Received: 31 January 2019 | Accepted: 18 July 2019 | Published: 9 September 2019
DOI:10.20870/oeno-one.2019.53.3.2379

OENO One
VINE AND WINE OPEN ACCESS JOURNAL

Yeast diversity on grapes from Galicia, NW Spain: biogeographical patterns and the influence of the farming system

David Castrillo, Eva Rabuñal, Noemi Neira and Pilar Blanco*

Estación de Viticultura e Enoloxía de Galicia (EVEGA-AGACAL),
Ponte San Clodio S/N, 32428 Leiro, Ourense, Spain

*Corresponding author: pilar.blanco.camba@xunta.gal

ABSTRACT

Aim: Organic viticulture has increased in recent decades, worldwide. Farming system influences grapes' yeast populations and, therefore, fermentation dynamics and wine quality. This work aims to study yeast diversity on grapes and musts from organic and conventional production in different areas within Galicia (NW Spain) and to evaluate the existence of yeast geographic patterns (*microbial terroir*) in this region.
Methods and results: During the 2015 vintage, 42 grape samples were collected from four Denominations of Origin (DOs) in Galicia. Representative cultivable yeasts from grapes and must samples were isolated and characterised at the species level in the *Estación de Viticultura e Enoloxía de Galicia* (EVEGA-AGACAL). Results were compared among DOs and between organic and conventional production. A total of 33 yeast species were identified. Organic production showed27.3% higher species richness than conventional production. Accordingly, diversity indexes (H′ and E) were higher under organic cultures compared to conventional production. The highest yeast diversity was found in Rías Baixas and Ribeira Sacra DOs. The predominant yeasts were *Aureobasidium* spp., *Metschnikowia* spp., *Hanseniaspora uvarum* and *Cryptococcus* spp., although their proportion varied depending on the farming system and location. Furthermore, important differences were observed in minor species, which appeared mainly in organic cultivation. In addition, a biogeographic pattern was found: *Issatchenkia terricola* and *Starmerella bacillaris* were isolated in Rías Baixas and Ribeira Sacra DOs, whereas *Lachancea thermotolerans* was ligated to Monterrei and Ribeiro DOs. ANOSIM, PERMANOVA and PCA analysis confirmed this differentiation.
Conclusions: The results indicated that an organic farming system favoured yeast diversity on grapes and musts. Moreover, species distribution made it possible to establish a biogeographic pattern in the yeast population that could be associated to a particular *microbial terroir* and wine typicality.
Significance and impact of the study: This study reports for the first time a survey on yeast diversity on grapes and musts from organic and conventional production in different areas within Galicia. The results highlighted the role of organic culture as a tool to preserve yeast richness and the existence of biogeographic patterns of yeast communities in vineyards in NW Spain, which could contribute to wine typicality of specific areas.

KEYWORDS

organic vineyards, grapes and musts, yeast diversity, biogeographic patterns, *microbial terroir,* Galicia

Additional tables can be downloaded from https://oeno-one.eu/article/view/2379

 © 2019 International Viticulture and Enology Society - IVES

INTRODUCTION

Organic viticulture is increasingly popular around the world because organic practices are more respectful to the environment and health (Azabagaoglus *et al.,* 2007; Fragoulis *et al.,* 2009; Mariani and Vastola, 2015). Spain is the largest organic vineyard in the world with the greatest growth during the last decade (European Commission, 2016; Willer and Lernoud, 2017). The wine industry plays an important economic and social role in Galicia (NW Spain); however, certified organic production methods are applied in less than 0.5% of the total area of Galicia vineyard(78.2 ha; 36 operators, of which 16 are processors) (CRAEGA, 2017). A wide climatic and orographic heterogeneity in Galicia favours the proliferation of vine pathogens and results in irregular yields as well as problems with pest management (Fernández-González *et al.,* 2012; Fraga *et al.,* 2014), which leads to a difficult and expensive implementation of organic viticulture.

Yeast diversity associated with grape berries has been widely studied (for a review see Barata *et al.,* 2012). Diversity and frequency of yeast populations on grapes are influenced by several factors including agronomical practices, climatic conditions, anthropogenic and biotic factors, maturation process and grapes sanitary conditions (Comitini and Ciani, 2008; Cordero-Bueso *et al.,* 2011; Drumonde-Neves *et al.,* 2016 and 2017; Garofalo *et al.,* 2016; Grangeteau *et al.,* 2017; Nemcová *et al.,* 2015; Renouf *et al.,* 2005). In particular, the influence of the farming system on yeast diversity has been reported in vineyards from several countries. The results varied among different studies and they are sometimes contradictory. Whereas some authors found a wider yeast diversity in organic vineyards (Bagheri *et al.,* 2015; Cordero-Bueso *et al.,* 2011; Martins *et al.,* 2014; Setati *et al.,* 2012 and 2015; Tello *et al.,* 2012), others reported higher yeast abundance in conventional grapes (Grangeteau *et al.,* 2017; Milanović *et al.,* 2013), while Guzzon *et al.* (2015) found no significant differences.

The application of more natural and environmentally friendly techniques in wineries implies an increase in the use of spontaneous fermentations for organic wine elaboration. These practices are believed to contribute to creating wines with unique *terroir*-related characteristics. The concept of *terroir* involves a number of factors, including climate, soils, grape varieties,

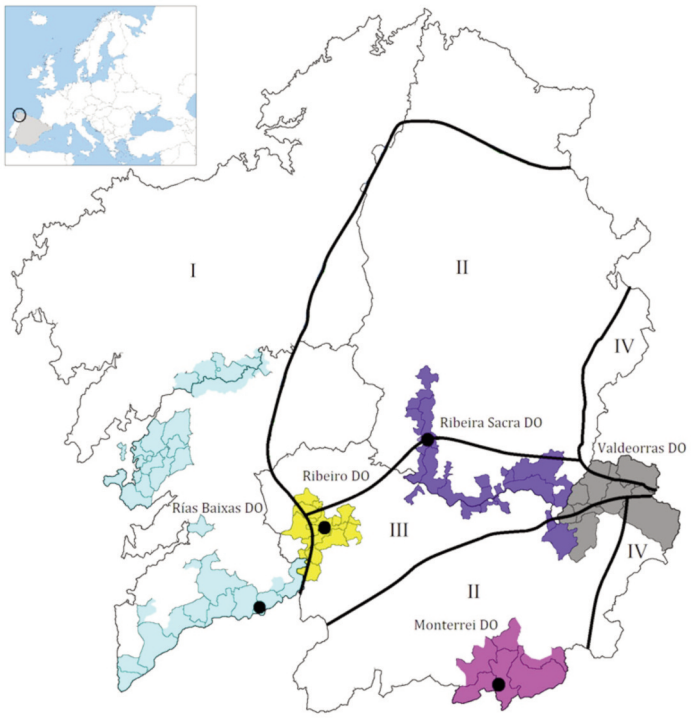


FIGURE 1. Denominations of Origin in Galicia and climatic zones: I, coastal oceanic climate; II, indoor oceanic climate; III, mediterranean oceanic climate; IV, mountain oceanic climate. Location of sampling sites.

 © 2019 International Viticulture and Enology Society - IVES

viticulture and oenological practices in a particular region. Furthermore, geographical differences found in vineyard yeast diversity have been associated with a *microbial terroir* responsible for distinct characteristics of wines from a given region (Belda *et al.,* 2017; Bokulich *et al.,* 2014 and 2016; Drumonde-Neves *et al.,* 2017; Nemcová *et al.,* 2015; Setati *et al.,* 2015). Therefore, the knowledge of yeast distribution patterns and the factors that influence their structure and diversity is of great importance because yeasts represent a valuable resource for the production of differentiated wines (Capozzi *et al.,* 2015; Tofalo *et al.,* 2013). In this sense, organic vineyards and wineries have been proposed as natural reservoirs of fermentative yeasts (Cordero-Bueso *et al.,* 2011; Tello *et al.,* 2012). Under these conditions, yeast biodiversity could be a competitive advantage in organic musts contributing to a more complex flavour and aroma in wines due to the role of non-*Saccharomyces* species involved (Capozzi *et al.,* 2015; Ciani and Comitini, 2015; Tofalo *et al.,* 2016).

Despite the importance of wine sector in Galicia, the information available about microorganisms associated with grapes and/or wineries is scarce. Data about yeast diversity during spontaneous fermentations evidenced differences between species during fermentations from the Atlantic and the interior regions, and also between vintages (Longo *et al.,* 1991). Apart from this, the influence of *Saccharomyces* and non-*Saccharomyces* yeast on the wine aroma profile has been reported (Lema *et al.,* 1996). Previous studies in our laboratory have been focused on strain diversity of *Saccharomyces cerevisiae* in an experimental winery (Blanco *et al.,* 2006). However, more research is required about yeast population associated with grapes from different areas of Galicia as well as the influence of the farming system on yeast diversity.

This study, carried out at *Estación de Viticultura e Enoloxía de Galicia* (EVEGA-AGACAL), reports for the first time a survey on yeast diversity in grapes and musts from organic versus conventional production in different areas within Galicia. It also verified the existence of biogeographic patterns of yeast populations in NW Spain vineyards.

MATERIALS AND METHODS

1. Origin of grapes and sampling design

Grapes were collected from vineyards within four Denominations of Origin (DO) from Galicia in 2015 before harvest (9 to 14 of September) (Figure 1). Representative grapevine varieties grown in Galicia were chosen: Albariño and Treixadura as white varieties and Brancellao and Mencía as red varieties. Table 1 shows the

TABLE 1. Location and characteristics of vineyards and grapevine varieties used in this study.

DO	Grapevine variety	Farming system*	Code	Coordinates	Elevation (m)	Rainfall** (mm)
Monterrei	Treixadura	Org	Mo-Trx	41°52'11.9"N,7°25'49.7" W	406	595
		Con		41°52'12.8"N, 7°25'54.5"W	402	
	Mencía	Org	Mo-Men	41°52'11.0"N, 7°25'51.9"W	404	
		Con		41°52'12.5"N, 7°25'56.9"W	399	
Ribeiro	Brancellao	Org	Ri-Bra	42º19'24.8" N, 8º6'7.8" W	270	752
		Con		42º19'22.3" N, 8º5'51,7" W	290	
	Treixadura	Org	Ri-Trx	42º19'27.6" N, 8º6'2.0" W	278	
		Con		42º19'22.0" N, 8º5'52.4" W	289	
Ribeira Sacra	Mencía	Org	RS-Men	42º34'11.5" N, 7º43'3.4" W	243	719
		Con		42º34'11.4" N, 7º43'3.5" W	244	
Rías Baixas	Albariño	Org	RB-Alb	42º5'47.9" N, 8º21'18.1" W	74	1071
		Con		42º5'46.8" N, 8º21'16.9" W	72	
	Treixadura	Org	RB-Trx	42º5'48.2" N, 8º21'15.1" W	75	
		Con		42º5'46.2" N, 8º21'17.7" W	71	

* Org, organic farming; Con, conventional farming. ** Average annual (2015) rainfall

© 2019 International Viticulture and Enology Society - IVES

location of vineyards, grapevine varieties sampled in each DO and their codes, plus the average annual rainfall and elevation for each vineyard. Date of grapes collection and maturation characteristics for each grapevine variety are summarised in Table S1.

For each variety, plots bordering or close to each other, but employing different farming systems, (organic and conventional) were selected. Organic producers used phytosanitary products according to legislation, which were mainly cooper, sulphur and plant extracts. In contrast, conventional producers applied these compounds and also synthetic fungicides to their vineyards. In every plot, three representative random blocks were established. Around 4 kg of grape bunches per variety were collected from at least 8 to 10 vines from each block.

2. Sample processing and yeast isolation

Grape samples were collected, transported to the laboratory in individual sterile plastic bags and processed within 2 h. A total of 42 samples were collected. To isolate yeast from the grapes, 20 healthy whole berries were aseptically separated from random bunches in the sample. Then the grapes were placed into sterile flasks with 100 mL of 2 % w/v buffered peptone water (Scharlau Microbiology, Barcelona, Spain) and incubated in an orbital shaker at 120 rpm for 2 h to dislodge yeast cells from the surface of the grape berries. In order to obtain must, the grapes were aseptically hand-destemmed and crushed. Both (grape washing solution and must) samples were diluted (10^{-1} to 10^{-5}) and 100 µL were homogeneously spread in duplicate on WL Nutrient Agar medium (Scharlau Microbiology) supplemented with 200 µg/mL biphenyl (Acros Organics by Thermo Fisher Scientific, Madrid, Spain) and 34 mg/L chloramphenicol (Merck KGaA, Darmstadt, Germany) to suppress moulds and bacteria, respectively. Plates were incubated at 28 °C until visible colonies appeared; those containing between 20 and 200 colonies were used for quantification of total viable cells. A representative number of yeasts from each sample were isolated in YEPD medium (1 % w/v yeast extract, 2% w/v peptone, 2 % w/v glucose, and 2 % w/v agar; all components were from Scharlau Microbiology) based on their colony aspect and frequency (10–20 for each sample). The isolates obtained were maintained at -80 °C in YEPD with 15 % (v/v) glycerol until further identification.

3. Molecular identification of yeast isolates

Yeast identification at species level was performed by PCR amplification of the 5.8S rRNA gene and the two internal (non-coding) ITS1 and ITS2 spacers using the ITS1 and ITS4 primers according to the method described by Esteve-Zarzoso *et al.* (1999).

PCR amplification and sequencing of D1/D2 region of 26S rDNA gene was used to confirm yeast identification. The D1 and D2 domains were amplified using NL-1 and NL-4 primers as described by Kurtzman and Robnett (1998). PCR products were purified using the PCR Extract Mini kit (5PRIME) according to the supplier's instructions (Fisher Scientific S.L., Madrid, Spain). The same primers were used for sequencing using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems by Thermo Fisher Scientific, Madrid, Spain) and a 3130xl Genetic Analyzer (Applied Biosystems). Sequences similarities were obtained using GenBank BLASTN search facilities (Altschul *et al.,* 1990). The identification was considered correct when the gene sequences identity was 98% or higher.

4. Statistical analysis of biodiversity

Yeast species diversity was estimated using different classical ecology indexes. The Shannon-Wiener index (H′) (Shannon, 2001) was calculated as follows:

$$H' = -\sum_{i=1}^{S} p_i ln(p_i)$$

where S is the species richness (number of species found) and $p_i = n_i/N$ is the relative abundance of species, calculated as the proportion of individuals (n) of species i with respect to the total of individuals (N).

Simpson's diversity index (1 – D) to evaluate yeast species dominance was calculated as follows (Simpson, 1949):

$$1 - D = 1 - \sum_{i=1}^{S} (p_i)^2 = 1 - \sum_{i=1}^{S} \left(\frac{n_i(n_i-1)}{N(N-1)}\right)$$

where D is the Simpson's index and S and p_i as described above.

Finally, in order to calculate the similarity or evenness of the different species abundance, a measure of Equitability (E) was calculated as follows (Pielou, 1969):

$$E = H/\ln(S) = H/H_{max}$$

 © 2019 International Viticulture and Enology Society - IVES

Differences in yeast populations between DOs and production systems were tested by one and two-way ANOSIM (non-parametric analysis of similarity) (Clarke, 1993) and PERMANOVA (permutational multivariate analysis of variance using distance matrices) (Anderson, 2001), using the PAST 3.20 (2018) software. In addition, the similarity percentages breakdown (SIMPER) between production systems/DOs was calculated to assess the average contribution of individual species to dissimilarity (Clarke, 1993). The Bray-Curtis similarity index with 9999 permutations was used in the tests, and in some cases, Bonferroni correction was also applied. Principal component analysis (PCA) was carried out by PAST 3.20, using the species frequencies to separate samples according to DOs and culture systems.

RESULTS

1. Influence of the farming system on yeast species richness

Representative yeasts were isolated from each sample and identified using genetic techniques. These isolates belonged to 33 different yeast species within 18 genera. Of those, 30 species were identified in musts and 24 on grapes. The results showed that total yeast richness (S) was higher on grapes from organic origin than conventional origin for all samples collected (Figure 2A). Musts followed the same trend, although two exceptions were found (samples from Treixadura in Monterrei and Ribeiro DOs) (Figure 2B). Considering the percentage of species with respect to the total (33 species), organic production exhibited 27.3% higher species richness than conventional production:

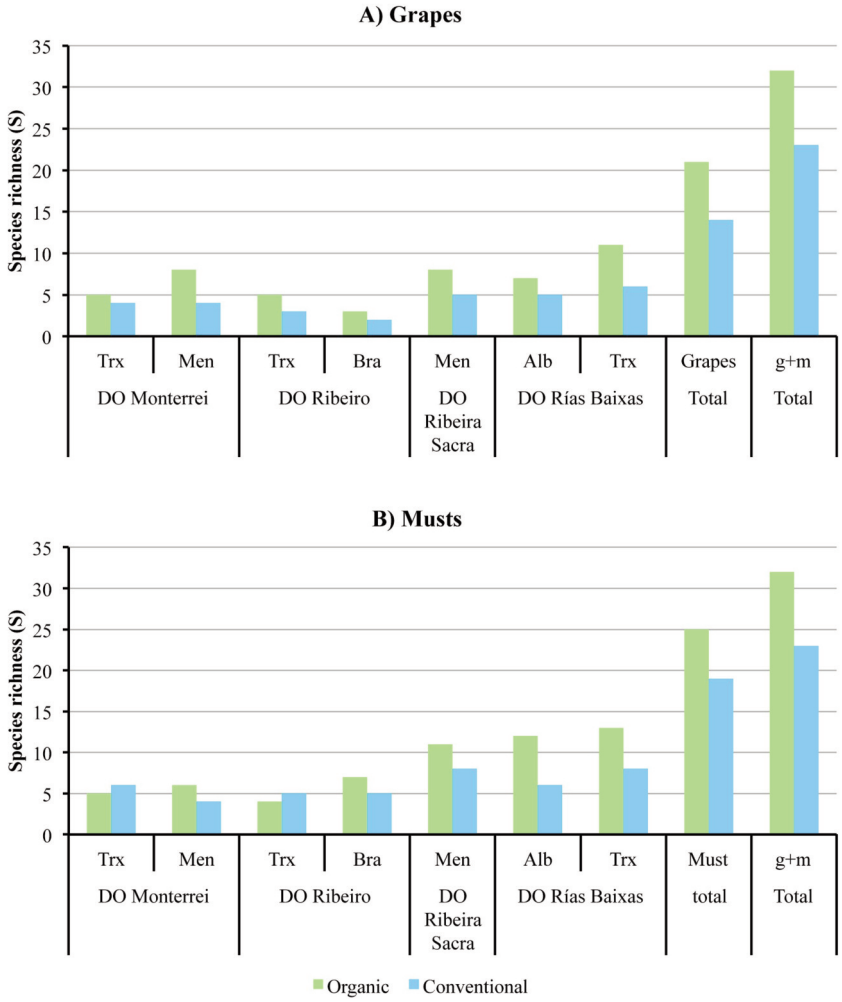


FIGURE 2. Yeast diversity, expressed as species richness S (number of species), found in organic and conventional samples from different DOs. A) grapes, B) musts. g+m = grapes + musts.

© 2019 International Viticulture and Enology Society - IVES

21.2% on grapes and 18.2% in musts. Within each group these percentages increased to 29.2% and 20.0 % for grapes and musts, respectively. Moreover, the results evidenced that the greatest yeast diversity was reached in musts from Rías Baixas DO under organic production; Ribeira Sacra DO also presented a high species richness (Figure 2B). In contrast, the lowest values of richness were observed in Ribeiro DO, especially with Brancellao grapes.

2. Frequency of yeast species under different farming system: geographical patterns

Frequencies of yeast species identified on grapes and in musts samples are summarised on Tables S2 and S3, respectively. *Metschnikowia* spp., *Hanseniaspora uvarum, Cryptococcus* spp., and yeast-like *Aureobasidium* spp. were the predominant species in all samples, although their frequency varied depending on the farming system and DO. Grape samples showed higher frequency of *Aureobasidium* and *Cryptococcus* genus compared to musts. In general, yeast-like *Aureobasidium* spp. reached higher frequencies in conventional grapes samples and *Cryptococcus* spp. in the organic samples, for instance, *Cryptococcus terrestris* in organic Brancellao (65.3%) (Figure 3). In contrast, an increase of fermentative yeast percentage in must samples was observed (Table S2 and S3, Figure 3).

Considering the total results for organic musts, the three predominant species *H. uvarum, Metschnikowia* spp. and *Aureobasidium* spp. showed a similar proportion (22.8%, 17.6% and

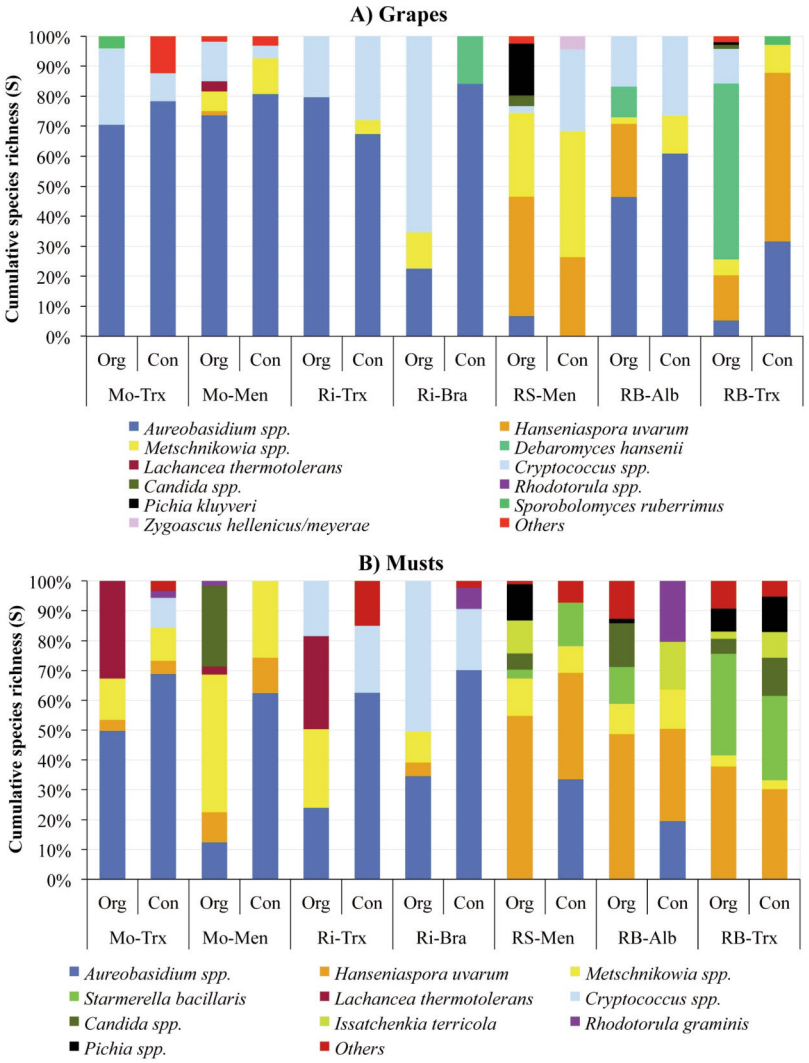


FIGURE 3. Cumulative yeast species richness (S) in organic and conventional samples from different Galician DOs (Mo, Monterrei DO; Ri, Ribeiro DO; RS, Ribeira Sacra DO; RB, Rías Baixas DO) and grapevine varieties (Trx, Treixadura; Men, Mencía; Bra, Brancellao; Alb, Albariño). A) grapes; B) musts.

 © 2019 International Viticulture and Enology Society - IVES

TABLE 2. Biodiversity indexes in organic (Org) and conventional (Con) grape and must samples from different Galician DOs and grapevine varieties.

DO-Grapevine variety	Farming system	Biodiversity indexes					
		H′	1 − D	E	H′	1 − D	E
		Grape samples			Must samples		
Mo-Trx	Org	0.85	0.46	0.62	1.11	0.62	0.80
	Con	0.67	0.36	0.61	1.07	0.50	0.60
Mo-Men	Org	1.10	0.48	0.53	1.36	0.69	0.76
	Con	0.67	0.33	0.48	0.90	0.53	0.82
Ri-Trx	Org	0.84	0.40	0.52	1.37	0.74	0.99
	Con	0.77	0.47	0.70	1.05	0.56	0.76
Ri-Bra	Org	0.87	0.51	0.79	1.50	0.74	0.83
	Con	0.44	0.27	0.63	0.98	0.48	0.61
RS-Men	Org	1.52	0.73	0.73	1.52	0.66	0.63
	Con	1.21	0.68	0.87	1.50	0.73	0.77
RB-Alb	Org	1.42	0.71	0.79	1.79	0.73	0.72
	Con	1.06	0.58	0.77	1.57	0.78	0.97
RB-Trx	Org	1.45	0.62	0.63	1.69	0.73	0.68
	Con	1.17	0.62	0.73	1.79	0.80	0.86
Total	**Org**	**1.96**	**0.77**	**0.65**	**2.32**	**0.87**	**0.74**
	Con	**1.53**	**0.64**	**0.61**	**1.93**	**0.75**	**0.67**

Values are the average of six data

17.2%, respectively). However, in conventional musts their proportion was different, with a higher incidence of *Aureobasidium* spp. (45.2%) (Table S3). *Metschnikowia* spp. and *H. uvarum* were also widely distributed in musts and, to a lesser extent, on grapes, in both culture systems. As an exception, grapes from Ribeira Sacra presented a high frequency of genus *Metschnikowia.*

In addition, the results evidenced a geographical pattern of yeast species, which allowed a clear differentiation between Monterrei-Ribeiro DOs and Ribeira Sacra-Rías Baixas DOs (Figure 3). Thus, yeast-like *Aureobasidium* spp. reached very high proportions in Monterrei and Ribeiro DOs, especially on grapes (Table S2; Figure 3A). In organic musts, the presence of *Aureobasidium* spp. was lower and increased the frequency of *L. thermotolerans*, which reached 32.6% and 31.3% in Monterrei and Ribeiro organic samples, respectively (Table S3; Figure 3B). Likewise, *Metschnikowia* spp. were found at high proportions in Monterrei must samples, whereas *Candida apicola* reached 27.1% in Mencía from this DO. The presence of *Metschnikowia* spp. was also important in Ribeiro DO (26.3%), together with *Cryptococcus* spp. in organic musts (Table S3).

In contrast, yeast-like *Aureobasidium* spp. was found at lower frequencies (even it was absent in several musts) in Ribeira Sacra and Rías Baixas DOs, except in Albariño grapes (Figure 3). *H. uvarum* was the predominant yeast species isolated in musts and grapes within these DOs (Tables S2 and S3; Figure 3), at proportions higher in organic than in conventional samples, except on grapes from Treixadura. A remarkable presence of *D. hansenii* (58.7%) was found in organic Treixadura grapes from Rías Baixas DO. *Candida* spp. and *Starm. bacillaris* (formerly *C. zemplinina*) reached a high frequency in musts, especially due to its presence in Treixadura (34.1%) and Albariño (12.4%) organic samples. This later one was important in conventional Treixadura musts from Rías Baixas DO (28.2%) and Mencía from Ribeira Sacra DO (14.5%) (Table S3). *Metschnikowia* spp. and *I. terricola* also appeared at frequencies higher than 10% in musts within these DOs. Regarding Ribeira Sacra, it is worth noting that the proportion of *Metschnikowia* spp. reached 42.1% and 28.0% in conventional and organic grapes, respectively. *Pichia kluyveri* was recorded in both grapes and musts, mainly in organic grapes (17.2%).

Finally, the farming system influenced the distribution of minor species, which were more abundant under organic production. Thus, *Candida bentonensis, Candida oleophila, Candida cf. sorbosivorans, Pichia sporocuriosa, Pichia membranifaciens* and *Zygosaccharomyces bailii* were isolated always in organic samples

 © 2019 International Viticulture and Enology Society - IVES

(Table S2 and S3). In contrast, species such as *Torulaspora delbrueckii* was isolated only in conventional musts. The presence of *Saccharomyces cerevisiae* was restricted to Mencía grapes from Monterrei DO (Table S2).

3. Yeast biodiversity indexes and statistics data analysis

The proportion of different species in both organic and conventional vineyards within each DO and for the total data in musts and grapes samples allowed the calculation of biodiversity indexes: Shannon-Wiener (H′), Simpson (1-D) and Equitability (E). The global results confirmed that organic production exhibited the highest diversity Shannon-Wiener index and Equitability in musts (H′ = 2.32 and E = 0.74) and grapes (H′ = 1.96 and E = 0.65) compared to conventional vineyards (H′ = 1.93 and E = 0.67 in musts, H′ = 1.53 and E = 0.61 and on grapes) (Table 2). Similarly, the Simpson's diversity index was higher in organic samples, i.e. the dominance was lower (D = 0.13 and D = 0.25 in organic and conventional musts, respectively; D = 0.23 and D = 0.36 in organic and conventional grapes, respectively). However, when particular DOs and/or grapevine varieties were considered, diversity indexes values were not always higher in organic samples. Although H' was higher in all cases, except in Treixadura must from Rías Baixas, E and (1 – D) were higher in some conventional musts and grapes. That was the case in Treixadura grapes from Ribeiro DO and in conventional musts from Rías Baixas and Ribeira Sacra DOs, which had presented the greatest yeast species richness.

Statistical analysis using ANOSIM and PERMANOVA proved the existence of differences in yeast population (species richness and frequency), especially between DOs and culture systems. Concerning species richness from the musts and grapes jointly analysed, global PERMANOVA showed significant differences among DOs (pPERMANOVA = 0.0480; F = 3.25); however, global ANOSIM results were not significant (p = 0.0652;

TABLE 3. Comparison of yeast population from different DO and production systems by analysis of similarity/distance measure (ANOSIM and PERMANOVA) in musts.

					ANOSIM			
	Mo-Org	Mo-Con	Ri-Org	Ri-Con	RS-Org	RS-Con	RB-Org	RB-Con
Mo-Org		0.2771	0.2925	0.5782	0.5641	0.2620	0.8301	0.5981
Mo-Con	*0.0140*		0.6165	0.6987	1	0.6355	1	0.8284
Ri-Org	*0.0084*	*0.0028*		0.8754	1	0.7445	0.9996	0.8573
Ri-Con	*0.0028*	*0.0028*	*0.0028*		1	0.7483	1	0.9463
RS-Org	*0.0028*	*0.0056*	*0.0028*	*0.0028*		0.5111	0.5278	0.1111
RS-Con	*0.6972*	*0.0084*	*0.0028*	*0.0028*	*0.0644*		0.4119	0.2503
RB-Org	*0.0028*	*0.0028*	*0.0028*	*0.0028*	*0.0476*	*0.0672*		0.3637
RB-Con	*0.0028*	*0.0028*	*0.0028*	*0.0028*	*1*	*0.9212*	*0.0028*	
					PERMANOVA			
	Mo-Org	Mo-Con	Ri-Org	Ri-Con	RS-Org	RS-Con	RB-Org	RB-Con
Mo-Org		5.759	2.476	9.696	6.832	2.772	12.2	6.956
Mo-Con	*0.0532*		15.64	10.39	58.51	9.095	74.34	35.63
Ri-Org	*1*	*0.0028*		21.77	18.43	5.941	26.87	14
Ri-Con	*0.0056*	*0.0028*	*0.0028*		70.65	12.71	86.21	42.4
RS-Org	*0.0056*	*0.0028*	*0.0056*	*0.0056*		3.529	6.041	4.731
RS-Con	*0.9044*	*0.154*	*0.0336*	*0.0728*	*0.574*		4.034	2.359
RB-Org	*0.0028*	*0.0028*	*0.0028*	*0.0028*	*0.1288*	*0.308*		4.826
RB-Con	*0.0028*	*0.0028*	*0.0028*	*0.0028*	*0.0728*	*1*	*0.1596*	

Statistical R- and F-values are shown above the diagonal and the level of significance (p-values) and the Bonferroni correction is shown below.

 © 2019 International Viticulture and Enology Society - IVES

R = 0.2295). The influence of culture system on global species richness was not significant (pPERMANOVA= 0.0522; pANOSIM= 0.0681). When the farming system and DO were grouped together, both tests presented more evident significant differences (pANOSIM = 0.0143, pPERMANOVA = 0.0061; R = 0.4554, F = 5.71). In addition, the highest dissimilarity (R = 1) was observed between Rías Baixas-Ribeira Sacra DOs and Monterrei-Ribeiro DOs binomials. In contrast, no significant differences in species richness were observed when the influence of the grapevine variety factor was determined ($p > 0.5942$ in both statistics).

Regarding the influence of DO, culture system and grapevine variety on species frequency in musts and grapes, significant differences ($p < 0.001$) were obtained in global ANOSIM and PERMANOVA tests for all factors and clusters. For example, in the cluster test for the two main factors (DO and culture system) significant differences ($p = 0.0001$) were found in both tests. ANOSIM and PERMANOVA values obtained for musts from different DOs and culture systems pairwise are summarised in Table 3. The results evidenced similarity (low R and F statistic values) between Rías Baixas and Ribeira Sacra DOs, but they were different from the other two DOs: Ribeiro and Monterrei (high R- and F-values). The highest F-values were obtained for organic samples of Rías Baixas (F = 74.34 and 86.21) and the Ribeira Sacra (F = 58.51 and 70.65) compared to the conventional Monterrei and Ribeiro DOs respectively. Furthermore, significant differences were found between the organic and conventional production systems for all DOs. In particular, the culture system strongly influenced yeast community in Ribeiro DO and, to a lesser extent, in Ribeira Sacra DO. Tests on musts for the DO-grapevine variety cluster also showed that the differences observed were due to DOs rather than to grapevine varieties. Tests on grapes shower lower differences in yeast population (Supplementary Table S4).

Moreover, the two-way ANOSIM and the two-way PERMANOVA were applied, which both allow the simultaneous study of two factors effects. The results showed significant differences in all the factors (DO, culture system and grapevine variety) for the frequency data (Table 4). Nevertheless, tests only revealed significant differences for DO factor (and only in the PERMANOVA test) when the species richness data were considered.

In addition, SIMPER analysis confirmed that the greatest contribution to differences between musts and grapes from organic and conventional cultivation was due to *Aureobasidium pullulans*, *H. uvarum* and *Metschnikowia* spp., previously described as the dominant species (Table 5). These species were the main contributors on grapes, but with higher percentage of *Cryptococcus* spp. and a remarkable presence of *D. hansenii* compared to musts. The first five species accounted for more than 70% of the cumulative percentage on grapes from both cultivation systems. In conventional samples, only *Aureobasidium* spp. represented half of the cumulative percentage of species, so diversity was lower than in organic samples. In contrast, the influence of *Starm. bacillaris, L. thermotolerans, Cr. terrestris* and *C. apicola* were important in musts. In this case, seven species were responsible for 70 % cumulative differences.

TABLE 4. Two-way ANOSIM and PERMANOVA for DO, culture system and grapevine variety factors in species frequency (%) and species richness (S).

	Factors	Two-way ANOSIM		Two-way PERMANOVA	
		R	*p*	*F*	*p*
Species frequency (%)	Variety	0.16428	0.0006	48.774	0.0001
	Farming system	0.51302	0.0001	86.021	0.0001
	DO	0.60001	0.0001	193.760	0.0001
	Farming system	0.37741	0.0001	169.410	0.0001
Species richness (S)	Variety	-0.2573	0.8716	0.2111	0.7069
	Farming system	1	0.5962	0.9762	0.1431
	DO	0.4074	0.0647	32.783	0.0174
	Farming system	0.7187	0.2301	48.473	0.0129

© 2019 International Viticulture and Enology Society - IVES

TABLE 5. Contribution of the predominant yeast species to diversity differences in organic and conventional grapes and musts (SIMPER analysis).

Species	Average dissimilarity	Contribution (%)	Cumulative contribution (%)	Mean organic	Mean conventional
			Grapes		
Aureobasidium pullulans	17.290	28.510	28.51	0.3910	0.5410
Hanseniaspora uvarum	8.399	13.850	42.36	0.1140	0.1140
Cryptococcus sp. (terrestris)	7.167	11.820	54.18	0.1160	0.0522
Metschnikowia spp.	5.654	9.324	63.50	0.0772	0.1110
Debaromyces hansenii	5.504	9.076	72.58	0.0979	0.0227
Cryptococcus carnescens	2.555	4.214	76.79	0.0511	0.0000
Cryptococcus stepposus	2.421	3.992	80.78	0.0133	0.0419
Aureobasidium proteae	2.326	3.836	84.62	0.0339	0.0285
Cryptococcus laurentii	1.991	3.283	87.90	0.0000	0.0398
Pichia Kluyveri	1.289	2.126	90.03	0.0258	0.0000
			Must		
Aureobasidium pullulans	16.950	24.620	24.62	0.1530	0.4220
Hanseniaspora uvarum	10.810	15.700	40.32	0.2280	0.1620
Metschnikowia spp.	6.595	9.582	49.91	0.1740	0.0884
Starmerella bacillaris	5.090	7.396	57.30	0.0707	0.0611
Lachancea thermotolerans	4.755	6.909	64.21	0.0951	0.0000
Cryptococcus terrestris	2.922	4.246	68.46	0.0484	0.0141
Candida apicola	2.528	3.674	72.13	0.0482	0.0069
Cryptococcus laurentii	2.251	3.271	75.40	0.0050	0.0429
Cryptococcus victoriae	2.248	3.266	78.67	0.0450	0.0000
Issatchenkia terricola	2.221	3.227	81.89	0.0191	0.0352

Finally, PCA of must samples using the frequency of main yeast species allowed the separation between DOs and farming systems. PC1 and PC2 explained 68% of the variance (Figure 4). PC1 separated Rías Baixas and Ribeira Sacra DOs samples (plotted on the negative side) from Ribeiro and Monterrei DOs samples (plotted on the positive side). PC2 separated organic samples (positive side) from conventional samples (negative side) in Ribeiro and Monterrei DOs, which agree with the distribution of the most relevant species as mentioned above within each DO. There was also a separation between organic and conventional production samples in the opposite areas of the PCA.

DISCUSSION

Organic viticulture is increasingly popular around the world; however, in Galicia its use as a farming system is not common. The existence of a great heterogeneity and adverse climatic and orography conditions seriously influence succession of this production system. The current study presents a comparison between yeast diversity in organic and conventional grapes/musts from four differentiated Galician areas.

The influence of farming systems (biodynamic, organic, conventional and/or integrated) on yeast species diversity have been approached in different winegrowing areas. In Galicia, our findings showed that yeast biodiversity tended to be higher in grapes and musts from organic production than in conventional production. These results agree with those reported for vineyards from Madrid, Spain, (Cordero-Bueso *et al.,* 2011; Tello *et al.,* 2012). In the same way, a higher yeast diversity has been described in biodynamic vineyards than in conventional and

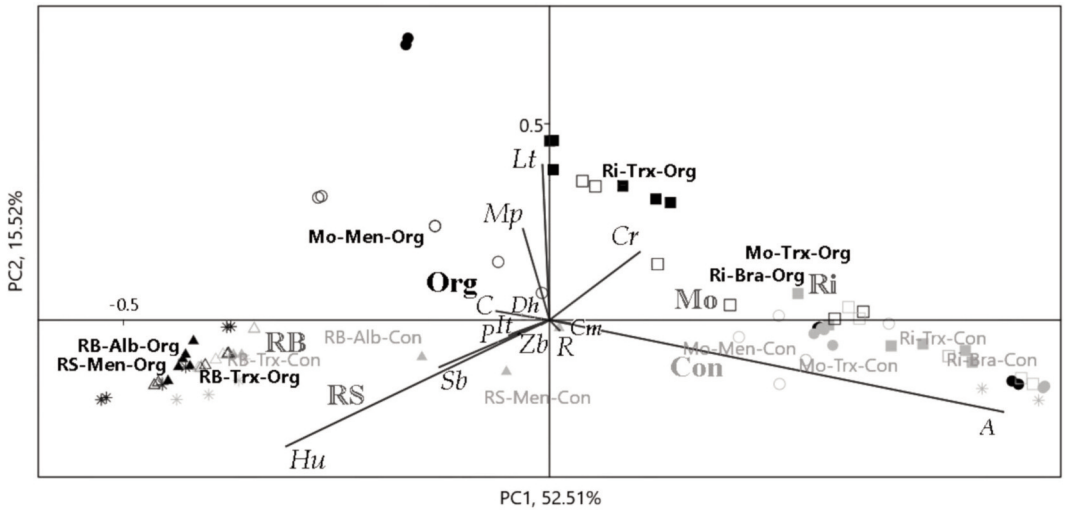


FIGURE 4. Principal component analysis (PCA) of organic (Org) and conventional (Con) musts based on main yeast species frequency.

Grey symbols, conventional; black symbols, organic; *, Ribeira Sacra DO; ○, Monterrei DO; □, Ribeiro DO; Δ, Rías Baixas DO. A, *Aureobasidium* spp.; C, *Candida* spp.; Cm, *Cy. macerans*; Cr, *Cryptococcus* spp.; Dh, *D. hansenii*; Hu, *H. uvarum*; It, *I. terricol*a; Lt, *L. thermotolerans*; Mp, *Metschnikowia* spp.; P, *Pichia* spp.; R, *Rhodotorula* spp.; Sb, *Starm. bacillaris*; Zb, *Zy. bailii*

integrated vineyards in South Africa (Bagheri *et al.,* 2015; Setati *et al.,* 2012 and 2015). With regard to studies performed in France, Martins *et al.* (2014) confirmed that diversity index (H′) were higher in organically farmed grapes. Conversely, Grangeteau *et al.* (2017) observed a lower diversity under organic modality, which could be explained by possible interactions between microorganisms, but also due to the use of higher doses of fungicides based on copper in organic vineyards. The same conclusions were reported by Milanović *et al.* (2013), who also found less yeast species diversity on grapes from organic Italian vineyards compared to conventional ones. In contrast, Comitini and Ciani (2008) demonstrated that conventional fungicide treatments applied on grapes during ripening also caused a drastic reduction in the population and diversity of yeasts. However, in some studies, no differences were found in microbial diversity between biodynamic and traditional vineyards, which appeared to be more related to grape variety than to agronomical practices (Guzzon *et al.,* 2015).

Yeast associated to grapes and musts has been widely reported (Barata *et al.,* 2012; Drumonde-Neves *et al.,* 2016; Grangeteau *et al.,* 2017). Yeast population on grapes was dominated by the ascomycetous yeast-like fungus *Aureobasidium* spp. and by basidiomycetous yeasts (for example, *Cryptococcus* spp., *Rhodotorula* spp. and *Sporobolomyces* spp.). These species have been described as normal microbiota associated to grapes (Barata *et al.,* 2012; Fleet, 2003; Grangeteau *et al.,* 2017; Martins *et al.,* 2014; Nemcová *et al.,* 2015; Prakitchaiwattana *et al.,* 2004; Setati *et al.,* 2012). All are oxidative yeasts which are technologically irrelevant because they are not detected for a long period during the alcoholic fermentation; so they do not have a negative effect on wine. These species were found as predominant yeasts in most samples in our study. Furthermore, the weakly fermentative yeasts *H. uvarum* and *Metschnikowia* spp. were also identified in certain grapes samples in a high proportion (between 1.4% and 42.1%). Both species have been associated with mature or damaged grapes (Drumonde-Neves *et al.,* 2017; Garofalo *et al.,* 2016; Guzzon *et al.,* 2014; Prakitchaiwattana *et al.,* 2004; Sipiczki, 2016). *H. uvarum* was especially abundant in Ribeira Sacra and Rías Baixas DOs, whereas *Metschnikowia* spp. appeared in samples from all DO, being especially high its frequency in Mencía grapes from Ribeira Sacra DO. A widely ranged distribution frequency in *H. uvarum* and *Metschnikowia* spp. has also been reported for grapes from Azores Archipielago (Drumonde-Neves *et al.,* 2017), Slovakia (Nemcová *et al.,* 2015) and France (Grangeteau *et al.,* 2017). These variations have been attributed to several factors, such as vintage, location, grape cultivar and phytosanitary treatments. In our study the differences are more related to location than grapevine variety and/or farming system,

 © 2019 International Viticulture and Enology Society - IVES

probably due to the particular climatic conditions in each DO and their influence on grape sanitary conditions.

Other minor oxidative and/or weakly fermentative species such as *C. apicola, C. oleophila, D. hansenii, P. kluyveri* and *L. thermotolerans* appeared, although not frequently, in some grapes samples, most of them from organic production. Their presence can be explained due to nutrient availability on the berry surface, which increased with ripening grapes (Barata *et al.,* 2012; Sipiczki, 2016). Some of these minor species as well as *Candida* spp., *Zygosaccharomyces* spp. and *I. terricola* were also widely identified, especially in the musts from Ribeira Sacra DO and Rías Baixas DO. *Candida* spp. and *Pichia* spp. are highly heterogeneous and ubiquitous film-forming yeasts regarded as common species on grapes and musts with the ability to produce off-flavours, but good manufacturing practices can prevent their activity (Barata *et al.,* 2012; Loureiro and Malfeito-Ferreira, 2003). The presence of *S. cerevisiae,* the major wine yeast in wineries and during fermentation, was restricted to scarce grape samples. Our results agree with the fact that *S. cerevisiae* appears with a lower frequency (less than 10–100 cfu/g of grapes), being rarely found in healthy berries (Fleet, 2003). These observations are the origin of a significant controversy about its origin in winemaking (Martini *et al.,* 1996; Mortimer and Polsinelli, 1999).

Regarding must samples, the yeast-like *Aureobasidium* spp. was also widely identified, although less frequently than on grapes; however, the frequency of fermentative yeasts such as *H. uvarum, Metschnikowia* spp, *Starm. bacillaris* or *L. thermotolerans* was higher than on grapes, and their distribution differed among DOs (Table S2, S3; Figure 3). In addition, other minor species were identified, mainly in organic samples. Grangeteau *et al.* (2017) had already described a decrease in *Aureobasidium* spp. in musts as well as the presence of some genera that had not appeared on grapes. *H. uvarum* showed high occurrence in musts and grapes from vineyards under different agronomic management (Bagheri *et al.,* 2015; Milanović *et al.,* 2013). These authors also detected *C. zemplinina* (*Starm. bacillaris*) associated to grapes and high-sugar musts. In our study, this species is related to Ribeira Sacra and Rías Baixas DOs samples, but not all had a high-sugar concentration (Table S1), and therefore the presence of *Starm. bacillaris* cannot be explained by this reason. Drumonde-Neves *et al.,* (2017) reported *H. uvarum* and *Metschnikowia* spp. as major species, but they also found *Pichia terricola* (synonym *I. terricola*) and *Starm. bacillaris* at high frequencies. In our study *I. terricola* was isolated exclusively in Rías Baixas and Ribeira Sacra DOs. However, an important incidence of *K. thermotolerans* (synonym *L. thermotolerans*) as well as *Candida stellata* (close to *Starm. bacillaris)* was found in the Madrid winegrowing area (Cordero-Bueso *et al.,* 2011; Tello *et al.,* 2012). These authors also suggested that phytosanitary treatment affects diversity; to be more specific, organic treatments favoured the presence of fermentative yeasts. Our results support the presence of yeast species with interesting fermentative properties in organic samples.

Concerning the influence of the farming system on the frequency of certain yeast species there are diverse, even contradictory, data available. Thus, yeast-like *Aureobasidium* was associated with organic samples by Martins *et al.* (2014), whereas Comitini and Ciani (2008) observed the opposite: a higher incidence of *A. pullulans* in conventional samples. Our results support a higher occurrence of *Aureobasidium* in conventional samples; however, the diversity and frequency of *Cryptococcus* spp. were wider under organic production, agreeing with Grangeteau *et al.* (2017). *Metschnikowia* and *Hanseniaspora* have been mainly associated with the conventional protection (Grangeteau *et al.,* 2017; Milanović *et al.,* 2013). However, these genera were isolated mainly in control (untreated) and organic plots by Comitini and Ciani (2008). The incidence of *Metschnikowia* spp. in Galicia was lower in organic grapes, but not in musts. *H. uvarum* appeared more frequently in organic musts from Ribeira Sacra and Rías Baixas. These differences in the incidence of certain species have been attributed to the impact of copper treatments in organic farming, but also to a possible interaction between species (Grangeteau *et al.,* 2017). In that sense, Setati *et al.* (2012) suggested that organic production involves an increase in richness species, including many yeasts with potential for biological control such as *H. uvarum*, *Metschnikowia* spp., *I. terricola* or *A. pullulans* (Guzzon *et al.,* 2014; Raspor *et al.,* 2010).

 © 2019 International Viticulture and Enology Society - IVES

The diversity indexes (H′, D, and E) obtained in this study for specific grape varieties and DOs were similar to those reported previously in grapes and musts (Cordero-Bueso *et al.,* 2011), whereas the global values were close to those found by Bagheri *et al.* (2015). Although in most cases, and at a global level, organic samples presented higher values of biodiversity indexes than their conventional counterparts, some exceptions were found. In all the exceptions, organic samples presented higher species richness, but the frequency of each species is an important factor for indexes calculation, especially for E′ and D.

Finally, statistical analysis of diversity data (species richness and species frequency) using ANOSIM and PERMANOVA showed significant differences among DOs and between culture systems; the highest values of R and F were obtained for DO factor. However, the low values of R and F in the one- and two-way tests indicated that grapevine variety does not have an important impact on yeast diversity. Therefore, these results confirmed that the factors that influenced diversity the most were the DO followed by the cultivation system and, to a lesser extent, the grapevine variety. Moreover, the results of two-way tests suggest that the species frequency found in each region had greater influence on yeast diversity than the species richness identified. In addition, PCA analysis (Figure 4) clearly separated samples of Rías Baixas and Ribeira Sacra DOs from those collected in Ribeiro and Monterrei DOs based on their species frequency. This differentiation is consistent with the values of similarity obtained by ANOSIM and PERMANOVA analysis (Table 3), and with the species patterns presented in figure 3. Differences in distribution of major yeasts and the association of certain species to specific DOs are responsible for these regional differences. Thus, our results suggest the existence of biogeographical patterns of yeast communities from different Galician DOs, although further studies including several vintages are required to confirm these findings. Recently, Bokulich *et al.* (2014) reported that grape microbial communities were closely related to the environmental conditions and the production area. Later studies evidenced an association between soil and grapes micro-organisms and environmental conditions (*microbial terroir*) that contribute to the regional characteristics of wines (Belda *et al.,* 2017, Bokulich *et al.,* 2016; Knight *et al.,* 2015; Mezzasalma *et al.,* 2018). The incidence of non-*Saccharomyces* fermentative yeast species, particularly in organic samples, showed that such yeasts are well adapted to these areas and could contribute to the complexity and distinctive characteristic of their wines.

CONCLUSION

The global results of this study confirmed that organic farming leads to an increase in the yeast diversity. The major species – *Metschnikowia* spp., *H. uvarum* and *Cryptococcus* spp. and yeast-like *Aureobasidium* spp. – were similar in almost all samples, although their proportions varied depending on the culture system and DO. The main differences in yeast biodiversity were attributed to a higher richness of minor species under organic production. In addition, certain species such as *L. thermotolerans* appeared in Ribeiro and Monterrei DOs whereas *Starm. bacillaris, Candida* spp. and *I. terricola* were associated to Rías Baixas and Ribeira Sacra DOs. These findings allowed the establishment of a geographical yeast species pattern (*microbial terroir*), which could influence wine typicality at regional level.

Acknowledgements : This study was supported by the RTA2012–00021-C03–01 grant of INIA (Instituto Nacional de Tecnología Agraria y Alimentaria) co-financed with FEDER funds (European Regional Development Fund). David Castrillo wishes to thank INIA and the European Social Fund for his FPI predoctoral contract. We would like to thank the producers: Esther Teixeiro Lemos, Roberto Regal, Cooperativa Cume do Avia, Víctor Diéguez, Begoña Troncoso, María del Carmen Álvarez, José Luis Mateo and José dos Santos Diz, for allowing us to collect samples from their farms.

REFERENCES

Altschul S.F., Gish W., Miller W., Myers E.W. and Lipman D.J, 1990. Basic local alignment search tool. *J. Mol. Biol.,* 215, 403–410. doi: 10.1016/s0022–2836(05)80360-2

Anderson M.J., 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecol.,* 26, 32–46. doi: 10.1111/j.1442-9993.2001.01070.pp.x

Azabagaoglus M.O., Akyol A. and Ozay A., 2007. The demand for organic wines and organic wine marketing. *J. Environm. Prot. Ecol*., 8, 171–178.

Bagheri B., Bauer F.F. and Setati M.E., 2015. The Diversity and Dynamics of Indigenous Yeast Communities in Grape Must from Vineyards Employing Different Agronomic Practices and their

 © 2019 International Viticulture and Enology Society - IVES

Influence on Wine Fermentation. *S. Afr. J. Enol. Vitic.,* 36, 243–251. doi: 10.21548/36-2-957

Barata A., Malfeito-Ferreira M. and Loureiro V., 2012. The microbial ecology of wine grape berries. *Int. J. Food Microbiol*., 153, 243–259. doi: 10.1016/j.ijfoodmicro.2011.11.025

Belda I., Zarraonaindia I., Perisin M., Palacios A. and Acedo A., 2017. From Vineyard Soil to Wine Fermentation: Microbiome Approximations to Explain the "*terroir*" Concept. *Front. Microbiol*., 8, 821. doi: 10.3389/fmicb.2017.00821

Blanco P., Ramilo A., Cerdeira M. and Orriols I., 2006. Genetic diversity of wine *Saccharomyces cerevisiae* strains in an experimental winery from Galicia (NW Spain). *Antonie Leeuwenhoek*., 89, 351–357. doi: 10.1007/s10482-005-9038-6

Bokulich N.A., Collins T.S., Masarweh C., Allen G., Heymann H., Ebeler S.E. and Mills D.A., 2016. These findings allowed the establishment of a geographical yeast species pattern (*microbial terroir*), which could influence on wine typicality at regional level. *mBio*, 7(3): e00631–16. doi:10.1128/mBio.00631-16.

Bokulich N.A., Thorngate J.H., Richardson P.M. and Mills D.A., 2014. Microbial biogeography of wine grapes is conditioned by cultivar, vintage, and climate. *Proc. Natl. Acad. Sci. U.S.A.,* 111, E139–E148. doi: 10.1073/pnas.1317377110

Capozzi V., Garofalo C., Chiriatti M.A., Grieco F. and Spano G., 2015. Microbial terroir and food innovation: The case of yeast biodiversity in wine. *Microbiol. Res*., 181, 75–83. doi: 10.1016/j.micres.2015.10.005

Ciani M. and Comitini F., 2015. Yeast interactions in multi-starter wine fermentation. *Curr. Opin. Food Sci*., 1, 1–6. doi: 10.1016/j.cofs.2014.07.001

Clarke K.R., 1993. Non-parametric multivariate analysis of changes in community structure. *Austral Ecol.,* 18,117–143. doi: 10.1111/j.1442-9993.1993.tb00438.x

Comitini F. and Ciani M., 2008. Influence of fungicide treatments on the occurrence of yeast flora associated with wine grapes. *Ann. Microbiol*., 58, 489–493. doi: 10.1007/bf03175547

Cordero-Bueso G., Arroyo T., Serrano A., Tello J., Aporta I., Vélez M.D. and Valero E., 2011. Influence of the farming system and vine variety on yeast communities associated with grape berries. *Int. J. Food Microbiol*., 145, 132–139. doi:10.1016/j.ijfoodmicro.2010.11.040

CRAEGA, 2017. Estadísticas. Memoria anual de actividades do ano 2015 do Consello Regulador da Agricultura Ecolóxica de Galicia. https://www.craega.es/

Drumonde-Neves J., Franco-Duarte R. Lima, T., Schuller D. and Pais C., 2016. Yeast Biodiversity in Vineyard Environments Is Increased by Human Intervention. *PLoS ONE,* 11 (8): e0160579. doi:10.1371/journal.pone.0160579

Drumonde-Neves J., Franco-Duarte R., Lima T., Schuller D. and Pais P., 2017. Association between Grape Yeast Communities and the Vineyard Ecosystems. *PLoS ONE*, 12 (1): e0169883. doi:10.1371/journal.pone.0169883

Esteve-Zarzoso B., Belloch C., Uruburu F.M. and Querol A., 1999. Identification of yeasts by RFLP analysis of the 5.8S rRNA gene and the two ribosomal internal transcribed spacers. *Int. J. Syst. Bacteriol*., 49, 329–337. doi: 10.1099/00207713-49-1-329

European Commission, 2016. Facts and figures on organic agriculture in the European Union. *Agric. Rural Dev. Eur. Comm*., 33. http://ec.europa.eu/agriculture/rica/pdf/Organic_2016_web_new.pdf

Fernández-González M., Rodríguez-Rajo F.J., Jato V., Aira M.J., Ribeiro H., Oliveira M. and Abreu I., 2012. Forecasting ARIMA models for atmospheric vineyard pathogens in Galicia and Northern Portugal: *Botrytis cinerea* spores. *Ann. Agric. Environm. Medicine.,* 19, 255–262. PMID: 22742797

Fleet G.H., 2003. Yeast interactions and wine flavour. *Int. J. Food Microbiol.,* 86, 11–22. doi: 10.1016/s0168-1605(03)00245-9

Fraga H., Malheiro A.C., Moutinho-Pereira J., Cardoso R.M., Soares P.M.M., Cancela J.J., Pinto J.G. and Santos J.A., 2014. Integrated Analysis of Climate, Soil, Topography and Vegetative Growth in Iberian Viticultural Regions. *PLoS ONE*, 9 (9): e0108078. doi:10.1371/journal.pone.0108078

Fragoulis G., Trevisan M., Di Guardo A., Sorce A., van der Meer M., Weibel F. and Capri E., 2009. Development of a management tool to indicate the environmental impact of organic viticulture. *J Environ Qual.,* 38, 826–835. doi:10.2134/jeq2008.0182

Garofalo C., Tristezza M., Grieco F., Spano G. and Capozzi V., 2016. From grape berries to wine: population dynamics of cultivable yeasts associated to "Nero di Troia" autochthonous grape cultivar. *World J. Microbiol. Biotechnol*., 32, 59. doi: 10.1007/s11274-016-2017-4

Grangeteau C., Roullier-Gall C., Rousseaux, S., Gougeon R.D., Schmitt-Kopplin, P., Alexandre H. and Guilloux-Benatier M., 2017. Wine microbiology is driven by vineyard and winery anthropogenic factors. *Microb. Biotechnol*., 10, 354–370. doi: 10.1111/1751-7915.12428

Guzzon R., Franciosi E. and Larcher R., 2014. A new resource from traditional wines: characterization of the microbiota of "Vino Santo" grapes as a biocontrol agent against *Botrytis cinerea*. *Eur. Food Res. Technol*., 239, 117–126. doi: 10.1007/s00217-014-2195-y

Guzzon R., Gugole S., Zanzoti R., Malacarne M., Larcher R., von Wallbrunn C. and Mescalchin E., 2015. Evaluation of the oenological suitability of grapes grown using biodynamic agriculture: the case

of a bad vintage. *J. Appl. Microbiol*., 120, 355–365. doi:10.1111/jam.13004

Knight S., Klaere S., Fedrizzi B. and Goddard M.R., 2015. Regional microbial signatures positively correlate with differential wine phenotypes: Evidence for a microbial aspect to *terroir*. *Sci. Rep*., 5, 14233. doi: 10.1038/srep14233

Kurtzman C.P. and Robnett C.J., 1998. Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. *Antonie Leeuwenhoek*, 73, 331–371. doi: 10.1023/A1001761008817

Lema C., García-Jares C., Orriols I. and Angulo L., 1996. Contribution of *Saccharomyces* and non-*Saccharomyces* populations to the production of some components of Albariño wine aroma. *Am. J. Enol. Vitic*., 47, 206–216.

Longo E., Cansado J., Agrelo D. and Villa T.G., 1991. Effect of climatic conditions on yeast diversity in grape musts from Northwest Spain. *Am. J. Enol. Vitic*., 42, 141–144.

Loureiro V. and Malfeito-Ferreira M., 2003. Spoilage yeast in the wine industry. *Int. J. Food Microbiol*., 86, 23–50. doi: 10.1016/S0168-1605(03)00246-0

Mariani A. and Vastola A., 2015. Sustainable winegrowing: Current perspectives. *Int. J. Wine Res.,* 7, 37–48. doi: 10.2147/ijwr.s68003

Martini A., Ciani M. and Scorzetti G, 1996. Direct enumeration and isolation of wine yeast from grape surfaces. *Am. J. Enol. Vitic*., 47, 435–440.

Martins G., Vallance J., Mercier A., Albertin W., Stamatopoulos P., Rey, P., Lonvaud A. and Masneuf-Pomarède I., 2014. Influence of the farming system on the epiphytic yeasts and yeast-like fungi colonizing grape berries during the ripening process. *Int. J. Food Microbiol.,* 177, 21–28. doi:10.1016/j.ijfoodmicro.2014.02.002

Mezzasalma V., Sandionigi A., Guzzetti L., Galimberti A., Grando M. S., Tardaguila J. and Labra M., 2018. Geographical and Cultivar Features Differentiate Grape Microbiota in Northern Italy and Spain Vineyards. *Front. Microbiol*., 9: 946. doi.org/10.3389/fmicb.2018.00946

Milanović V., Comitini F. and Ciani M., 2013. Grape berry yeast communities: influence of fungicide treatments. *Int. J. Food Microbiol*., 161, 240–246. doi:10.1016/j.ijfoodmicro.2012.12.019

Mortimer R.K. and Polsinelli M., 1999. On the origins of wine yeast. *Res. Microbiol.,* 150, 199–204. doi: 10.1016/s0923–2508(99)80036–9

Nemcová K., Breierová E., Vadkertiová R. and Molnárová J., 2015. The diversity of yeasts associated with grapes and musts of the Strekov winegrowing region, Slovakia. *Folia Microbiol.,* 60, 103–109. doi: 10.1007/s12223–014–0347-x

Pielou E.C., 1969. An Introduction to Mathematical Ecology. New York: Wiley. 169, Issue 3940, pp. 43–44. doi: 10.1126/science.169.3940.43-a

Prakitchaiwattana C.J., Fleet G.H. and Heard G.M., 2004. Application and evaluation of denaturing gradient gel electrophoresis to analyse the yeast ecology of wine grapes. *FEMS Yeast Res.,* 4, 865–877. doi: 10.1016/j.femsyr.2004.05.004

Raspor P., Miklič-Milek D., Avbelj M. and Čadež N., 2010. Biocontrol of grey mould desease on grape caused by *Botrytis cinerea* with autochthonous wine yeasts. *Food Technol. Biotechnol.,* 48, 336–343. Hrčak ID: 57565, URI: https://hrcak.srce.hr/57565

Renouf V., Claisse O. and Lonvaud-Funel A., 2005. Understanding the microbial ecosystem on the grape berry surface through numeration and identification of yeast and bacteria. *Aust. J. Grape Wine Res*., 11, 316–327. doi: 10.1111/j.1755-0238.2005.tb00031.x

Setati M.E., Jacobson D., Andong U-C. and Bauer F., 2012. The Vineyard Yeast Microbiome, a Mixed Model Microbial Map. *PLoS ONE*, 7 (12), e52609. doi:10.1371/journal.pone.0052609

Setati M.E., Jacobson D. and Bauer F.F., 2015. Sequence-based analysis of the *Vitis vinifera* L. cv Cabernet-Sauvignon grape must mycobiome in three South African vineyards employing distinct agronomic systems. *Front Microbiol*., 6, 1358. doi: 10.3389/fmicb.2015.01358

Shannon C.E., 2001. A Mathematical Theory of Communication. *Mob. Comput. Commun. Rev.,* 5, 3–55. doi: 10.1145/584091.584093

Simpson E.H., 1949. Measurement of diversity. *Nature*, 163, 688. doi: 10.1038/163688a0

Sipiczki M., 2016. Overwintering of vineyard yeasts: Survival of interacting yeast communities in grapes mummified on vines. *Front. Microbiol.,* 7, 212. doi: 10.3389/fmicb.2016.00212

Tello J., Cordero-Bueso G., Aporta I., Cabellos J.M. and Arroyo T., 2012. Genetic diversity in commercial wineries: effects of the farming system and vinification management on wine yeasts. *J. Appl. Microbiol.,* 112, 302–315. doi:10.1111/j.1365-2672.2011.05202.x

Tofalo R., Patrignani F., Lanciotti R., Perpetuini G., Schirone M., Di Gianvito P., Pizzoni D., Arfelli G. and Suzzi G., 2016. Aroma profile of Montepulciano d'Abruzzo wine fermented by single and co-culture starters of autochthonous *Saccharomyces* and non-*Saccharomyces* yeasts. *Front Microbiol*., 7, 610. doi: 10.3389/fmicb.2016.00610

Tofalo R., Perpetuini G., Schirone M., Fasoli G., Aguzzi I., Corsetti A. and Suzzi G., 2013. Biogeographical characterization of *Saccharomyces cerevisiae* wine yeast by molecular methods. *Front Microbiol*., 4, 166. doi: 10.3389/fmicb.2013.00166

Willer H. and Lernoud J., 2017. The World of Organic Agriculture. Statistics and Emerging Trends. FiBL & IFOAM – Organics International. Frick and Bonn. 78, 118–119. https://shop.fibl.org/CHen/mwdownloads/download/link/id/785/?ref=1

© 2019 International Viticulture and Enology Society - IVES