation of must and on the surface the of wine-making equipment [21, 23].

Definitely, greater number of technologically important strains was isolated in 2017 from both Nitra and South Slovakian wine regions, representing only two species – *S. cerevisiae* and *K. apiculata*. In this year, also a greater number of yeast strains with good technological properties were isolated from both monitored wine regions. Yeasts isolated directly from the vineyard are believed to be more competitive than commercial active dry wine yeasts, because they are adapted to the given environment as well as to local vinicultural conditions. The technological parameters of yeasts are very important from the point of view of proper fermentation process in winemaking. Differences in total counts of isolated yeasts could be caused by climate conditions in combination with a protection system of grapevine. In further research, the isolated yeast strains will be definitively identified by DNA sequencing.

CONCLUSION

Indigenous yeasts were isolated, pure cultures were prepared and their technological properties were studied. Pure yeast cultures were obtained from grapes, must, fermenting must and young wines. The isolated strains were classified to *Saccharomyces*, *Kloeckera*, *Candida* and *Rhodotorula* genera, together with the unique *Lachancea* genus. Some of the strains isolated in this study can be potentially used for the production of typical (terroir) wines in the given wine-producing region or for the preparation of suitable starter cultures in wine production.

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