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Biodiversity of epiphytic yeasts on post-harvest table grapes in markets of Tabriz, Iran

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Abstract: Several reports are available on species diversity of yeasts on grape berries in different grapevine producing countries, including Iran. However, there is a paucity of knowledge on species diversity of yeasts on post-harvest table grapes worldwide. Hence, this study was performed to explore the species diversity of epiphytic yeasts on post-harvest table grapes in markets of Tabriz, northwest Iran. Towards this aim, 120 grape samples, mostly Keshmesh, Shahani, Gezeluzum and Shast-arous cultivars, were purchased from selected main markets in Tabriz and subjected to yeast isolation. Total number of 180 epiphytic yeast isolates were recovered. The isolates were preliminary grouped based on the morphological characteristics and DNA fingerprinting profiles using MSP-PCR fingerprinting technique. The D1/D2 domain of the 26S rDNA was amplified and sequenced for one or two isolates representing each fingerprinting group. Totally, 20 isolates were sequenced and the phylogeny inferred from sequence data of D1/D2 region revealed a rich diversity of yeast species on post-harvest table grape berries. Sixteen yeast species belonging to both ascomycetes and basidiomycetes were identified. The majority of identified yeast species (75%) belonged to ascomycetes. *Aureobasidium pullulans*, *Hanseniaspora uvarum* and *Metschnikowia sinensis* are reported as the most frequently isolated yeasts. In this study, *Clavispora lusitaniae* and *Cyberlindnera fabianii* are newly reported on grape berries worldwide and *C. lusitaniae*, *C. fabianii*, *Wickerhamomyces anomalus* and *Yamadazyma mexicana* represent new records for the mycobiota of Iran.

Key words: biodiversity, yeast, grape, D1/D2 domain

INTRODUCTION

Grapevine (*Vitis vinifera* L.) is one of the major horticultural crops worldwide, including in Iran, with a wide range of uses such as fresh and dried fruit, production of wine, jam, concentrate and seed oils (Reisch et al. 2012). This fruit is a primary source of microbial communities, including fungi and bacteria, playing important role in the yield and quality of the products and health of the plant itself (Martins et al. 2013). Several studies have been performed on biodiversity of microbial communities on grape berries including bacteria and yeasts (Barata et al. 2012b; Leveau & Tech 2010; Martins et al. 2013; Sabate et al. 2002; Setati et al. 2012). Yeasts play significant roles in our daily life ranging from the production of bread and fermented beverages to pharmaceuticals industries and biochemical synthesis (Demain et al. 1998). A number of yeast species possess clinically importance, causing disease in human, especially in immunosuppressed patients (Noverr et al. 2001), while some other species are known as plant pathogens (Schisler et al. 2011). The importance of yeast species as biological control agents of post-harvest, field and soil born plant pathogens, have been documented in several studies (Bleve et al. 2006; Calderone & Fonzi 2001; Ebrahimi et al. 2013; Klaasen et al. 2006; Nally et al. 2013). Hence, ongoing efforts are being made by biologists to further discover potential of yeast species as biological control agents of plant pests, especially for detoxification and reduction of toxins secreted in agricultural products by fungal plant pathogens (Wachowska et al. 2017).

Several studies have been conducted on species diversity of yeasts on grape berries worldwide (Combina et al. 2005; Mortimer & Polsinelli 1999; Nisiotou & Nychas 2007; Rosini et al. 1982). Rich

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diversity of yeast species has been reported on grape berries. In the majority of investigations ascomycetous yeasts such as *Hanseniaspora* spp. Zikes, *Candida* spp. Berkhout, *Metschnikowia* spp. Kamienski and *Pichia* spp. E.C. Hansen, basidiomycetous yeasts *viz.*, *Cryptococcus* spp. Vuill., *Rhodotorula* spp. F.C. Harrison, *Sporobolomyces* spp. Kluyver & C.B. Niel and the yeast-like fungus, *Aureobasidium pullulans* (de Bary) G. Arnaud, have been reported as common and predominant yeasts on grape berries (Barata et al. 2012b). Very recently, the biodiversity of epiphytic and endophytic yeasts on grape berries has been studied in Iran and twenty-three species were reported, with *Hanseniaspora*, *Candida*, *Metschnikowia* and *Pichia* as the most commonly isolated genera (Ghanbarzadeh et al. 2020). However, different biotic (e.g. grape variety and age) and abiotic (including climatic conditions, geographic location, degree of grape maturity and physical damage of the grapes) factors and agricultural practices such as nutrition, fungicide application and viticulture practices influence the distribution of yeasts on grapevines (Combina et al. 2005; Mortimer & Polsinelli 1999; Nisiotou & Nychas 2007; Rosini et al. 1982). For example, upon maturity of the berries, the yeast communities on grape surfaces increase and basidiomycetous yeasts are replaced by ascomycetous ones (Fleet 2003; Prakitchaiwattana et al. 2004; Rosini et al. 1982). Basidiomycetous yeasts, in general, are predominant in chilly climatic regions and late crop varieties and ascomycetous yeasts (especially *Hanseniaspora uvarum* (Niehaus) Shehata, Mrak & Phaff are common in grape varieties from mild climate regions (Yanagida et al. 1992). Branda et al. (2010) reported *Cryptococcus* and *Rhodotorula* as the most frequently isolated genera in the southernmost glacier of Europe. Ghanbarzadeh et al. (2020) reported the ascomycetous yeasts as dominant group (with 73 percent of isolation frequency) in vineyards of northwest Iran.

In the past, identification and classification of yeast species largely relied on conventional methods including diagnostics physiological and biochemical tests, such as assimilation of different carbon and nitrogen sources, fermentation, vitamin requirements, growth rate at various temperatures, hydrolysis of urea, and antibiotic resistance (Barnett et al 2000; Kurtzman and Fell 1998). However, traditional methods for identification of yeast species have proven difficult and not reliable in some cases, resulting in erroneous species identification. Nowadays, molecular methods have been developed for detection, identification and classification of different microorganisms including yeasts. Sequence data of the D1/D2 region of 26S rDNA and ITS1/ITS2 regions have been successfully used for identification of yeasts at the species level, enabling researchers for quick and accurate identification of yeast species, without the need for diagnostic physiological tests (Kurtzman 2014). In recent years the development of a reference library of DNA barcodes and the increasing availability of reference sequence data in GenBank have eased species identification in yeasts. Besides, traditional methods for yeast identification have largely been replaced by sequence-based methods (Kurtzman et al. 2011; Mokhtarnejad et al. 2016). However, physiological and biochemical tests remain as useful means for understanding of yeast autecology and functional characteristics (Kurtzman et al. 2011; Mokhtarnejad et al. 2016).

Given the importance of abiotic factors on biodiversity of yeasts on grape berries, this study was aimed to explore species diversity of yeasts on post-harvest table grape in markets of Tabriz by means of morphological and molecular data.

MATERIALS AND METHODS

Sampling

Healthy grape bunches were purchased from selective main markets located in Tabriz in September 2016, when grapes were completely ripe and sweet. The samples were transferred to the laboratory in clean plastic bags, kept at refrigerator at 4°C and analyzed until 48 hours. The main supplier of fresh table grape in Tabriz markets are different counties in East Azarbaijan province and neighboring provinces including West Azarbaijan (the Urmia region) and Ardabil (the Meshginshar region) provinces.

Yeast isolation

Epiphytic yeasts were isolated according to the protocol explained in Ghanbarzadeh et al. (2020); in brief, 15–20 grape berries of each bunch were put in a 250 ml Erlenmeyer flask together with 100 ml sterile distilled water. After shaking for 30 min (180 rpm), the solution was centrifuged for 10 min at 5000 xg. Water was removed and the remained sediment was re-suspended in 1 ml of Yeast Extract Peptone Dextrose (YEPD) medium (2% ᴅ-glucose, 2% bacto-peptone and 1% yeast extract (Nally et al. 2013). Sample dilutions of 1/10 to 1/1000 were prepared, spread each on YEPD agar medium and incubated at ±26°C for 3–4 days.

Purification and maintenance of yeast isolates

Isolates were streaked on YEPD agar medium to get single colonies. Single colonies were picked up and transferred to fresh culture medium. For long storage, the purified isolates were mixed with some glass beads (2 mm diameter) and glycerol 20% and maintained in 5 ml vials at -80°C. All of the isolates were deposited in to PYCC, Portuguese Yeast Culture Collection, at university of Nova de Lisboa, Faculty of science and technology, department of life science, Lisbon, Portugal.

Grouping yeast isolates based on morphological characteristics

For preliminary identification, the isolates were divided into groups based on morphological characteristics. Thus, some main characteristics of the colonies such as color: whether can be red, yellow, orange, white or from white through cream to tan, texture: whether can be mucoid, fluid or viscous, butyrous, friable, or membranous, size: whether the colonies are large, medium or tiny, surface: the surface of the colonies can be smooth or rough, sectored, folded, ridged, or hirsute, margin: which can be entire, undulating, lobed, erose, or fringed with hyphae or pseudohyphae, were examined on YEPD agar medium (Kurtzman et al. 2011).

DNA extraction and MSP-PCR Fingerprinting

Total genomic DNA was extracted following the protocol of Sampaio et al. (2001). The quality and quantity of DNA was checked by electrophoresis on 1% agarose gel and spectrophotometer, respectively. Then, Microsatellite/Minisatellite Primed (MSP)-PCR fingerprinting technique with M13 primer was used to analyze each morphological group for molecular identification and among the isolates with the same fingerprinting pattern, one or two isolates were selected for sequencing the D1/D2 region of 26S rDNA (Ramírez-Castrillón et al. 2014). For amplification, one µl portion of the diluted DNA sample (80 ng/ul) was used in a 24 µl PCR mixture containing 1X PCR buffer, dNTPs (10 mM), MgCl2 (2.5 mM), 10 µM of M13 primer and 1U of Taq polymerase. Amplification was performed in Biometra T Professional Basic PCR Thermocycler (Germany) as follows: 5 min at 96°C (denaturation), followed by 35 cycles of 30 s at 96°C, 1 min at 50°C and 2 min at 72°C and a final extension step of 7 min at 72°C. For negative control, DNA was replaced by sterile distilled water. Amplified DNA fragments were separated on 1.5% agarose gel and stained with Gel red 1X solution and visualized under UV light.

Molecular identification of the selected isolates

The D1/D2 domain of 26Sr DNA of the isolates were amplified using ITS5 and LR6 primers (Sampaio & Gonçalves 2008). PCR reactions were performed in a 50 µl volume containing 5µl portions of each diluted DNA samples (80 ng/µl), 10X PCR buffer, dNTPs (1.25 mM), $MgCl_2$ (2.5 mM), 10 µM of each primer and 1U of Taq polymerase. The program started at 95°C for 5 min followed by 34 cycles at 95°C for 30s, 54°C for 30s, and 72°C for 2 min, with final extension at 72°C for 7 min. The PCR products were examined by electrophoresis on a 1.2% (w/v) agarose gel stained with GelRed for visualization under UV light. The PCR products were purified and sequenced by STABVIDA institute (Oeiras, Portugal). All the sequenced data are registered in GenBank and accession numbers were obtained for each of them.

Finally, the obtained sequences were edited using the BioEdit v.5.0.6 software (Hall 1999) and then compared with those available in GenBank (National Center for Biotechnology Information, NCBI, USA), using the BLAST algorithm. The obtained sequences from GenBank, with high similarity, together with the new yeast sequences generated in this study, were aligned using the multiple sequence alignment online interface MAFFT (Katoh et al. 2005). The best evolutionary model was obtained using the software MrModelTest v.2.3. (Nylander 2004). An initial Bayesian inference (BI) analysis was performed with MrBayes v.3.2.1 (Ronquist & Huelsenbeck 2003) as explained in Arzanlou et al. (2015).

RESULTS AND DISCUSSION

A total of 180 epiphytic yeast isolates were isolated from 120 grape samples purchased from different markets in September, harvest time of grapes in northwestern Iran. The cultivar of most grape samples was Keshmesh, however other cultivars such as Shahani, Gezeluzum and Shast-arous were also purchased from the markets. Isolation of yeast was not successful for some of the grape samples while some others had plenty of different yeast colonies. Therefore, the percentage of isolation in general was calculated as 47.5%.

Yeast isolates showed a wide range of differences in morphological features including colony size, color, texture, surface and margin (Fig. 1). Based on the morphological characteristics, all isolates were divided into 13 different groups which most of the isolates were placed in WNMSEG, WNMSED and PNMSEG groups (Table 1). M13 fingerprinting pattern was obtained for all of the morphological groups. In each group, for the isolates with the same pattern, one or two (depending on the number of identical isolates) isolates were selected for sequencing (Table 1). The fingerprinting patterns for PNMSEG and WNMSED groups were very heterogenic as, after sequencing of the D1/D2 region, different yeast species were identified in WNMSED group such as *Cyberlindnera fabianii* (Wick.) Minter, *Clavispora lusitaniae* Rodr. Mir., *H. uvarum*, *Meyerozyma caribbica* (Vaughan-Mart., Kurtzman, S.A. Mey. & E.B. O'Neill) Kurtzman & M. Suzuki and *Torulaspora delbrueckii* (Lindner) Lindner (Fig. 2a). In contrast, all members of PNMSEG group, despite having different fingerprinting patterns, were identified as *Metschnikowia sinensis* M.L. Xue & L.Q. Zhang. As it is shown in figure 2b, the isolates of *M. sinensis* were classified in five different groups showing different fingerprinting patterns: 1) group one including 7, 8, 23A, 60, 71, 78, 86B isolates, 2) group two including 95B and 93P isolates, 3) group three including 33 and 48B isolates, 4) group four including 58B, 47B, 45B, 54 and 92B isolates and 5) group five including 103P and 108B isolates. Therefore, the fingerprinting patterns of *M. sinensis* isolates indicated that there should be significant genetic variation among the isolates of this species or *M. sinensis* is a complex of closely related species that could not be resolved using the D1/D2 sequence, solely. Additional gene sequence data supplemented with biochemical and physiological tests are required to prove this provisional. No genetic diversity was observed in the fingerprinting patterns of *H. uvarum* isolates (Fig. 2c). MSP-PCR fingerprinting method has been widely used for differentiation of yeast species, analyzing the diversity of yeasts and description of new yeast genus and species (Caruso et al. 2002; Ghanbarzadeh et al. 2020; Mokhtarnejad et al. 2016; Naumov & Naumova 2009; Suh et al. 2013).

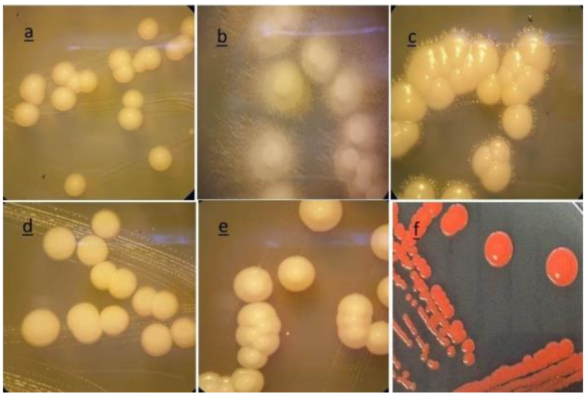

Fig. 1. Some of the morphological characteristics of the yeast colonies used for classification of the isolates into different morphological groups including a) WNMSEG, b) NMRFD, c) WNMSfG, d) WNMHED, e) WNMBEG and f) OVMSEG morphological groups. Key to abbreviations: W: white, P: pink, O: orange, C: brown-cream (colony color); N: mucoid, V: viscous (colony texture); L: large, M: medium, T: tiny (colony size based on visual scale); S: smooth, R: rough, L: liny=sectored, B: bulgy, H: hirsute (colony surface); E: entire, F: hyphae, f: pseudohyphae (colony edge); D: dull, G: glistening.

Phylogenetic analysis based on the sequence data of D1/D2 region of the isolates obtained in this study together with the sequence data from GenBank (Table 2) clustered our isolates with the representative type strains of known yeast species with high posterior probability (Fig. 3, 4). Phylogenetic analysis revealed a rich diversity among yeast isolates from post-harvest table grape in this study as sixteen species belonging to 14 genera could be identified. Both ascomycetous and basidiomycetous yeasts were isolated from different samples. However, the isolation frequency of ascomycetous yeasts was 75% (compared with 18.7% for basidiomycetous yeasts). *Naganishia adeliensis* (Scorzetti, I. Petrescu, Yarrow & Fell) Xin Zhan Liu, F.Y. Bai, M. Groenew. & Boekhout, *Naganishia albida* (Saito) Xin Zhan Liu, F.Y. Bai, M. Groenew. & Boekhout and *Rhodotorula mucilaginosa* (A. Jörg.) F.C. Harrison were the only basidiomycetous yeasts identified in this study.

Table 1. Morphological groups and the isolates in each group of the yeasts that were identified by sequencing of D1/D2 domain of the 26S rDNA.

Morphological Group*	**Isolate****	**PYCC*****	**Species**	**GenBank accession ımbers (D1/D2 domain of the 26S rDNA)**
WNMSEG	81B, 35-1, 23 (1°, 23P, 29-1, 24B, 32B, 46, 48P, 56, 58A, 63A, 83A, 89A, 93A, 94A, 104A, 108A)	PYCC 8661, PYCC 8659, PYCC 8658	*Hanseniaspora uvarum*	MT032424, MT032423, MT032422
WNMSED	105, (100A, 101P, 102P, 103A, 79A) 110, (102A, 103B, 106P) 92A, (92P) 17A, (3) 14B, (109A, 88A)	PYCC 8662, PYCC 8663, PYCC 8664, PYCC 8665, PYCC 8660	*Cyberlindnera fabianii* *Torulaspora delbrueckii* *Meyerozyma caribbica* *Clavispora lusitaniae* *Hanseniaspora uvarum*	MT032439, MT032427, MT032437, MT032430, MT032421
WNMSFD	84B	PYCC 8666	*Candida membranifaciens*	MT032425
WNMRFD	17B, (31)	PYCC 8667,	*Pichia kudriavzevi*	MT032440,
WNMBEG	90	PYCC 8668	*Yamadazyma mexicana*	MT032431
WNMRED	83B, (35B)	PYCC 8669	*Pichia kluyveri*	MT032426
WNMSfG	85, (84P, 88BA)	PYCC 8670	*Meyerozyma guilliermondii*	MT032429
WNMHED	107A, (104B, 41)	PYCC 8671	*Wickerhamomyces anomalus*	MT032428
PNMSfG	52B	PYCC 8672	*Naganishia adeliensis*	MT032432
OVMSEG	114A, (100B)	PYCC 8673	*Rhodotorula mucilaginosa*	MT032438
CVMSEG	114B, (106A)	PYCC 8674	*Naganishia albida*	MT032433
PNMSEG	33, 108B, 60 (2, 7, 8, 23A, 29-2, 33, 35-2, 45B, 47B, 48B, 54, 57B, 58B, 60, 63B, 71, 76A, 76B, 78, 79P, 86B, 88BB, 89B, 92B, 93B, 94B, 95B, 101B, 102B, 103P, 106B, 107B, 108B, 109B, 112)	PYCC 8675, PYCC 8676, PYCC 8677	*Metschnikowia sinensis*	MT032435, MT032436, MT032434
Morphologically identified	82, 1, 12, 13, 19, 45A, 48A, 52A, 66, 72A, 79B, 86A, 95A, 101A, 107P	-	*Aureobasidium pullulans*	-

*The isolates underlined have been sequenced and the others identified by comparing the fingerprinting patterns.
**Morphological characteristics are respectively as below:
W: white, P: pink, O: orange, C: brown-cream (colony color)
N: mucoid, V: viscous (colony texture)
L: large, M: medium, T: tiny (colony size based on visual scale)
S: smooth, R: rough, L: liny=sectored, B: bulgy, H: hirsute (colony surface)
E: entire, F: hyphae, f: pseudohyphae (colony edge)
D: dull, G: glistening
***PYCC: Portuguese Yeast Culture Collection

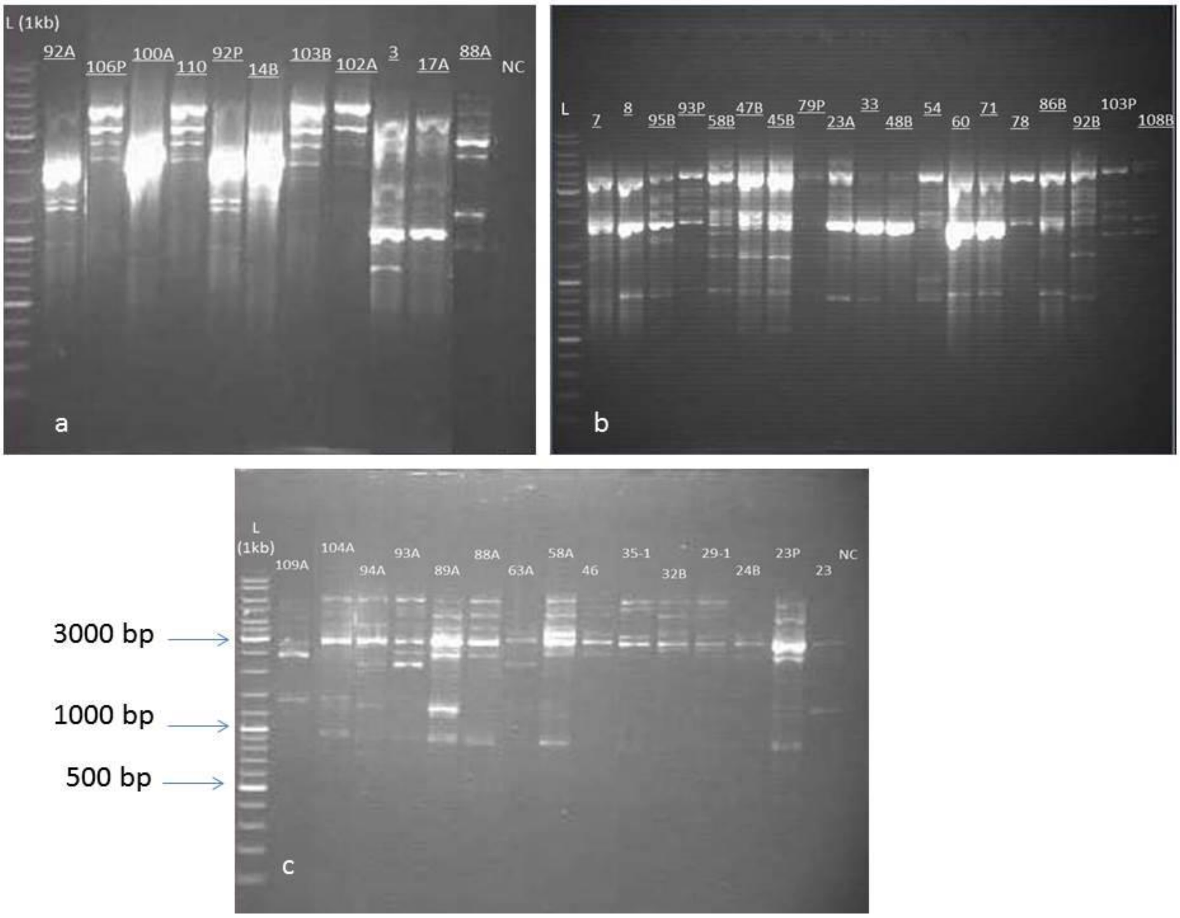

Fig. 2. a. M13 fingerprinting pattern for some isolates in the morphological group of WNMSED. Isolates with different patterns identified as *M. caribbica* (92A and 92P isolates), *T. delbrueckii* (106P, 110, 103B and 102A isolates), *C. fabianii* (100A and 14B isolates), *C. lusitaniae* (3 and 17A isolates) and *H. uvarum* (88A isolate), b. The representative isolates of *M. sinensis*, morphological group of PNMSEG, with different fingerprinting patterns, c. The representative isolates of *H. uvarum* with the same fingerprinting patterns. L: ladder (1kb, Thermo Scientific, USA) and NC: negative control.

Our results were in agreement with previous studies on species diversity of yeasts on mature and ripe grape berries (Combina et al. 2005; Prakitchaiwattana et al. 2004; Raspor et al. 2006). Like in our findings, ascomycetous yeasts have been reported as the dominant yeasts on grape berries at harvest time. In a recent study on diversity of yeast species on grape berries in Iran, Ghanbarzadeh et al. (2020) reported 15 ascomycetous yeast species, compared with five basidiomycetous species belonging to the genera *Filobasidium* L.S. Olive, *Naganishia* Goto, *Papiliotrema* J.P. Samp., M. Weiss & R. Bauer, *Rhodotorula* and *Trichosporon* Behrend. Raspor et al. (2006) reported species of the genera *Naganishia* (*Cryptococcus*), *Rhodotorula* and *Sporobolomyces* as basidiomycetous yeasts on grape berries at harvest time.

Grape maturity is one of the factors affecting the biodiversity of yeasts on grape berries. It has been shown that the mycobiota of immature grape berries is very similar to that of other plant substrates especially the leaves as basidiomycetous yeasts (such as *Cryptococcus*, *Rhodotorula* and *Sporobolomyces* and black yeast *A. pullulans*) are most dominant (Barata et al. 2012a; Fleet 2003). However, maturation causes weakness in the peel and resulting diffusion of juice on berry surface. The availability of rich sugary medium on the surface of grape berries would increase the growth of oxidative or low fermentative yeast populations such as *Candida*, *Hanseniaspora*, *Metschnikowia* and *Pichia* (Fleet 2003; Loureiro & Malfeito-Ferreira 2003; Sabate et al. 2002). In this study, grape samples were purchased at harvest time when they were completely ripe and as a result, the isolation of ascomycetous yeasts was higher than in basidiomycetous yeasts.

Among the identified species, *M. sinensis* and *H. uvarum*, with 34 and 23 representative isolates respectively, were the most dominant isolated yeast species. In various studies, *H. uvarum* and *M. pulcherrima* have been reported as the dominant species on grape berries (Combina et al. 2005; Nisiotou & Nychas 2007; Raspor et al. 2006), although *H. uvarum* has been only isolated in the last week before ripening (Rosini et al. 1982). All of the *Metschnikowia* isolates recovered in this study were identified as *M. sinensis* concordant with Kachalkin et al. 2015.

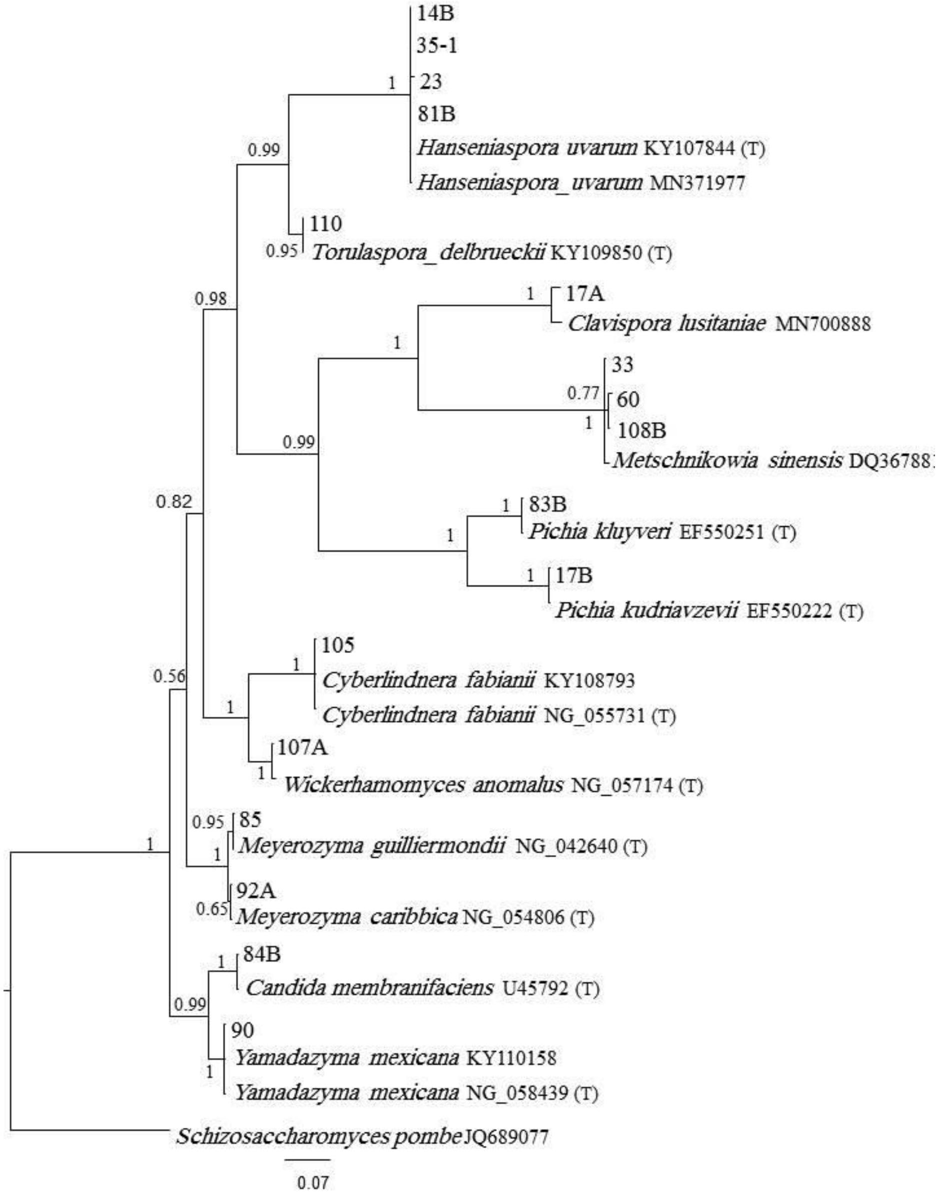

Fig. 3. Phylogenetic tree representing some isolates of post-harvest table grape ascomycetous yeasts and their closest related species (T means type strain). The tree was constructed by Bayesian analysis of D1/D2 sequence alignment using MrBayes v.3.2.1. The scale bar indicates 0.07 expected changes per site. The tree was rooted to *Schizosaccharomyces pombe* JQ689077.

In this study, the black yeast, *A. pullulans* was also determined as another dominant species on grape berries. It is the widespread saprophyte in phyllosphere of various plants and is considered as one of the biocontrol agents of post-harvest diseases specially gray rots of sweet cherries and table grapes, caused by *Botrytis cinerea* Pers. (Schena et al. 2003). In agreement with our results, *A. pullulans* has been reported as the main species isolated from mature and immature grapes and both damaged and undamaged grape berries (Prakitchaiwattana et al. 2004; Sabate et al. 2002).

The frequency of other identified species was very low and they were only isolated from one or two grape samples; however, six isolates of *C. fabianii* and four isolates of *T. delbrueckii* were obtained. *Saccharomyces cerevisiae* Meyen, the main fermentative species mainly found in various fermented beverages and wines, was not isolated in this study. Other studies also stated that *S. cerevisae* and other fermentative species of *Saccharomyces* Meyen ex Hansen are rarely isolated from healthy and undamaged berries (Pretorius 2000; Sabate et al. 2002).

In our previous study on grapes collected directly from selective vineyards in northwestern Iran, 23 yeast species were found on grape berries (Ghanbarzadeh et al. 2020) while in this study, 16 yeast species were identified on post-harvest grape berries. This can be attributed to differences in sampling method as well as different locality and date of sampling.

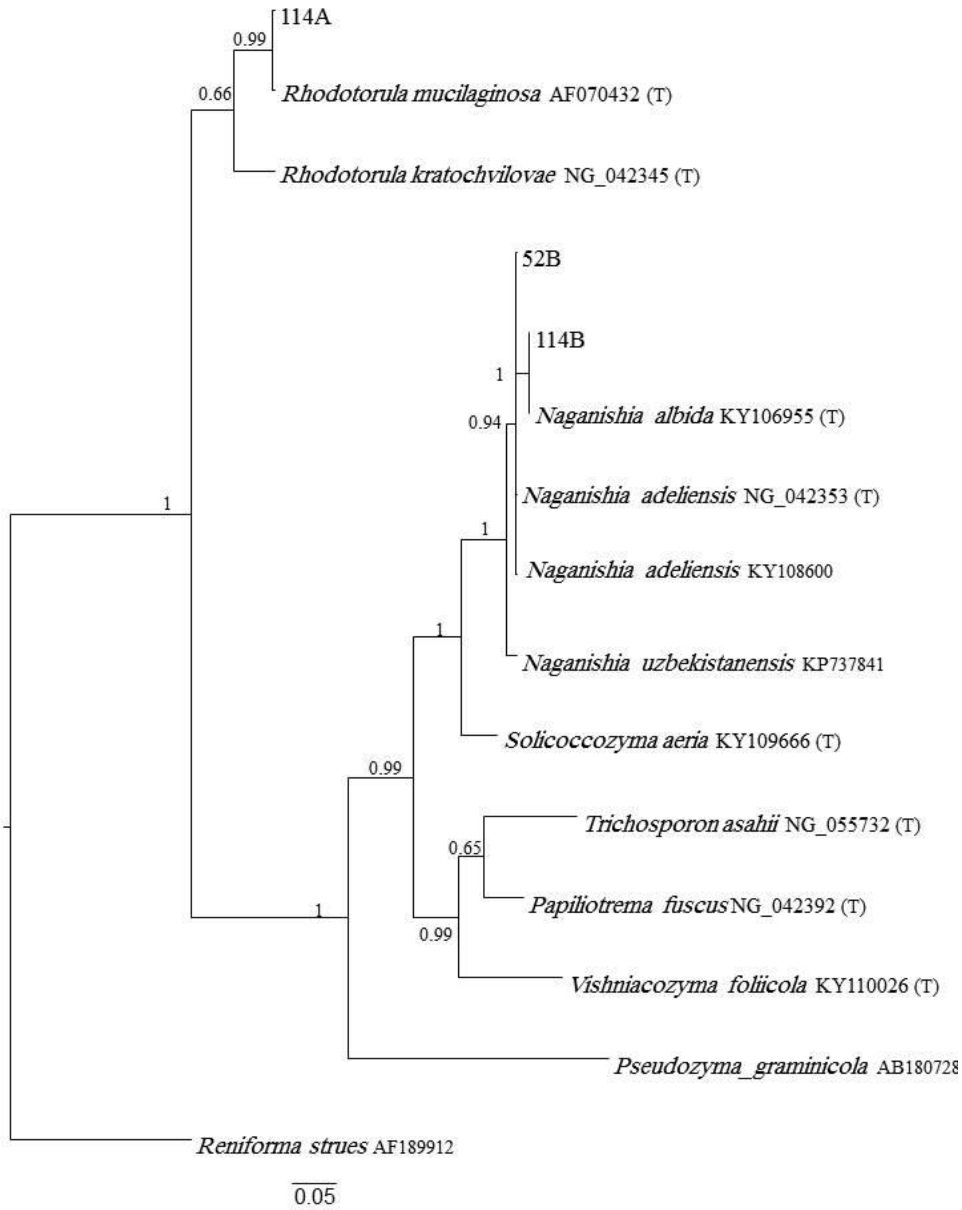

Fig. 4. Phylogenetic tree representing some isolates of post-harvest table grape basidiomycetous yeasts and their closest related species (T means type strain). The tree was constructed by Bayesian analysis of D1/D2 sequence alignment using MrBayes v.3.2.1. The scale bar indicates 0.05 expected changes per site. The tree was rooted to *Reniforma strues* AF189912.

However, some species were identified in both studies such as *A. pullulans*, *Candida membranifaciens* (Lodder & Kreger) Wick. & K.A. Burton, *H. uvarum*, *Meyerozyma guilliermondii* (Wick.) Kurtzman & M. Suzuki, *M. sinensis*, *Pichia kluyveri* Bedford, *Pichia kudriavzevii* Boidin, Pignal & Besson and *R. mucilaginosa*. The species that were identified as dominant species in this study were also identified in the previous study with the highest frequency compared to other species.

All of the species identified in this study, except *C. fabianii* and *C. lusitaniae*, have been previously reported on grape samples. *Cyberlindnera fabianii* is a ubiquitous yeast isolated from soil, water and different plant substrates (Mukisa et al. 2012). It has been reported from leaves of sugarcane (Limtong et al. 2014) and rice (Limtong & Kaewwichian 2015) and also from fermentation of sorghum and millet beverages (Mukisa et al. 2012). Therefore, its association with grape is not unlikely. This species was isolated from six different grape samples and this is the first report of *C. fabianii* as an inhabitant of grape in the world. Some strains of *C. lusitaniae* in GenBank have been reported to occur on grape (isolate number: XJ-72) and grape juice in Pakistan (isolate number: QG1), and kiwi in Iran (isolate number: MGK01), but there is not any officially published paper of these reports. Therefore, here we report this species as the mycobiota of grape berries, for the first time.

According to the yeast studies performed in Iran, *C. membranifaciens* and *M. guilliermondii* have been identified as the biocontrol agents of *Botrytis cinerea* on grape berries (Kasfi et al. 2018). Other studies conducted in Iran have been also reported the yeasts on Gum trees (*Eucalyptus* spp.) (Kamari et al. 2017), pigeon feces (Pakshir et al. 2019) and soil (Jamali et al. 2016; Mokhtarnejad et al. 2015; Mokhtarnejad et al. 2016). In this study, we report C. *fabianii*, *C. lusitaniae*, *W. anomalus* and *Y. mexicana* as new species for the mycobiota of Iran.

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شناسایی مخمرهای جداسازی شده از انگورهای تازه پس از برداشت در بازار کلان شهر تبریز

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چکیده: گزارشات متعددی از تنوع گونه‌ای مخمرهای انگور در کشورهای مختلف تولید کننده انگور و از جمله ایران وجود دارد. با این وجود، مطالعه جامعی در مورد تنوع گونه‌ای مخمرها در سطح انگورهای پس از برداشت وجود ندارد. بنابراین، این مطالعه جهت بررسی تنوع گونه‌ای مخمرهای اپیفیت انگورهای برداشت شده موجود در بازارهای تبریز، شمال‌غرب ایران، انجام شد. بدین منظور، ۱۲۰ نمونه انگور، بیشتر ارقام کشمش، شاهانی، قزل‌اوزوم و شصت عروس، از برخی میوه‌فروشی‌های اصلی تبریز خریداری شد و مخمرهای آن‌ها جداسازی شدند. در کل، ۱۸۰ جدایه مخمر اپیفیت جداسازی شد. این جدایه‌ها براساس خصوصیات ریخت‌شناختی و الگوهای انگشت‌نگاری DNA با روش MSP-PCR گروه‌بندی شدند. سپس از هر گروه، یک یا چند جدایه برای توالی‌یابی ناحیه D1/D2 از ژن 26S rDNA انتخاب شدند. در کل ۲۰ جدایه توالی‌یابی شدند و بررسی‌های فیلوژنتیکی بیانگر تنوع گونه‌ای قابل ملاحظه مخمرها در حبه‌های انگور برداشت شده بود. شانزده گونه مخمر متعلق به هر دو گروه آسکومیست و بازیدیومیست شناسایی شدند. اکثر گونه‌های مخمر شناسایی شده (۷۵٪) متعلق به آسکومیست‌ها بودند. گونه‌های *Aureobasidium pullulans*، *Hanseniaspora uvarum* و *Metschnikowia sinensis* به عنوان فراوان‌ترین مخمرهای جداسازی شده، بودند. در این مطالعه، گونه‌های مخمری *Cyberlindnera fabianii* و *Clavispora lusitaniae* برای اولین بار از روی انگور در دنیا و گونه‌های *C. fabianii*، *C. lusitaniae*، *Wickerhamomyces anomalus* و *Yamadazyma Mexicana* به عنوان آرایه‌های مخمری جدید برای مایکوبیوتای ایران گزارش می‌شوند.

کلمات کلیدی: تنوع زیستی، مخمر، انگور، ایران

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