



Molecular characterization of yeasts isolated from traditional Turkish cheeses

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Abstract

Thirty-two yeast strains were identified by means of molecular methods isolated from traditional Turkish cheeses (*Tulum*, *Kashkaval*, *Mihalic*, *Orgu*, *White*, *Sepet*, and *Goat*). *Debaryomyces hansenii* and *Torulaspora delbrueckii* were found as predominant species in cheese samples. Other species which were identified were *Kluyveromyces lactis*, *Candida parapsilosis*, *Clavispora lusitaniae*, *Saccharomyces cerevisiae*, *Kluyveromyces marxianus*, *Rhodotorula mucilaginosa*, *Meyerozyma guilliermondii* (formerly *Pichia guilliermondii*), *Candida zeylanoides* and *Candida albicans*. *Rhodotorula mucilaginosa* and *D. hansenii* strains, from *Kashkaval* cheese, showed antilisterial activity, whereas only one *K. lactis* strain from *Orgu* cheese exhibited proteolytic activity.

Keywords: *Tulum*, *Kashkaval*, *Mihalic*, *Orgu*, *White*, *Sepet*, and *Goat* cheeses; *Debaryomyces hansenii*; *Torulaspora delbrueckii*; *Kluyveromyces lactis*; *Rhodotorula mucilaginosa*; characterization; antimicrobial activity.

Practical Application: The wild yeast microflora from some traditional Turkish cheeses were identified by means of molecular techniques. These information may be useful for quality and shelf life of Turkish cheeses.

1 Introduction

Yeasts are an essential part of cheese microflora because they can tolerate low water activity and pH, high salt concentrations and low storage temperatures and are resistant to some chemicals such as sanitizers and cleaning compounds (Ferreira & Viljoen, 2003; Banjara et al., 2015). Certain yeasts may have the ability to use lactose, proteins, lipids, and some organic acids and may be applied for ripening by generating some flavor components and modifying the texture of cheeses and dairy products. Traditional fermented dairy products such as kefir and kumys are also produced by lactic acid bacteria and yeast fermentation (Jakobsen & Narvhus, 1996; Vasdinyei & Deak, 2003; Ferreira & Viljoen, 2003; Büchl & Seiler, 2011; Banjara et al., 2015).

At the same time, some yeast strains can cause spoilage by producing gas, off-flavors, softening of texture and discoloration changes in dairy products, especially with low pH such as yogurt, cream cheese, fermented milk, sour cream, etc. (Vasdinyei & Deak, 2003; Kavas et al., 2006; Büchl & Seiler, 2011; Corbaci et al., 2012).

Yeasts may enter cheese production process from a variety of sources, such as the starter microorganisms, brine, environmental air, workers, and processing equipment (Banjara et al., 2015). The most commonly isolated yeast genera from dairy products are *Debaryomyces*, *Yarrowia*, *Candida*, *Zygosaccharomyces*, *Cryptococcus*, *Geotrichum*, *Kluyveromyces*, *Trichosporon*, *Rhodotorula*, *Torulaspora*, and *Saccharomyces*. *Kluyveromyces marxianus*, *Debaryomyces hansenii*, and *Saccharomyces cerevisiae* species are mostly presented in dairy samples. Besides, *Yarrowia lipolytica* and *D. hansenii* may also be used as adjunct cultures

for flavor enhancement during cheese ripening (Hansen & Jakobsen, 2001; Ferreira & Viljoen, 2003; Vasdinyei & Deak, 2003; Capece & Romano, 2009).

Turkish White cheese, *Kashkaval*, and *Tulum* cheeses are the best known and the most commonly consumed cheese varieties which have a national and economic value in Turkey. *Mihalic*, *Orgu*, *Sepet*, and *Goat* cheese are the other crucial Turkish cheese varieties (Hayaloglu et al., 2002; Kamber, 2008; Aday & Karagul Yuceer, 2014).

In the researches about yeast microflora of Turkish cheeses, *D. hansenii*, *Pichia amethionina* var. *amethionina*, *K. lactis* and *Candida* spp., were identified in Turkish White cheeses (Hayaloglu et al., 2002); *Candida*, *Geotrichum*, *Kluyveromyces*, *Pichia*, *Saccharomyces* and *Zygosaccharomyces* genera were found in *Tulum* cheeses (Karasu-Yalcin et al., 2012); *Debaryomyces* spp., *Candida* spp., *Kluyveromyces* spp., *Trichosporon* spp., *Saccharomyces* spp. and *Geotrichum* spp. were obtained from *Tulum*, white pickled and, *Kasar* (*Kashkaval*) cheeses (Kavas et al., 2006); *Candida* (especially *C. famata* var. *famata*), *Geotrichum* and *Trichosporon* spp. were identified as dominant yeast flora in *Mihalic* cheeses (Karasu-Yalcin et al., 2017); and *D. hansenii* was found as predominant yeast species in homemade cheeses (Corbaci et al., 2012).

It has also been mentioned that certain species of yeasts may have inhibition characteristics against spoilage or pathogenic microorganisms in cheeses. *D. hansenii* may have

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antibacterial activity against *Clostridium butyricum* and *C. tyrobutyricum* in cheese brines (Ferreira & Viljoen, 2003). The antimicrobial activity of yeasts has been known for years. It is thought that antagonistic effects of yeasts are obtained through competition for nutrients, production of ethanol and organic acid, antimicrobial compounds such as extracellular proteins or glycoproteins called mycocins (Hatoum et al., 2013; Buyuksirit & Kuleasan, 2014). Certain yeast genera, such as *Saccharomyces*, *Debaryomyces*, *Cryptococcus*, *Candida*, *Hanseniaspora*, *Kluyveromyces*, *Pichia*, *Torulopsis*, *Williopsis* and *Zygosaccharomyces*, may produce mycocins (Hatoum et al., 2012; Buyuksirit & Kuleasan, 2014).

In this research, the wild yeast strains isolated from traditional Turkish cheeses were identified by means of molecular techniques. The antagonistic effect and the proteolytic and lipolytic activities of the isolates were also evaluated.

2 Materials and methods

2.1 Yeast isolation

The cheese samples belonging to *Tulum*, *Kashkaval*, *Mihalic*, *Orgu*, *White*, *Sepet*, and *Goat* cheese varieties were collected (n = 50). Samples (25g) were weighed in sterile bags and homogenized in 225 mL buffered peptone water using a peristaltic blender (Stomacher, ISOLAB, Turkey). The decimal dilutions of the samples were spread on YEPD (yeast extract-peptone-dextrose) agar (Merck, Darmstadt, Germany) medium with 0.1 g/L chloramphenicol (Merck, Darmstadt, Germany) added and incubated during 2-6 days at 28 °C. One to three colonies were selected randomly from the YEPD agar, subcultured to obtain pure cultures on YEPD medium (Capece & Romano, 2009).

2.2 Yeast identification

Genomic DNA extraction was performed using a synthetic resin (Instagene Bio-Rad Matrix), following the protocol described by Capece et al. (2011). The presumptive identification of the yeasts obtained from traditional Turkish cheeses was performed by PCR amplification of the internal transcribed spacers between the 18S and 26S rDNA genes (ITS1-5.8S-ITS2) and subsequent restriction analysis, as reported by Esteve-Zarzoso et al. (1999). PCR products were digested without further purification with restriction enzymes *HaeIII* and *HinfI* (Promega, USA). Restricted fragments were analyzed by electrophoresis on 2% agarose gels, and 1.0X TBE buffer, stained with SyberSafe DNA gel stain (Invitrogen, USA) and the obtained profiles were visualized and photographed with Gel Doc™ XR+ (Bio-Rad, USA) under UV light. A 100-bp DNA ladder marker (Promega, USA) served as the standard size. Isolates showing the same restriction pattern were grouped, and one or two representative yeasts for each group were delivered to Eurofins Genomics Srl (Vimodrone, Italy) for sequencing. The sequences received were compared with those deposited in the GenBank DNA database (National Center for Biotechnology Information, 2015) using the basic BLAST search tools (Altschul et al., 1997).

2.3 Molecular characterization using MSP-PCR

The genetic variability among the analyzed strains was evaluated by means of microsatellite-primed PCR (MSP-PCR) experiments. The primers employed in MSP-PCR assays were the minisatellite M13 (GAGGGTGGCGTTCT) and the microsatellite synthetic oligonucleotide (GTG)₅. Amplification reactions were done in a total volume of 50 µL containing 10 µL of Taq Polymerase 5X Buffer (Promega, USA), 4.0 µL of 25 mM MgCl₂ (Promega, USA), 1 µL of 10 mM dNTP (Promega, USA), 5 µL of 5 µM primer, 0.25 µL (5 U/µL) of Taq DNA polymerase (Promega, USA) and 5 µL of the extracted DNA, by adding sterile water until final volume. The thermal cycler was programmed as follows: initial denaturation at 95 °C for 5 min, followed by 35 cycles at 94 °C for 1 min, 1 min at 52 °C, 2 min at 72 °C for (GTG)₅, while the initial denaturation was followed by 40 cycles at 95 °C for 40 sec, 1 min at 52 °C, 1 min at 72 °C; the final extension step for both primers was at 72 °C for 5 min for M13. The amplification was conducted in duplicate for each sample to assess the reproducibility of the obtained patterns. The PCR products were determined by agarose gel electrophoresis, as previously described. The conversion, normalization and further analysis of the patterns were carried out with FP Quest™ software ver. 4.5 (Bio-Rad, USA).

2.4 Antagonistic effect of the yeast strains

The antagonistic effect of the yeast strains against *Listeria monocytogenes* ATCC 7644, *Staphylococcus aureus* ATCC 6538 and *Enterococcus faecalis* ATCC 29212 were evaluated by using agar spotting method.

The 3 µL of 24 h cultures of the yeast isolates were spotted onto the surface of YEPD agar and incubated during 48-72 h at 30 °C. Then, the plates were covered by ten milliliter of BHI soft agar (0.7% agar) inoculated with 10 µL of one of the *Listeria monocytogenes* ATCC 7644, *Staphylococcus aureus* ATCC 6538, and *Enterococcus faecalis* ATCC 29212 test cultures. After incubation during 18-24 hours at 37 °C, the test plates were checked for clear inhibition zone diameters at around test cultures (Harris et al., 1989). The antagonistic effects of yeasts were also confirmed using neutralized supernatants (supernatant pH adjusted as 7.0) from the cultures.

2.5 Proteolytic and lipolytic activity of the yeast strains

Skim milk agar (Oxoid, UK) was used for determination of proteolytic activity of yeasts. Lipolytic activity was analyzed by spotting 24 h cultures of the yeasts on Tributyrin Agar (Oxoid, UK) and incubating at 30 °C (Lanciotti et al. 2004).

3 Results

3.1 Isolation and characterization of yeasts

The yeast loads were between 1.47 and 4.71 log cfu g⁻¹ in 24 of 50 (48%) analyzed cheese samples in the study (Table 1). The yeast counts were of 2.87-4.71 log cfu g⁻¹ for *Tulum*, 1.47-3.81 log cfu g⁻¹ for *Kashkaval*, 3.61-4.36 log cfu g⁻¹ for *Mihalic*, 2.68-4.71 log cfu g⁻¹ for *Kelle*, 3.44-4.17 log cfu g⁻¹ for *Orgu*, 3.39 log cfu g⁻¹

for White, 2.91 log cfu g⁻¹ for *Sepet* and 4.53 log cfu g⁻¹ for *Goat* cheese samples. (Table 1). Thirty-two yeast strains isolated from Turkish cheeses in the present study were ascribed to 11 different species by RFLP analysis and sequencing of ITS region. Table 2 shows the size of digested PCR products with *Hae*III and *Hin*fl

and the results of ITS sequencing, performed on representatives of different RFLP profiles found. All the sequenced yeast isolates showed high similarity (100% for all the isolates, except those of *Rhodotorula mucilaginosa*) to the sequences in Genbank (Table 2). The most frequent species was, as expected, *Debaryomyces hansenii*, with 16 isolates belonging to this species, followed by *Torulaspora delbrueckii* (3 isolates), whereas the species *K. lactis*, *C. parapsilosis* and *Clavispora lusitaniae* were present with two isolates for each one. Other species, such as *S. cerevisiae*, *K. marxianus*, *Rhodotorula mucilaginosa*, *Meyerozyma guilliermondii* (formerly *Pichia guilliermondii*), *C. zeylanoides* and *C. albicans*, were represented by only one isolate (Table 2).

Table 1. The yeast counts and number of positive cheese samples.

Cheese Type	Yeast counts (minimum-maximum) (log cfu g ⁻¹)	Number of Positive sample/Analyzed samples (n/n)
<i>Tulum</i>	2.87-4.71	5/10
<i>Kashkaval</i>	1.47-3.81	6/14
<i>Mihalic</i>	3.61-4.36	4/6
<i>Kelle</i>	2.68-4.71	4/6
<i>Orgu</i>	3.44-4.17	2/5
<i>White</i>	3.39	1/3
<i>Sepet</i>	2.91	1/5
<i>Goat</i>	4.53	1/1

3.2 Molecular characterization using MSP-PCR

Based on the identification results, yeast species including at least two isolates were submitted to molecular characterization at the strain level. The genetic polymorphism was analyzed using MSP-PCR with M13 and (GTG)₅ primers.

Table 2. Molecular identification results of yeasts isolated from Turkish cheeses.

Isolate	Source	ITS-5.8S RFLP (base pair)			GenBank Acc. Number	Species
		AP*	<i>Hae</i> III (bp)	<i>Hin</i> fl (bp)		
39-1	<i>Tulum</i> cheese	800	No restriction	390+410	-	<i>T. delbrueckii</i>
40-1	<i>Tulum</i> cheese	850	320+230+180+150	120+380	CP006446.1	<i>S. cerevisiae</i>
40-2	<i>Tulum</i> cheese	650	400+150+90	325+325	-	<i>D. hansenii</i>
43-1	<i>Tulum</i> cheese	700	400+150+90	325+325	-	<i>D. hansenii</i>
45-1	<i>Tulum</i> cheese	690	400+150+90	325+325	-	<i>D. hansenii</i>
47-1	<i>Tulum</i> cheese	800	No restriction	390+410	-	<i>T. delbrueckii</i>
1-1	<i>Kashkaval</i> cheese	750-800	650+80	90+120+200+260	KU311151.1	<i>K. marxianus</i>
2-1	<i>Kashkaval</i> cheese	690	400+150+90	300+320	-	<i>D. hansenii</i>
4-1	<i>Kashkaval</i> cheese	600	450+120	280+290	KP675554.1	<i>C. albicans</i>
11-1	<i>Kashkaval</i> cheese	650	No restriction	90+120+230+220	AF444635.1	<i>Rhodotorula mucilaginosa</i>
11-2	<i>Kashkaval</i> cheese	650	430+120+90	300+320	KP053703.1	<i>Meyerozyma guilliermondii</i>
21-1	<i>Kashkaval</i> cheese	550	110+400	280+290	KC556814.1	<i>C. parapsilosis</i>
49-1	<i>Kashkaval</i> cheese	690	400+150+90	325+325	-	<i>D. hansenii</i>
49-2	<i>Kashkaval</i> cheese	690	400+150+90	325+325	-	<i>D. hansenii</i>
12-1	<i>Mihalic</i> cheese	700	400+150+90	325+325	KP835570.1	<i>D. hansenii</i>
12-2	<i>Mihalic</i> cheese	700	400+150+90	325+325	-	<i>D. hansenii</i>
13-1	<i>Mihalic</i> cheese	650	400+150+90	325+325	-	<i>D. hansenii</i>
25-1	<i>Mihalic</i> cheese	400	No restriction	200+200	-	<i>Clavispora lusitaniae</i>
25-2	<i>Mihalic</i> cheese	690	400+150+90	325+325	-	<i>D. hansenii</i>
33-1	<i>Mihalic</i> cheese	690	400+150+90	325+325	-	<i>D. hansenii</i>
14-2	<i>Kelle</i> cheese	400	No restriction	200+200	-	<i>Clavispora lusitaniae</i>
19-1	<i>Kelle</i> cheese	690	400+150+90	300+320	-	<i>D. hansenii</i>
28-1	<i>Kelle</i> cheese	550	110+400	280+290	-	<i>C. parapsilosis</i>
29-1	<i>Kelle</i> cheese	690	400+150+90	325+325	-	<i>D. hansenii</i>
14-2	<i>Kelle</i> cheese	400	No restriction	200+200	-	<i>Clavispora lusitaniae</i>
30-1	<i>Orgu</i> cheese	690	400+150+90	325+325	-	<i>D. hansenii</i>
36-1	<i>Orgu</i> cheese	750-800	650+80	100+120+200+300	KU218504.1	<i>K. lactis</i>
36-2	<i>Orgu</i> cheese	750-800	650+80	100+120+200+300	-	<i>K. lactis</i>
34-1	<i>White</i> cheese	800	No restriction	390+410	KT029803.1	<i>T. delbrueckii</i>
34-2	<i>White</i> cheese	650	400+150+90	325+325	KR089899.1	<i>Candida zeylanoides</i>
31-1	<i>Sepet</i> cheese	690	400+150+90	325+325	-	<i>D. hansenii</i>
32-1	<i>Goat</i> cheese	690	400+150+90	325+325	-	<i>D. hansenii</i>

*AP = size (base pairs) of the ITS-5.8S rDNA PCR product; RFLP = Restriksiyon Fragment Length Polymorphism.

The numerical analysis (UPGMA clustering) of the combined MSP-PCR patterns with the two oligonucleotides resulted in the dendrogram reported in Figure 1. The reproducibility of the MSP-PCR was higher than 90% (data not shown). Clusters were arbitrarily identified at a similarity level of 60%. The isolates were grouped in 6 main clusters and one single strain (56-2). The analyzed isolates were grouped according to the species, except *D. hansenii* isolates, which were separated into two clusters, 1 and 4. Cluster 1 was composed of one biotype, whereas cluster 4 could be further subdivided (at a similarity level of about 85%) in three different subclusters, 4a, 4b, and 4c, plus two single strains (49-1 and 19-1), indicating a significant polymorphism among *D. hansenii* strains. Certain biodiversity was also found in *T. delbrueckii*; in fact, one of the four strains grouped separately (56-2). The 56-1 and 56-2 strains were not cheese-derived. The others are included in cluster 5, in which two

biotypes can be individuated, the first one, shared by the isolates 34-1 and 39-1, and the other one, exhibited by 47-1. The two *Cl. lusitaniae* strains (cluster 3) showed different biotypes, as did the two *K. lactis* strains, composing cluster 6. Otherwise, the two *C. parapsilosis* isolates, belonging to cluster 2, showed the same biotype. MSP-PCR revealed a remarkably high genetic diversity within our set of strains; however, the low correlation with the source of isolation was found.

3.3 Antagonistic effect of the yeast strains

Only three of the yeast strains isolated from *Kashkaval* cheese samples (11-1, 49-1, 49-2) showed antagonistic effect (halos between 8.0-12.0 mm) against *Listeria monocytogenes* ATCC 7644. These strains were identified as *Rhodotorula mucilaginosa* (11-1) and *D. hansenii* (49-1, 49-2). The most effective antilisterial activity

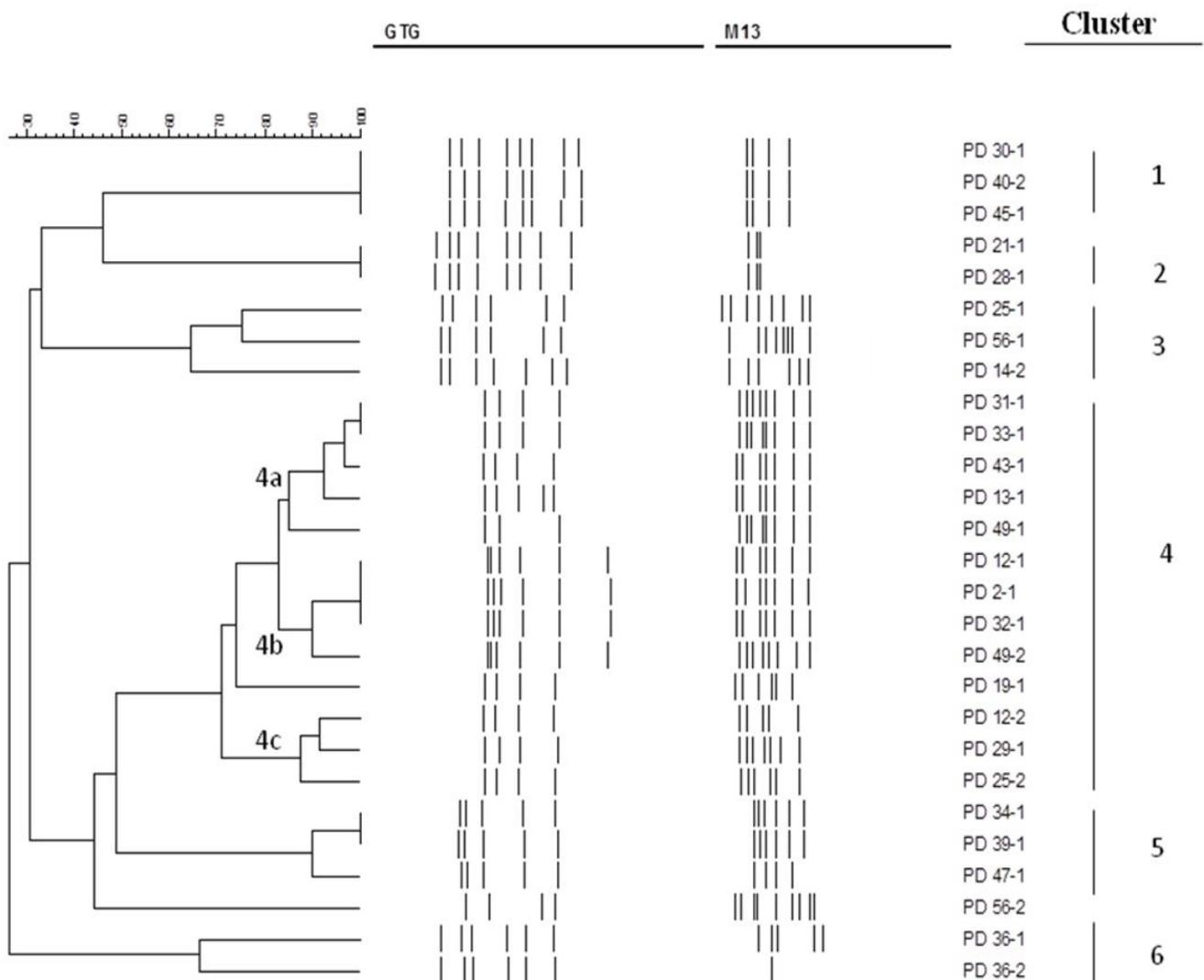


Figure 1. Cluster analysis of the profiles obtained by means of MSP-PCR with M13 and (GTG)₅ oligonucleotides (PD 30-1; *D. hansenii*, PD 40-2; *D. hansenii*, PD 45-1; *D. hansenii*, PD 21-1; *C. parapsilosis*, PD 28-1; *C. parapsilosis*, PD 25-1; *Clavispora lusitaniae*, PD 56-1; *Clavispora lusitaniae*, PD 14-2; *Clavispora lusitaniae*, PD 31-1; *D. hansenii*, PD 33-1; *D. hansenii*, PD 43-1; *D. hansenii*, PD 13-1; *D. hansenii*, PD 49-1; *D. hansenii*, PD 12-1; *D. hansenii*, PD 2-1; *D. hansenii*, PD 32-1; *D. hansenii*, PD 49-2; *D. hansenii*, PD 19-1; *D. hansenii*, PD 12-2; *D. hansenii*, PD 29-1; *D. hansenii*, PD 25-2; *D. hansenii*, PD 34-1; *T. delbrueckii*, PD 39-1; *T. delbrueckii*, PD 47-1; *T. delbrueckii*, PD 56-2; *T. delbrueckii*, PD 36-1; *K. lactis*, PD 36-2; *K. lactis*).

(12 mm) was determined in the *Rhodotorula mucilaginosa* (11-1) isolates (Table 1). The antagonistic effect against other tested pathogen bacteria was not shown.

3.4 Proteolytic and lipolytic activities of the yeast strains

Only the strain 36-1, identified as *K. lactis* and isolated from an *Orgu* cheese sample (Table 1), showed proteolytic activity. Lipolytic activity was not observed on tributyrin agar.

4 Discussion

Yeast counts are determined at a high level in cheeses (e.g., 10^6 - 10^9 cfu/g), even in cheeses produced by bacterial starters (Capece & Romano, 2009; Karasu-Yalcin et al., 2017). In the present study, the yeast loads were found as too low (1.47 - 4.71 log cfu g⁻¹) to affect in cheese ripening. The regulation limiting yeast and mold counts (1 - 2 log cfu g⁻¹) (Turkish, 2010) and limited artisanal cheese production are thought to be the reasons of the low yeast counts in this study. The yeast count of *Kashkaval* cheese, a kind of *pasta filata* cheese, was determined in this study to range between 1.47 and 3.81 log cfu g⁻¹. Çetinkaya & Soyutemiz (2006) reported the yeast and mold counts of *Kashkaval* cheese between 3.41 and 3.91 log cfu g⁻¹. There are some factors during the processing of *Kashkaval* cheese for reducing yeast count like heat treatment (75 ± 1 °C for 5 min) of the curd during the stretching process (Çetinkaya & Soyutemiz, 2006), and some chemical preservatives, which are allowed in limited amounts such as sorbates and benzoates, may also reduce yeast count (Ozdemir & Demirci, 2006).

Yeasts are widely isolated from cheeses because of ability to live at acidic pH, high salted environments, and storage at low temperatures of these foods. Yeasts may cause spoilage on dairy products contaminated through raw materials, additives, and other ingredients, and that may also occur due to environmental contamination, including surfaces of equipment (Fleet, 1990; Jakobsen & Narvhus, 1996). However, some yeast strains have a desirable flavor and taste development during the ripening stage (Fleet, 1990).

In this study, the predominant isolated species from cheese samples was *D. hansenii*, especially in *Tulum* and *Mihalic* cheese. *T. delbrueckii*, *K. lactis*, *C. parapsilosis*, *Cl. lusitanae*, *S. cerevisiae*, *K. marxianus*, *R. mucilaginosa*, *Meyerozyma guilliermondii*, *C. zeylanoides*, and *C. albicans* were found as other identified yeast species. It was mentioned that yeast species belonging to *Debaryomyces*, *Candida*, *Kluyveromyces*, *Trichosporon*, *Saccharomyces*, *Pichia*, *Zygosaccharomyces* and *Geotrichum* genera were the most generally isolated species from Turkish white pickled, *Tulum* and *Kashkaval* cheeses (Hayaloglu et al., 2002; Kavas et al., 2006; Karasu-Yalcin et al., 2012). *Candida*, *Geotrichum*, and *Trichosporon* genera were also identified as dominant yeast flora for *Mihalic* cheese (Karasu-Yalcin et al., 2017). It was mentioned that *D. hansenii* was the most widely found species in almost all cheeses types (Fleet, 1990; Capece & Romano, 2009; Karasu-Yalcin et al., 2017). *Debaryomyces hansenii* has excellent tolerance to salty environments and proteolytic and lipolytic enzyme activity that are important for cheese ripening (Jakobsen & Narvhus, 1996; Capece & Romano, 2009).

Karasu-Yalcin et al. (2017) emphasized that it was expected to find *D. hansenii* as dominant yeast microflora of *Mihalic* cheese, a salty traditional cheese in Turkey.

Some yeast strains may show an antagonistic effect against pathogen bacteria by producing antibacterial compounds called mycocins (killer toxins). These compounds are glycoproteins or extracellular proteins which damage the cell membrane function in the targeted microorganisms (Hatoum et al., 2013). Although the antimicrobial effects of yeasts against other yeasts by killer toxins are well analyzed (Goerges et al., 2006; Buyuksirit & Kuleasan, 2014), studies conducted to find out the antibacterial effects of yeasts against *Listeria monocytogenes* and *Staphylococcus aureus* are limited (Goerges et al., 2006; Roostita et al., 2011; Hatoum et al., 2013). Goerges et al. (2006) reported that *C. intermedia*, *K. marxianus*, and *Pichia norvegensis* yeast strains isolated from cheeses showed strong antilisterial activity. Roostita et al. (2011), reported that a *C. parapsilosis* isolate from yoghurt with fruits showed antibacterial activity against *Pseudomonas aeruginosa*, *Escherichia coli* and *S. aureus*. It has been reported that *Candida catenulata*, *C. parapsilosis*, *C. tropicalis*, *D. hansenii*, *G. candidum*, *P. fermentans*, and *P. anomala* strains obtained from raw milk and cheese products showed antilisterial activity against *Listeria ivanovii*. In this study, one *R. mucilaginosa* (11-1) and two *D. hansenii* (49-1, 49-2) strains exhibited antibacterial activity against *L. monocytogenes*. These three strains were isolated from *Kashkaval* cheese samples. Although a study about antilisterial activity potential of *D. hansenii* (Hatoum et al., 2013) has been published, to our knowledge, this research is the first report describing the antimicrobial activity of *Rhodotorula mucilaginosa* against *Listeria monocytogenes*. *R. mucilaginosa* has commonly been found in the microbial flora of some foods and beverages such as cherries, peanuts, fresh fruits, apple cider, fruit juice, sausages, cheese, crustaceans and edible mollusks, and has also been found in air, seawater, freshwater, and goat's milk (Wirth & Goldani, 2012).

As a result of screening the proteolytic and lipolytic activity potential of the yeast isolates, proteolytic activity was only found in one *K. lactis* from *Orgu* cheese, and none of the isolates showed lipolytic activity in this study. Flores et al. (1999) also described proteolytic *Kluyveromyces lactis* strains.

5 Conclusion

Debaryomyces hansenii and *Torulaspora delbrueckii* were identified as predominant yeast species in traditional Turkish cheese samples. Although antilisterial and proteolytic activity potentials were determined in a limited number of strains, there is a need for more extensive studies to determine the technological properties of the yeast strains from natural yeast microflora of different traditional Turkish cheeses with a view to its possible use in the dairy industry.

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