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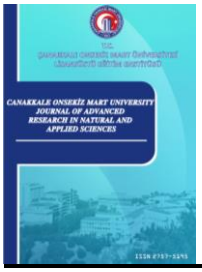

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Phylogenetic Analysis and Extracellular Enzyme Profiles of Yeast Strains Isolated from Raspberry Fruits

Tülay Turgut Genç[1*], Melih Günay[2]

[1]Department of Biology, Faculty of Arts and Science, Biology, Çanakkale Onsekiz Mart University, Çanakkale, Turkey
[2]Department of Biology, Graduate School of Natural and Applied Sciences, Çanakkale Onsekiz Mart University, Çanakkale, Turkey

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Research Article

Abstract – Raspberry fruit contains phenolic compounds, flavones, flavonoids, vitamins, and antioxidant substances that are important properties for health and pharmacological sciences. Edible berries provide also a suitable habitat for the growth of various microorganisms. In this study, yeast biota associated with raspberry fruits was determined by molecular identification techniques. Raspberry fruits were collected from Çanakkale, Gelibolu (Gallipoli). Yeast strains were isolated and then identified by using the analysis of ITS1-5.8S-ITS2 rDNA gene sequences. The phylogenetic analysis of all yeast strains was carried out by using the MEGA–X phylogenetic analysis tool. The extracellular enzyme profiles of identified yeast species were determined by the API-ZYM kit system. The distribution of yeast species on the raspberry fruits was determined as *Hanseniaspora uvarum*, *Metschnikowia viticola*, *Aureobasidium pullulans*, and *Metschnikowia pulcherrima*. It was observed that yeast strains belong to *Metschnikowia* genus were dominant on raspberry fruits. All yeast strains in *Metschnikowia* genus showed different enzyme profiles against seven extracellular enzymes. These enzymes may be the discriminatory enzymes for the yeast strains in the *Metschnikowia* genus. When the phylogenetic relationships among all yeast strains were investigated, all strains were divided into two main clades. While the first clade consists of only *Metschnikowia* genus, second clade includes *H. uvarum* and *A. pullulans* yeast species. Our results indicated that restriction patterns and also extracellular enzyme profiles could be utilized for differentiation of yeast strains within the genus. *M. pulcherrima*, *H. uvarum*, and *A. pullulans* can be used for industrial applications for future researches.

Keywords *– Extracellular enzymes, PCR-RFLP, Raspberry, Yeast biota*

1. Introduction

Raspberry fruit (*Rubus idaeus* L.) belongs to *Rosacea* family, naturally distributed in Canada, the USA, and Europe (Wang, Laamanen, Uosukainen, & Valkonen, 2005). Raspberry plants can grow about 1000 m altitude and humid regions throughout in northern line of Turkey (Göktaş, 2011). Raspberry fruits contain a great amount of valuable nutrients such as (poly)phenolic compounds, flavones, flavonoids, and vitamins. The phytochemicals and antioxidants contents of raspberry berries are important for diminishing biological oxidative stress in mammalian cells (Kähkönen et al., 1999; Halvorsen et al., 2001; Weber and Liu, 2002). Glycosylated phenol salidroside, one of the bioactive compounds of raspberry fruits, has therapeutic potential as it shows activity against the pathological processes of Huntington's disease (HD) (Kallscheuer et al., 2019). HD is a neurodegenerative disorder caused by the loss of striatal neurons due to the aggregation of huntingtin proteins encoded by the HTT gene (The Huntington's Disease Collaborative Research Group, 1993). Many studies were conducted antimicrobial potential of different parts of raspberry fruits (Puupponen-Pimia et al., 2005; Krisch, Galgóczy, Papp, & Vagvolgi, 2009; Riaz, Ahmad, & Rahman, 2011;

[1] tturgutgenc@comu.edu.tr
[2] melihgunay@comu.edu.tr
*Corresponding Author

Velićanski, Cvetković, & Markov, 2012). In addition, raspberry fruits are used for wine production, due to having different acids and anthocyanin pigment as well as fructose (Duarte et al., 2010).

Microbial enzymes are utilized in many fields such as biofuel production, animal feeds and, the medical industry. In the food industry, these enzymes are used in mainly dairy products, wine production, and bakery. Microbial enzymes are effective in enhancing the flavor and nutrient values of the products during the fermentation process (Wang, Li, Yang, Yang, & Zhu, 2012). Yeasts are a significant microorganism for determining food microbial quality and producing fermented products. Most of the microbial enzymes were obtained from different yeast species such as *Saccharomyces cerevisiae*, *Kluyveromyces lactis*, *Geotrichum candidum*, *Yarrowia lipolytica*, *Rhodotorula* spp., *Kluyveromyces marxianus*, *Komagataella pastoris*, *Debaryomyces hansenii*, *Cryptococcus* spp., *Cryptococcus laurentii*, *Sporobolomyces salmonicolor* (Johnson, 2013a, 2013b).

Microbial diversity on raspberry fruits was limited on molds and bacteria in different researches (Tournas & Katsoudas, 2005; Leff & Fierer, 2013). The yeasts associated with raspberry fruits have not been identified and characterized with molecular techniques yet. Therefore, in this research yeast strains were isolated from raspberry fruits collected from Gallipoli, Çanakkale-Province, and identified by using analysis of PCR-RFLP and ITS1-5.8S-ITS2 gene sequences. The phylogenetic analysis of all yeast strains was carried out by using the MEGA–X phylogenetic analysis tool. The extracellular enzyme profiles of yeast strains were determined with the API-ZYM kit system. *Hanseniaspora uvarum*, *Aureobasidium pullulans*, *Metschnikowia viticola* and *Metschnikowia pulcherrima* were identified on raspberry fruits. *Metschnikowia* genus was dominated more than half of yeast microbiota on raspberry fruit. All identified *H. uvarum* and *A. pullulans* yeast strains and nine of the isolated *M. pulcherrima* yeast strains were displayed a high level industrially important β-glucosidase activity.

2. Materials and Methods

2.1. Yeast Strains

Raspberry fruits were collected aseptically from Gelibolu-Turkey (40°51'50" North Latitude, 26°37'20" East longitude) taking into account of ripening period. Raspberry fruits were weighed and homogenized in 2% sodium citrate solution, were spread onto YGC-Agar medium (40 gr/l Yeast Extract Glucose Chloramphenicol Agar) including 0.1% sodium propionate. Plates were incubated at 30 °C for 2-3 days, the growing yeast colonies were counted to determine the colony forming units (CFU/gr). Yeast strains, having different colony morphology, were selected randomly and transferred to YEPD medium (10 gr/l Yeast Extract, 20 gr/l Bacto-peptone, 20 gr/l Agar, 2 % Dextrose). After incubation of plates at 30 °C for 2-3 days, the isolated yeast strains were stored at -80°C for further use.

2.2. PCR-RFLP Analysis

Genomic DNA extraction of yeast strains was carried out by a previously developed DNA extraction procedure (Sherman, Fink, & Hicks, 1986). ITS1-5.8S-ITS2 rDNA gene regions of all strains were amplified by using universal primers, ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'), for yeast strains and amplification was studied by previously reported conditions (White et al., 1990). PCR products were electrophoresed and the length of PCR amplicons was calculated by Gel-Pro Analyzer v4.0 software. PCR products of ITS1-5.8S-ITS2 rDNA gene regions were purified GeneJet PCR Purification Kit (Thermo Scientific – K0702) and were digested with Hae III, Hinf I, Msp I, Hha I, and Alu I restriction endonucleases, according to supplier's instructions. The length of restriction fragments was calculated by using Gel-Pro Analyzer v4.0 software The yeast strains were classified with respect to restriction patterns.

2.3. Sequencing and Phylogenetic Analysis

PCR products of all yeast strains were sequenced by utilizing the Applied Biotechnologies 3500xl Genetic Analyzer. The attained ITS1-5.8S-ITS2 rDNA gene sequences were analyzed using the BLAST (Basic Local Alignment Search Tool) online tool on NCBI (National Center for Biotechnology Information) webserver. All sequences of ITS1-5.8S-ITS2 region were uploaded to GenBank with the accession numbers MN556577-MN556603. ITS1-5.8S-ITS2 rDNA sequences of all yeast strains were studied by using MEGA-X (Molecular Evolutionary Genetics Analysis) software (Kumar et al., 2018). The nucleotide sequences of the ITS1-5.8S-ITS2 gene region of all yeast strains and two outgroups (*Saccharomyces cerevisiae* and *Pichia guilliermondii*) were aligned with ClustalW (v1.6) algorithm in MEGA-X. Maximum parsimony tree was constructed by using a bootstrap method and Subtree-Pruning-Regrafting (SPR) parameters for the determination of phylogenetic relationships of yeast strains. 1000 bootstrap replicates were used to defined branch support and bootstrap values above 50% were given.

2.4. Extracellular Enzyme Profiles

Extracellular enzyme profiles of identified yeast strains were determined by using the API-ZYM kit system (Bio-Mérieux, France). API-ZYM kit system is a minimized and semi-quantitative test system and utilized for screening 19 different enzyme activities (Alkaline phosphatase, Esterase (C 4), Esterase Lipase (C 8), Lipase (C 14), Leucine arylamidase, Valine arylamidase, Cysteine arylamidase, Trypsin, α-chymotrypsin, Acid phosphatase, Naphthol-AS-BI-phosphohydrolase, α-galactosidase, ß-galactosidase, ß-glucuronidase, α-glucosidase, ß-glucosidase, N-acetyl-ß-glucosaminidase, α-mannosidase, α-fucosidase). All yeast strains were grown in YEPD medium at 30 °C for 12 hours with constant shaking (120 rpm/rev). The 65 µL from the saturated yeast culture were transferred to each microwells of the API-ZYM strip. The API-ZYM strips were incubated at 37 °C for 4 hours. After that, ZYM A and ZYM B reagents were added to each cupule and all the strips were incubated at room temperature for 5 minutes. Enzyme profiles of yeast strains were defined by a color scalar of API-ZYM kit system (0-5 scalar).

3. Results and Discussion

3.1. Identification of Yeast Strains

In our study, the diversity of yeasts species on raspberry fruits has been investigated and the total yeast counts were calculated as $1.2X10^3$ CFU/gr. Depending on the colony morphology differences twenty-seven yeast strains were selected randomly for further identifications. Yeast strains were classified into five groups according to their colony morphology features such as colony color, colony margin, colony top, etc. (Table 1). The amplification of ITS1-5.8S-ITS2 rDNA gene region of yeast strains resulted in three PCR groups with the length of ~400bp (18 yeast strains) ~650bp (2 yeast strains) and ~800bp (7 yeast strains) (Table 2). When the yeast strains present in PCR groups and the colony morphology groups were compared, the yeast strains (R-3, R-6, R-23, and R-25) having different colony morphologies localized within the same PCR group.

Table 1
Grouping of yeast strains according to colony morphologies**.**

Group No	Yeast Strains
1	R-1, R-2, R-4, R-5, R-7, R-8, R-9, R-10, R-12a, R-13, R-14, R-15, R-24, R-26
2	R-3, R-25
3	R-6
4	R-11, R-12b, R-16, R-17, R-18, R-19, R-20, R-22, R-23
5	R-21

The analysis of the restriction profile of ITS1-5.8S-ITS2 gene region has been used to identify yeast strains isolated from natural sources and to determine strains discrepancy (Carvalho, Meirinho, Estevinho, & Choupina, 2010). Therefore, all yeast strains were cleaved with five restriction enzymes (Hinf I, Hae III, Msp I, Alu I, and Hha I) and were grouped again for the PCR-RFLP analysis (Table 2). The yeast strains in the first PCR group displayed two different restriction profiles. The PCR products of all yeast strains in the first group were not digested with Alu I, and three yeast strains (R-3, R-6, and R-25) were not digested with Msp I restriction enzyme. The yeast strains present in the second PCR groups showed similar restriction patterns with the group members. By means of PCR-RFLP analysis, four different restriction profiles were attained. We assumed that each restriction profile may represent to different yeast strains.

As indicated in previous reports, the colony morphology and/or PCR size alone is not sufficient for distinguishing yeast strains and, the RFLP analysis was essential for discrimination of yeast strains. The PCR-RFLP results of ITS1-5.8S-ITS2 gene region are similar to previous studies which are especially used Hae III, Hinf I and Hha I (Cfo I) restriction endonucleases (Guillamon, Sabate, Barrio, Cano, & Querol, 1998; Rodriguez-Vico, Clemente-Jimenez, Mingorance-Cazorla, Martinez-Rodriguez, & Las Heras-Vazquez, 2003; Romano, Capece, Siesto, & Romaniello, 2009; Gibson et al., 2011). The Hae III, Hinf I, and Hha I restriction pattern of second group members was similar to *M. pulcherrima* yeast species recorded in previous studies (Esteve-Zarzoso, Belloch, Uruburu, & Querol, 1999; Rodriguez-Vico et al., 2003).

Table 2
PCR-RFLP results of yeast strains

PCR Length (~bp)	Restriction Profiles	Yeast Strains	Restriction Product Length (~bp)				
			Hae III	Hinf I	Msp I	Alu I	Hha I
~400	1	R-3, R-6, R-25	289-120	215-186	-	-	217-99-90
	2	R-1, R-2, R-4, R-5, R-7, R-8, R-9, R-10, R-12a, R-13, R-14, R-15, R-23, R-24, R-26	286-109	192-188	224-120-60	-	208-96-90
~650	3	R-20, R-22	441-150	278-168-129	270-184-141	387-207	183-179-108-94
~800	4	R–11, R–12/b, R–16, R–17, R-18, R–19, R–21	750	353-184-164	743	353-184-164	331-324

In this research, the ITS1-5.8S-ITS2 rDNA gene region of all yeast strains was sequenced. The obtained sequences were analyzed by the BLAST tool on the NCBI web server. The nucleotide sequences of the ITS1-5.8S-ITS2 rDNA gene region were submitted to the GenBank Database on NCBI and attained acces-

sion number for all sequences (Accession Number: MN556577-MN55603) (Table 3). According to the BLAST analysis of ITS1-5.8S-ITS2 rDNA gene region, all yeast strains displayed 95-100% similarity with their reference yeast strains except the R-4 yeast strain. It was determined that all yeast strains in the first PCR group (~400 bp) were the member of *Metschnikowia* genus. Three yeast strains (R-3, R-6, and R-25) showed 98-99% sequence similarity with *M. viticola* reference yeast strain (KY104213.1). *M. viticola* was firstly isolated from wild grapes (Peter, Tornai-Lehoczki, Suzuki, & Dlauchy, 2005). In our research, *M. viticola* yeast species was recorded first time on raspberry fruits. Also, RFLP profile of *M. viticola* was displayed for the first time in our research. Restriction digestion with Msp I can be used for distinguishing the *M. viticola* species from *M. pulcherrima* yeast species.

All other yeast strains in the first PCR group showed nearly 95% similarity with *M. pulcherrima* reference yeast strain (NR_164379.1) except R-4 (91% similarity). The taxonomic identification of *M. pulcherrima* yeast strains by comparing their rDNA sequences with those deposited in databases can easily lead to false results. Because of the sequences of *M. pulcherrima* yeast strains were deposited in the database without an expert taxonomic verification. Because of the non-homogenized rDNA repeats and the frequent heterozygosity of barcode genes, the type strains of the species of the *M. pulcherrima* clade are not separated by clear barcode gaps (reviewed in Sipiczki, 2020). The colony morphologies, pigment secretion pattern, and RFLP profile of all identified *M. pulcherrima* yeast species were similar in our research. However, the yeast species defined as *M. pulcherrima* should be analyzed taxonomically, as indicated in Sipiczki's review.

Table 3
Blast analysis of ITS1-5.8S-ITS2 rDNA gene region.

Yeast Strains	Similarity (%)	Identified Yeast Strains (Ref. Acc. Number)	GenBank Accession Number
R-1	97.92%	*M. pulcherrima* (NR_164379.1)	MN556577
R-2	97.33%	*M. pulcherrima* (NR_164379.1)	MN556578
R-3	98.75%	*M. viticola* (NR_077083.1)	MN556579
R-4	91.20%	*M. pulcherrima (*NR_164379.1)	MN556580
R-5	97.80%	*M. pulcherrima* (NR_164379.1)	MN556581
R-6	97.11%	*M. viticola* (KY104213.1)	MN556582
R-7	97.31%	*M. pulcherrima* (NR_164379.1)	MN556583
R-8	97.04%	*M. pulcherrima* (NR_164379.1)	MN556584
R-9	95.54%	*M. pulcherrima* (NR_164379.1)	MN556585
R-10	95.25%	*M. pulcherrima* (NR_164379.1)	MN556586
R-11	99.02%	*H. uvarum* (KY103569.1)	MN556587
R-12a	96.47%	*M. pulcherrima* (NR_164379.1)	MN556588
R-12b	98.88%	*H. uvarum* (KY103569.1)	MN556589
R-13	97.33%	*M. pulcherrima* (NR_164379.1)	MN556590
R-14	96.75%	*M. pulcherrima* (NR_164379.1)	MN556591
R-15	96.75%	*M. pulcherrima* (NR_164379.1)	MN556592
R-16	100.00%	*H. uvarum* (KY1035671.1)	MN556593
R-17	99.25%	*H. uvarum* (KY1035671.1)	MN556594
R-18	100.00%	*H. uvarum* (KY1035671.1)	MN556595
R-19	100.00%	*H. uvarum* (KY1035671.1)	MN556596
R-20	99.42%	*A. pullulans* (MG890282.1)	MN556597
R-21	95.68%	*H. uvarum* (KY103569.1)	MN556598
R-22	98.90%	*A. pullulans* (KY552634.1)	MN556599
R-23	96.44%	*M. pulcherrima* (JN229413.1)	MN556600

R-24	96.78%	*M. pulcherrima* (NR_164379.1)	MN556601
R-25	98.56%	*M. viticola* (NR_077083.1)	MN556602
R-26	94.97%	*M. pulcherrima* (NR_164379.1)	MN556603

R-20 and R-22 yeast strains present in the second PCR group showed 98-99% sequence similarity with *A. pullulans* yeast species. There is no reference type strain for *A. pullulans* in the GenBank Database on NCBI. Therefore, the highest percent sequence similarity results were given in Table 3 for *A. pullulans* yeast species. Seven yeast strains (R–11, R–12b, R–16, R–17, R-18, R–19, and R–21) in the third PCR group displayed 96-99% sequence similarity with *H. uvarum* reference yeast species (KY103569.1). These results revealed that the restriction endonucleases utilized for RFLP analysis were suitable for the discrimination of yeast strains from each other. The percent distribution of yeast species on the raspberry fruits was determined as 55.6% *M. pulcherrima*, 25.9% *H. uvarum*, 11.1% *M. viticola*, and 7.4% *A. pullulans* (Figure 1). It is observed that yeast strains belong to the *Metschnikowia* genus were dominant on raspberry fruits collected from Gelibolu.

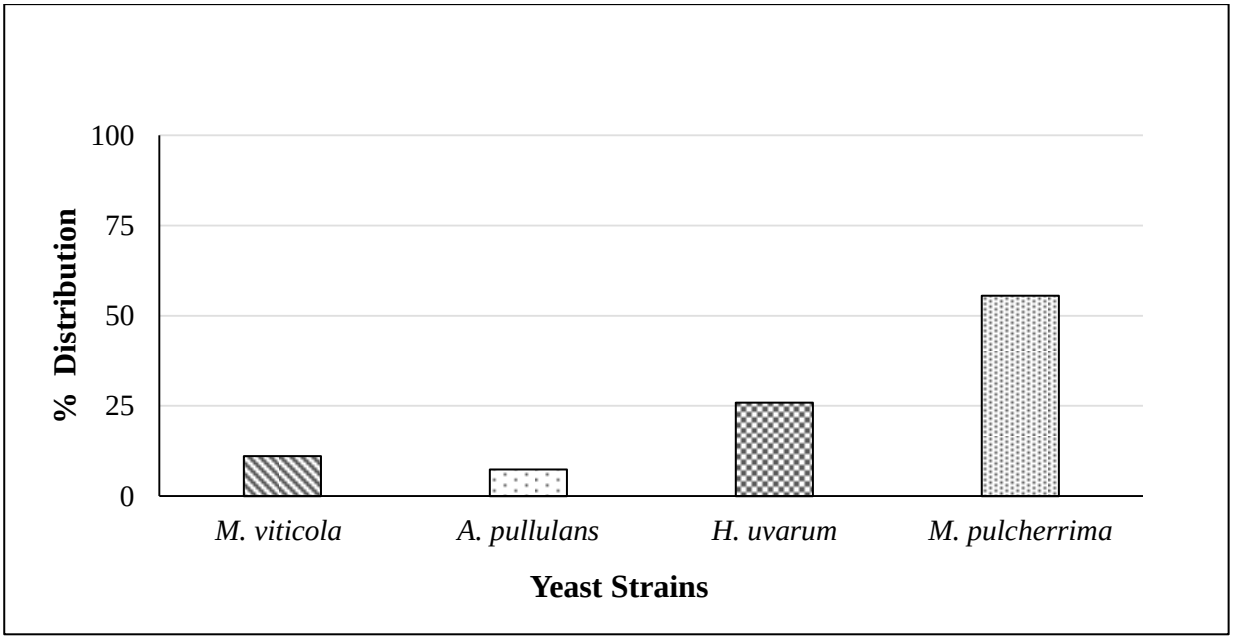

Figure 1. The percent distribution of yeast species on raspberry fruits.

The phylogenetic analysis of all yeast strains was carried out by using the MEGA–X phylogenetic analysis tool. ITS1-5.8S-ITS2 rDNA gene sequences of yeast strains were aligned by the ClustalX v1.6 algorithm and the Maximum parsimony tree was constructed by using default parameters. 1000 bootstrap replicates were used to defined branch support. The percentage of trees is shown next to the branch and frequencies under 50% are not given. *S. cerevisiae* and *P. guilliermondii* yeast species were selected as outgroup. According to the maximum parsimony tree, 27 yeast strains were separated into two main clades. It was determined that the first clade consisted of *Metschnikowia* genus (*M. pulcherrima* and *M. viticola*), the second clade contained *H. uvarum* and *A. pullulans* yeast species (Figure 2). As shown in the MP tree, all *M. pulcherrima* yeast species localized in the first sub-clade. The second sub-clade includes *M. viticola* yeast species that were also discriminated as a different group with RFLP results. The second main clade is divided into two sub-clades. All *H. uvarum* and *A. pullulans* yeast species localized in the third and fourth sub-clades, respectively. These yeast strains also showed two different restriction patterns in the RFLP results. All these results indicated that, RFLP results and MP analysis consistent with each other.

The non-*Saccharomyces* yeast strains are frequently utilized for biotechnological research along with their industrially importance. Yeast strains are generally used for various research, such as biomedical studies, biocontrol agents, bioremediation and fermentation process (Johnson & Echavarri-Erasun, 2011). *M. pulcherrima, H. uvarum,* and *A. pullulans* are good antagonists for post-harvest diseases in many fruits. It was reported that these yeast strains have protective features for *Botrytis cinerea*-based diseases (Spadaro, Vola, Piano, & Gullino, 2002; Vero, Garmendia, Gonzalez, Garat, & Wisniewski, 2010; Liu et al., 2010). *A. pullulans* yeast species are used as an indicator for environmental pollutions. The pullulan polysaccharide, produced by *A. pullulans*, have been utilized for different applications, such as the production of commercial capsules for applying diet of diabetic patients (Deshpande, Rale, & Lynch, 1992; Sena, Costelli, Gibson, & Coughlin, 2006; Cheng, Demirci, & Catchmark, 2011). Therefore, the identified yeast species, *M. pulcherrima, H. uvarum* and *A. pullulans* could be used for various commercial applications in the future.

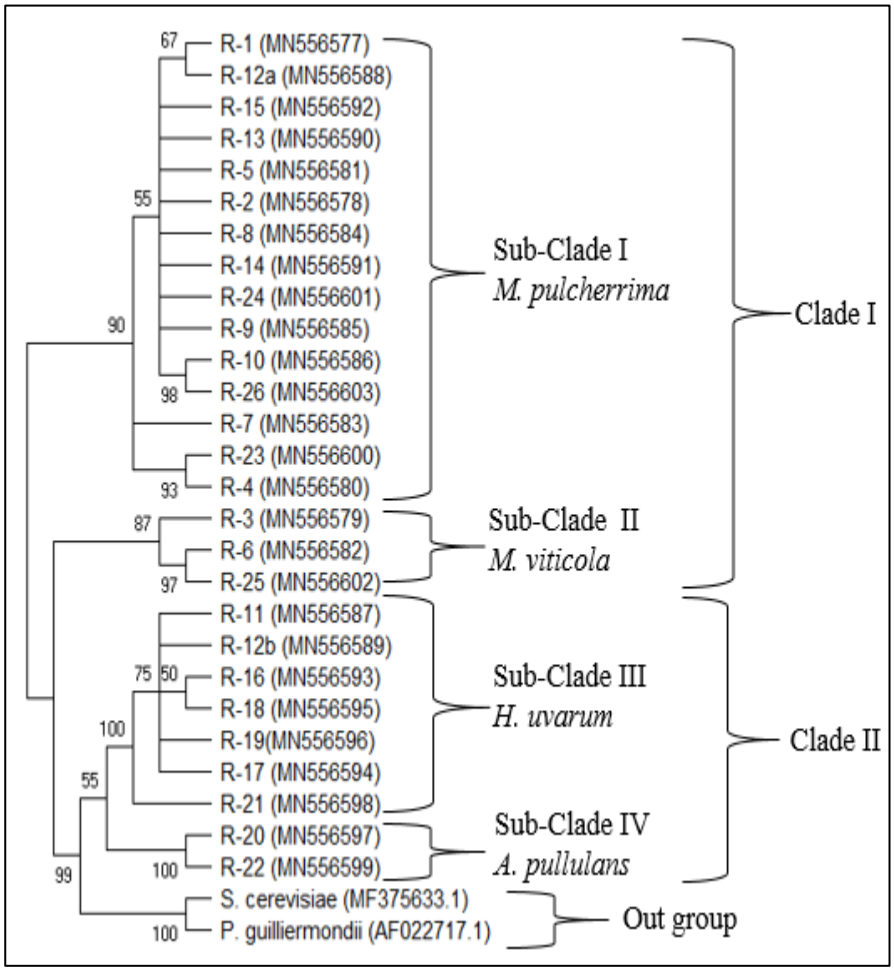

Figure 2. Maximum parsimony tree of yeast strains isolated from raspberry. Maximum parsimony tree of yeast strains isolated from raspberry. MP tree was constructed by using the bootstrap method and Subtree-Prunning-Regrafting (SPR) parameters in the MEGA-X software. 1000 bootstrap replicates were used to define branch support and above 50% bootstrap values were given. *S. cerevisiae* and *P. guilliermondii* yeast strains were selected as outgroup.

3.2. Extracellular Enzyme Profiles

The extracellular enzymes produced by yeast strains were used to obtain various fermented products. Therefore, the extracellular enzyme activities of all identified yeast strains were investigated. The extracellular enzyme profile of yeast strains was analyzed by using the API-ZYM kit system and the results were given in Table 4. The lipase (C-14), trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-

glucuronidase, N-acetyl-glucosaminidase, α-mannosidase, and α-fucosidase activities were not recorded in all yeast strains, so, these results were not given in Table 4. All yeast strains showed high leucine arylamidase activity.

It was shown that *M. viticola, H. uvarum* and *A. pullulans* yeast strains have high leucine arylamidase and β-glucosidase enzyme activity. Interestingly, *M. pulcherrima* yeast strains showed two different extracellular enzyme profiles. In the first profile, six *M. pulcherrima* yeast strains (R-1, R-4, R-5, R-7, R-12a, R-26) exhibit moderate levels of enzyme activities given in the table. In the second enzyme profile, *M. pulcherrima* yeast strains (R-2, R-8, R-9, R-10, R-13, R-14, R-15, R-23, R-24) showed a low level of esterase, esterase lipase, acid phosphatase, and naphthol-AS-BI-phosphohydrolase activity and, high level of β-glucosidase activity. No activity was recorded for the alkaline phosphatase, valine arylamidase, cystine arylamidase, and α-glucosidase enzymes. The extracellular enzyme profiles are utilized to define and discriminate yeast strains (Garcia-Martos et al., 2001). In our results, *M. pulcherrima* yeast strains have different enzyme activities even if they were identified as the same species.

Table 4
Extracellular enzyme profile of yeast strains determined with API ZYM test

Yeast Strains	C	1	2	3	4	5	6	7	8	9	10
M. pulcherrima (R-1, R-4, R-5, R-7, R-12a, R-26)	0	3	3	2	4	4	2	3	2	3	2
M. pulcherrima (R-2, R-8, R-9, R-10, R-13, R-14, R-15, R-23, R-24)	0	0	1	1	4	0	0	1	1	0	5
M. viticola (R-3, R-6, R-25)	0	0	1	1	4	0	0	2	1	1	4
H. uvarum (R–11, R–12/b, R–16, R–17, R-18, R–19 ve R–21)	0	0	1	1	4	1	0	1	0	0	5
A. pullulans (R-20, R-22)	0	0	1	0	4	0	0	0	0	0	4

C, Control; 1: Alkaline Phosphatase; 2: Esterase (C4); 3: Esterase Lipase (C8); 4: Leucine arylamidase; 5: Valine arylamidase; 6: Cystine arylamidase; 7: Acid Phosphatase; 8: Naphthol-AS-BI-phosphohydrolase; 9: α-glucosidase; 10: β-glucosidase. Results were expressed on a scale from no activity (0) to maximum activity (5).

A. pullulans, *H. uvarum* and some of *M. pulcherrima* yeast strains have β-glucosidase activity. β-glucosidase (EC 3.2.1.21) breaks the β-1-4 glycosidic bond in oligosaccharides. The β-glucosidase enzyme produced by microorganisms contributes to increasing flavor, aroma, and quality of the wine (Esen, 2003). Another enzyme, leucine arylamidase, is used to increase wine quality of aroma and taste (Dodor & Tabatabai, 2007; Delfini & Formica, 2001; Nikolaou, Andrighetto, Lombardi, & Nikolaos, 2007; Nikolaou, Soufleros, Boulompasi, & Tzanetakis, 2006; Fleet, Charoenchai, Henschke, & Todd, 1997). The leucine arylamidase (EC 3.4.11.2), belongs to the aminopeptidase enzyme family, hydrolyzes N-terminal ends of amino acids and, all isolated yeast strains have high leucine arylamidase activity in our research.

4. Conclusion

Yeast strains have generally used for biotechnological surveys, such as pharmacology, medicine, drug, enzyme, and food industry. Therefore, the isolation and identification of yeast species from natural habitats are more significant for manufacturers. In this study, the isolated and identified *M. pulcherrima*, *A. pullulans*, and *H. uvarum* yeast strains are good candidates for different industrial applications. *M. pulcherrima* yeast

species can be used as biocontrol agents to pathogenic microorganisms because of their strong antimicrobial activity. Many antagonistic strains of *Metschnikowia* have been patented and also some *Metschnikowia*-based commercial products were used as fermentation additives and biocontrol agents against plant pathogens and post-harvest diseases (reviewed in Sipiczki, 2020). Similarly, *A. pullulans* yeast species known as black yeast were commercially used for the production of different enzymes (amylase, xylanase, pectinase, etc), and pullulan is the important polysaccharide for the production of biodegradable plastics (reviewed in Gaur et al., 2015). The apiculate yeast species *H. uvarum* (anamorph *Kloeckera apiculata*) are also commercialized as oenological starter cultures for the production of volatile compounds in wine (Masneuf-Pomarede et al., 2016). The identified yeast species in our research, *M. pulcherrima, H. uvarum*, and *A. pullulans* could be used for different commercial purposes in the future.

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Author Contributions

Tülay Turgut Genç: Conceived and designed the analysis, edited the data, wrote and finalized the paper.

Melih Günay: Performed the experimental studies and obtained the data, wrote the paper.

Conflicts of Interest

The authors declare no conflict of interest.

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