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ORIGINAL ARTICLE

Determination of the microbial flora in traditional İzmir Tulum cheeses by Denaturing Gradient Gel Electrophoresis

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Abstract In this study, it was aimed to determine microbial flora members in three traditional Tulum cheeses (C1, C2 and C3) produced in different villages and settlement areas in İzmir, Turkey. For this purpose, culture depended and 16S rRNA based culture independent methods were used. According to the results of culture depended method, *Lactococcus* spp., *Enterococcus* spp., *Staphylococcus* spp., *Lactobacillus* spp., *Pediococcus* spp. and yeast-mold were detected in all samples at different levels. In order to determine and identify both of the culturable and non-culturable microorganisms, denaturing gradient gel electrophoresis (DGGE) method was used. DGGE results have shown that there were eight different dominant microorganisms (*Streptococcus thermophilus*, *Lactococcus lactis* subs. *lactis*, *Streptococcus infantarius* subs. *infantarius*, *Lactobacillus gallinarum*, *Streptococcus equinus*, *Enterococcus faecalis*, *Enterococcus faecium*, *Lactococcus garvieae*) in three regionally cheese samples. Further more, total bacterial loads were monitored with real-time PCR (qPCR) method. According to the results, 3.5×10^8, 3.8×10^8, 8.4×10^8 copy number of DNA was detected in C1, C2 and C3 cheese samples, respectively. This study is the first description for the dynamics of microbial composition of Izmir Tulum cheese after the production and brining processes.

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Introduction

Milk, a nutritionally essential food, provides many beneficial compounds in its composition for the development of skeletal bone structure and other organs from childhood to elderly. Not only milk but also dairy products (cheese, yoghurt and kefir etc.) have quite rich calcium, phosphorous, magnesium and protein content, which are all essential for healthy bone growth and development and commonly used in daily nutrition all around the world. Research on the processing, biochemistry and microbiology in their production is being done as there are more than 1000 different cheeses spread in the world, across various cultures and regions (Jany and Barbier 2008). Cultural and regional differences have added to different taste and texture characteristics to the cheese world. The first fermented milk-product, cheese is almost 8000 years old and it was born in Middle East region. There are essential stages for the production of cheeses such as renneting, coagulation, curd processing techniques, salting and ripening conditions. In spite of the raw material being the same in every cheese, differences in the cheese processing stages bring different characteristics to the cheeses (Jany and Barbier 2008).

Microorganisms are an essential component of cheese flora and play important roles during cheese manufacture. It is well-known that an important part of microorganisms is naturally found to contribute specific flavor, aroma and texture to traditional cheeses. Generally, two microflora groups have important roles in cheese production. One of them which is named starter—lactic acid bacteria (LAB)

that play a mediating role in acidification and ripening during the process. Second group consist of the non-lactic acid bacteria, other bacteria, yeasts and molds which are major player for cheese ripening (Jany and Barbier 2008; Serhan et al. 2009). These groups and environmental factors contribute to the cheese characteristics. It is known that dynamics of the microbial ecosystem are influenced through environmental changes (Jany and Barbier 2008; Casalta et al. 2009). The important factors are the moisture, curd processing techniques and conditions, salt concentration, pre-ripening, pH or redox potential, ripening time—temperature and the microbial interactions (Jany and Barbier 2008).

Today, 11 million tons/year milk is produced and 40% of it is (4–5 million tons/year) manufactured as cheese which is about 700–800 thousand tons/year in Turkey (Kamber 2007). More than 50 varieties of cheeses are produced in Anatolia but three of them are most preferable and popular, such as white pickled cheese, Kaşar cheese and different types of Tulum cheese (Hayaloglu et al. 2007; Cakmakci et al. 2008). Percentages of the major three cheeses production is 60, 17, 12% (includes Tulum and Mihaliç cheese), respectively, and 11% are other kinds of local cheeses (Kamber 2008). Tulum cheeses are the traditional ones that spreaded out of Anatolia from east to west (Bayar and Özrenk 2011). Generally, Tulum cheese, as long time ripened cheese varieties, is characterized with white or cream colour, high fat content, crumbly and, semihard and hard texture. Taste of Tulum cheese is almost like buttery and pungent (Hayaloglu et al. 2007). These cheeses called "Tulum" because they are made from cow, sheep or goat milk and ripened in goat or sheep skin bags. Skin bags are air and water permeable, and give strong structure to the cheese, and also unique taste and odor as well. In the past, goat skin bags were commonly used, but nowadays alternative containers also using such as wooden, plastics or earthenware containers for ripening are used (Hayaloglu et al. 2007; Bayar and Özrenk 2011). There are more than ten Tulum cheese varieties in Turkey such as Erzincan Tulum cheese, Karin Kaymak cheese, İzmir brined Tulum cheese, Simi Tulum cheese, Divle Tulum cheese, etc. (Hayaloglu et al. 2007; Hayaloglu and Karagul-Yuceer 2011).

İzmir Tulum cheese is produced from raw milks of ewes or mixture of milks that includes ewes and goats or cows. These cheeses are mostly manufactured and consumed in west Anatolia such as İzmir, Aydın, Muğla, Manisa and Denizli, and other parts of Turkey. One of the minor difference from other Tulum cheeses is, İzmir brined cheese has high level of salt and ripened under brine (Hayaloglu and Karagul-Yuceer 2011). Production of traditional İzmir Tulum cheese process can be summarize with some main steps. Milk mixtures in Tulum cheese are heated at 63 °C for 30 min and then it is cooled to 35–37 °C. 120–125 ml rennet per 1000 L is added; then it is coagulated for 45–50 min. The curds containing whey are hung and the whey drained off for 3–5 h. Then, the curds are cut into blocks with a knife and dry salted (waiting 24 h for oxidation). In production, lacquered tin packaging is used and added brine (16%). Cheese samples are Pre-ripened (14–15 °C for 1 week), stored and ripened (4–6 °C) (Fig. 1).

Microbial profiling of dairy products is currently carried out with culture-independent molecular techniques. These techniques are faster and more reliable than conventional culture dependent techniques (Giraffa and Neviani 2001). DGGE technique has been used to characterize the microbial diversity in many dairy products (Ercolini et al. 2001). DGGE was developed to analyse microbial communities, based on the sequence-specific distinctions of 16S rRNA amplicons (Muyzer and Smalla 1998). Separation is based on the decreased electrophoretic mobility of partially melted double-stranded DNA molecules in polyacrylamide gels with gradient of urea and formamide as denaturing agents. The GC clamp, which is attached to the 5′ end of one of the primers, prevents complete separation of the two DNA strands (Muyzer et al. 1993).

The aim of this study was to determine the microbial flora of the Tulum cheese by using culture dependent and

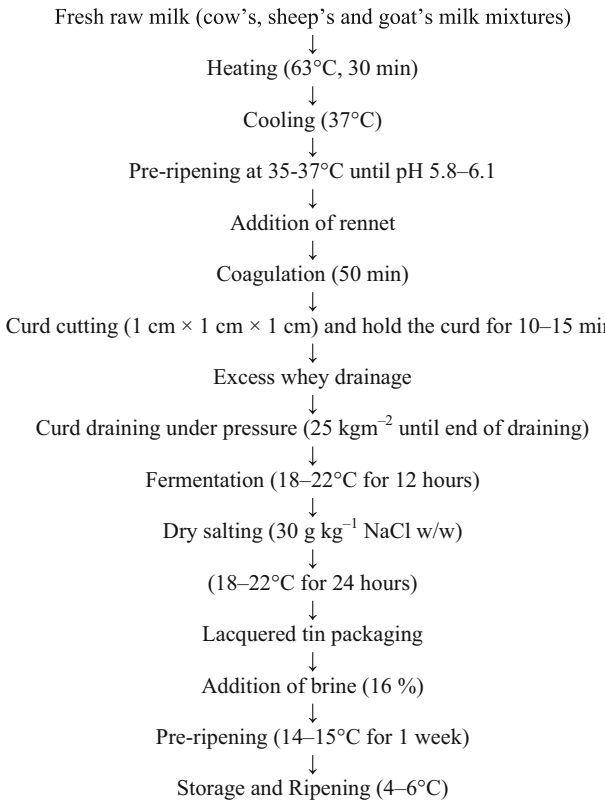

Fig. 1 Flow diagram of traditional Izmir Tulum cheese production

culture independent techniques. The cheese samples used in this study were produced with the same production technique. The differences between the samples were due to the fact that the producers are located in different regions of İzmir. Thus, it was thought that different climatic and plant cover affected the basic characteristics of the milk and properties of Tulum cheeses. Researches on Tulum cheeses are very limited, especially about traditional İzmir Tulum cheese, and hence there was a need for this study.

Materials and methods

Cheese properties and sampling conditions

İzmir Tulum cheese samples (12 months maturated) were produced from cow's, sheep's and goat's milk mixtures collected randomly from several markets in Aegean region of Turkey. Tulum cheese samples were collected from three independent and geographically separated manufacturers in İzmir (named as C1, C2 and C3) and sampled according to FIL-IDF standard 50B and transported to the laboratory under refrigerated conditions. Although there was no significant difference between the production and content of cheese samples, the effects of different settlements on microbial flora was examined.

Culture-depended microbial counts

Before microbiological analyses, 25 g of each different cheese were homogenized in sterile plastic bags with using a stomacher and then transferred to 250 ml erlenmeyer flasks included 225 ml sterile distilled water.

Natural culturable cheese producing microbiota such as bacteria, yeasts and molds requires specific growth media. Hence, seven different growth media were used for culture of the specific microorganisms (Alegria et al. 2009). Microorganisms and their required media with specific growth conditions are listed in Table 1.

After homogenization, 0.1 ml of cheese samples were inoculated to specific media (Table 1) by spread plate method for determining the bacterial type and number. Inoculated plates were incubated overnight at the specified temperatures and colonies counted for triplicates for each sample dilution. The cfu/g was calculated [(number of colonies × dilution factor)/volume of inoculation] and observations were plotted as $\log_{10}$ cfu/g.

1 ml of each three cheese homogenate were stored in 1.5 ml microcentrifuge tubes at − 20 °C for molecular biological analyses, until use.

Table 1 Specific media and growth conditions for selected microorganisms

Microorganism	Medium	Growth conditions
Lactococcus spp.	M17 Agar	37 °C, 48 h
Streptococcus spp.		
Enterococcus spp.	LB Kanamycin Agar	
Coliform groups	Violet Red Bile Agar (VRB Agar)	
Staphlococcus spp.	Baird Parker Agar (BPA)	
Micrococcus spp.		
Lactobacillus spp.	DE MAN, ROGOSA, SHARPE Agar (MRS Agar)	37 °C (5% CO_2), 48 h
Pediococcus spp.	Tomato Juice Agar	27 °C, 4–5 days
Total yeast and mold	Yeast Extract Glucose Chloramphenicol Agar (YGC Agar)	

Culture-independent microbial community analyses

Microbial diversity of cheese samples were determined by DGGE and quantified by real-time PCR analyses. All DNA extractions were carried out by using High Pure PCR template Preparation Kit (Roche Applied Science, Mannheim, Germany) according to the manufacturer's protocol. DNA samples were stored at − 20 °C for further analyses.

Nested PCR–DGGE analyses

Nested PCR method was chosen to increase the specific detection of the genera. Amplification of putative bacterial DNA fragments was carried out using 27F (5′-GAGTTTGATCCTGGCTCAG-3′) and 1522R (5′-AAGGAGGTGATCCANCCRCA-3′) 16S rRNA universal primer sets (Lanoil et al. 2000) and HDA1 (338F) (5′-ACTCCTACGGGAGGCAGCAGT-3′) HDA2 (518R) (5′-GTATTACCGCGGCTGCTGGCAC-3′) inner primer sets targeting the 16S rRNA V3 variable gene region (Lopez et al. 2003; Diez et al. 2001). 40 base pair GC-clamp was added to the 5′ end of the HDA1 primer for DGGE analysis.

All polymerase chain reactions were carried out by Techne TC-Plus thermal cycler (Bibby Scientific, US) in a total volume of 50 µl including 2 µl of sample DNA (approx. 20 ng μl^{-1}) as template, each primer 0.5 µM, each deoxynucleoside triphosphates at 200 µM, 5 µl of 10× reaction buffer, 2.5 µl of 10× Tune Up Buffer and 1.25 unit of HelixAmp^{TM} HyperSense-Taq polymerase (Nanohelix, South Korea). Thermal profile for PCR was as follows; a single denaturation step of 2 min at 95 °C, a 3-step amplification program set for 30 cycles with

denaturation for 20 s at 95 °C, annealing for 40 s at 52 °C and extension for 70 s at 72 °C with a final extension step of 72 °C for 5 min. PCR products were analyzed on a 2% agarose gel electrophoresis and visualized in UVP Biospectrum Bioimaging Systems (Ultra Violet Products Ltd., Cambridge UK) before DGGE analyses.

The DCode universal mutation detection system (Bio-Rad Laboratories, Richmond, CA, USA) was used for DGGE analysis. The gel solution consist of 8% polyacrylamide gel (w/v, acrylamide: bisacrylamide) with 35–55% denaturing gradient of urea-formamide (100% correspondent to 7 M urea and 40% [v/v] formamide) for separating the bacterial amplified DNA samples. The electrophoresis conditions were 135 V in 1 × TAE buffer at 60 °C for 5 h.

Sequence analyses

Separated DNA bands were excised with sterile scalpels and incubated in 50 µl sterile water at 4 °C for overnight (Bonetta et al. 2008). After incubation, 5 µl of the supernatant was used as template in reamplification reaction using the HDA1 and HDA2 primer sets (without GC-clamp) and same reaction conditions described above.

PCR products were sequenced by Life Sciences Application and Research Center Laboratories, Gazi University, Ankara, Turkey. Sequenced partial 16S rRNA genes obtained from DGGE bands were modified and edited using DNA Baser V.3.5.0 and BioEdit software. Sequence comparisons were performed using the basic local alignment search tool (BLASTn) program within the National Center for Biotechnology Information (http://www.ncbi.nml.nih.gov/BLAST) database.

Real-time PCR analyses

All quantitative PCR amplifications were carried out with LightCycler 1.5 (Roche Diagnostics, Mannheim, Germany). Reaction mixture was prepared using LightCycler® FastStart DNA Master SYBR Green I kit (Roche Diagnostics GmbH, Mannheim, Germany). Based on literature the primer sets were selected for qualitative and quantitative detection of total bacteria. Forward primer sequence was 5′-GTG STG CAY GGY TGT CGT CA-3′ and Reverse primer sequence was 5′-ACG TCR TCC MCA CCT TCC TC-3′ (Maeda et al. 2003). PCR conditions were constructed via manual instructions; initial denaturation at 95 °C for 10 min., amplification for 45 cycles with denaturation at 95 °C for 10 s, annealing at 60 °C for 5 s, extension at 72 °C for 8 s, respectively. In addition, melting curve analyses were performed to control that the fluorescent signal obtained in a simultaneous real-time PCR assay originated from specific PCR products and not from artifacts like primer dimers. The quantification of the amplified DNA fragments was based on the standard curve generated by PCR products of the same gene region of *E. coli.*

Results and discussion

Enumeration of microorganisms

According to the results of the culturable method, the presence of 5 microorganisms in the natural flora of cheese samples was determined. These microorganisms are thought to be responsible for cheese ripening and possibly its natural microflora (Table 2).

Results of the culture based analysis as shown in Table 2, *Lactococcus* genus is the top resident microorganism in three cheese samples. It has been known that the culturable microbiota of the milk products are primarily members of lactic acid bacteria such as *Lactobacillus*, *Streptococcus*, *Enterococcus*, *Lactococcus*, *Leuconostoc*, *Weissella* and *Pediococcus.* Also, *Propionibacterium*, *Staphylococcus*, *Corynebacterium*, *Brevibacterium*, yeasts and molds exist as well (Serhan et al. 2009; Quigley et al. 2011). It was seen that the results of *Enterococcus* spp. and *Pediococcus* spp. colony counts are almost equal to each other. On the other hand, the Staphylococci populations were at the lowest rates with 2.0×10^3–4.0×10^3 cfu/g in the C1 and C2 samples, and significantly higher (1.2×10^5 cfu/g) in the C3. Similarly, the colony count of yeasts and molds were quiet high with C3 sample showing 4.0×10^7 cfu/g.

Casalta et al. 2009 have mentioned that some species of Staphylococci are resident bacteria during the ripening and they may contribute to the aromatic characteristics of cheese. *Staphylococcus* genus is known as pathogenic. Specifically, *S. aureus* causes the mastitis in goats. Therefore, no dairy producer wants this organism to be found in terms of animal health. From the point of view of the cheese samples examined, it is not surprising that these results are found because these cheeses are not produced

Table 2 Colony numbers of isolated microorganisms by traditional culture depended method

Isolated microflora members	Estimated organism number (cfu/g)		
	C1	C2	C3
Enterococcus spp.	1.1×10^7	7.0×10^5	1.3×10^8
Lactobacillus spp.	1.1×10^7	1.4×10^6	5.8×10^7
Lactococcus spp.	9.0×10^7	2.8×10^8	2.5×10^8
Pediococcus spp.	1.6×10^7	9.3×10^5	1.4×10^8
Staphylococcus spp.	2.0×10^3	4.0×10^3	1.2×10^5
Yeasts and molds	7.1×10^4	5.4×10^4	4.9×10^7

commercially but are produced homemade by traditional techniques.

PCR–DGGE analyses

A bacterial mix, including *Lactobacillus casei* ssp. *casei* NRRL B 1922, *Streptococcus thermophilus* ST 36, *Lactobacillus casei* ssp. *rhamnosus* NRRL B 442 and *Enterococcus hirae* UWWE 3080-31024 strains was prepared and used as a marker in polyacrylamide gel (Fig. 2). As seen in Fig. 2, eight different DNA bands were detected in total for the three cheese samples. When the sequence of these eight DNA bands and BLAST analysis results were examined, it was determined that they were *Streptococcus thermophilus*, *Lactococcus lactis* subs. *lactis*, *Streptococcus infantarius* subs. *infantarius*, *Lactobacillus gallinarum*, *Streptococcus equinus*, *Enterococcus faecalis*, *Enterococcus faecium*, *Lactococcus garvieae*, respectively (Table 3). Two of these organisms (*Enterococcus hirae* and *Streptococcus thermophilus*) were detected in all samples (Fig. 2).

Figure 3 Phylogenetic tree was constructed using with Mega 5.1 Beta version. Bacterial sequences labeled with "▲" indicate those isolated, sequenced and corrected bacterial DNA from the three cheese samples, C1, C2 and C3.

El-Baradei et al. (2007) studied bacterial diversity of the traditional Egyptian Domiati cheese by PCR-temporal temperature gel electrophoresis (TTGE) and DGGE. *Leuconostoc mesenteroides*, *Lactococcus garvieae*, *Aerococcus viridans*, *Lactobacillus versmoldensis*, *Pediococcus inopinatus*, *and Lactococcus lactis* were found as dominant species.

Real time PCR analyses

Real time PCR technology was used in order to determine current total microbial loads in cheeses for experimental reliability and efficiency. According to the results, total bacterial loads were calculated about 3.5×10^8, 3.8×10^8, and 8.4×10^8 copy number of DNA, respectively.

These traditional cheeses were supported to growth of about five bacterial species and yeasts as the media used were specific for these groups. However, by using molecular techniques for detecting and profiling the microorganisms (Alegria et al. 2009) we obtained many more that were normally not seen with culture based methods. In addition, the molecular techniques allowed the identification up to the species level of the organisms seen in the three cheeses (Fig. 3). To solve the problem of identifying non-culturable organisms, researchers have developed and adopted many molecular methods based on DNA or RNA in recent years (Jany and Barbier 2008; Bonetta et al. 2008; Quigley et al. 2011; Su et al. 2012). In this study, in order

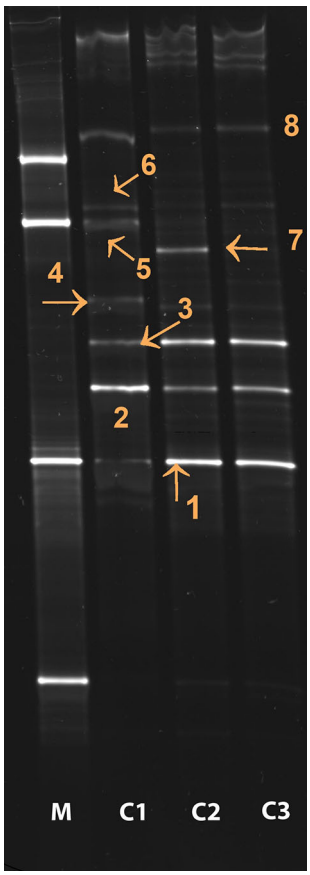

Fig. 2 DGGE profile of Tulum cheeses. M: marker (from top to bottom; *Lactobacillus casei*, *Enterococcus hirae*, *Streptococcus thermophilus*, *Lactobacillus rhamnosus*, respectively). C1, C2 and C3; Tulum cheese samples collected from different locations in İzmir, Turkey. Of the four bacteria, *Enterococcus hirae* and *Streptococcus thermophilus* occurred in all samples. Additional amplicons obtained indicated presence of other species that were analysed by sequencing

to determine microbial flora in samples, DGGE method was chosen and focused for detailed molecular analysis.

Therefore, we have focused on DGGE and real-time PCR as effective methods in this study. DGGE was effective method for the microbial profiling, but a few challenges that we faced were retrieval of DNA amplicons from acrylamide gels, especially for the faint fragments. Hence the dominant and clearly visible bands were selected and taken for the further molecular analyses such as sequencing. The other problem seen was deciding the percentage amount of denaturation gradients of the gel. However most researchers have chosen the DGGE denaturing gradient rates as 40–60% urea-formamide (Alegria et al. 2009; Bonetta et al. 2008; Su et al. 2012; Florez and Mayo 2006). In this study, 35–55% urea-formamide concentrations were found to be better for separation and providing clear DNA fragments.

Many kinds of microorganisms were identified in the different stages of Tulum cheeses ripening such as

Table 3 Sequence analysis results of DGGE bands

Band ID	Most closely related organism	NCBI accession number and similarity (%)
1	*Streptococcus thermophilus*	100/CP019935.1
2	*Lactococcus lactis* subsp. *lactis*	100/JN863615.1
3	*Streptococcus infantarius subs. infantarius*	100/KY810597.1
4	*Lactobacillus gallinarum*	100/JN851792.1
5	*Streptococcus equinus*	100/HE646371.1
6	*Enterococcus faecalis*	98/CP015410.2
7	*Enterococcus faecium*	99/KT626401.1
8	*Lactococcus garvieae*	99/KY800383.1

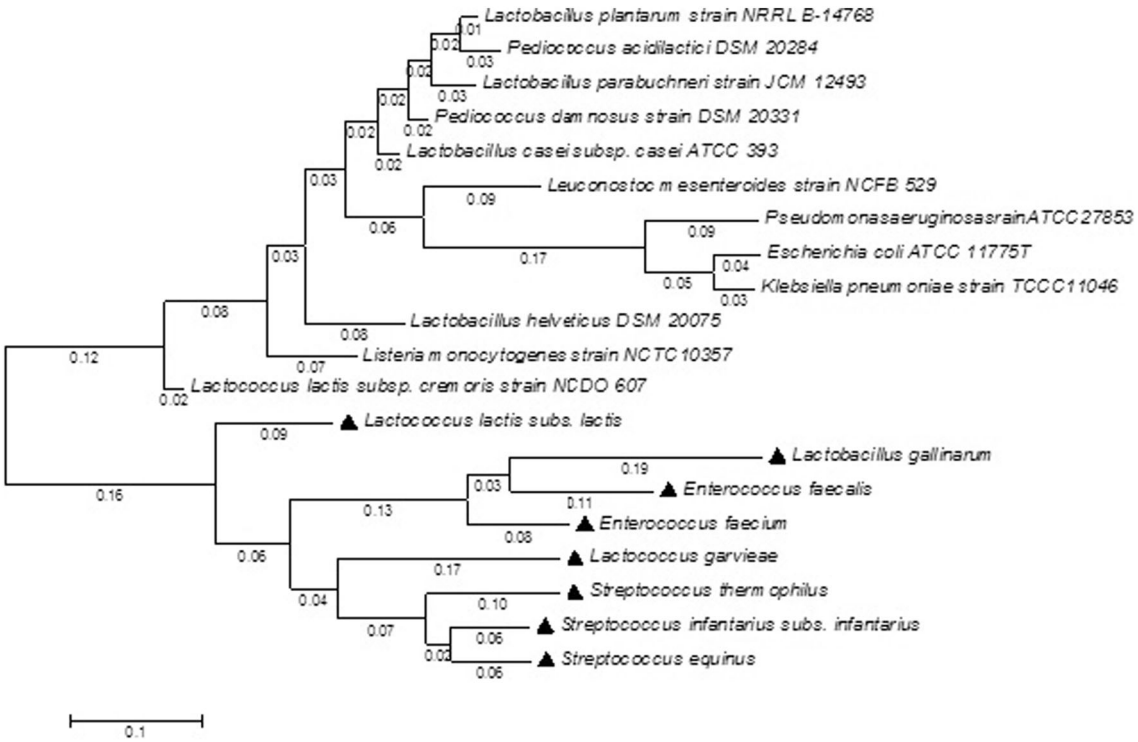

Fig. 3 Phylogenetic tree was constructed using with Mega 5.1 Beta version. Labeled bacteria with "▲" indicate isolated, sequenced and corrected bacterial DNA from C1, C2 and C3 cheese samples

Streptococcus lactis, *S. cremoris*, *S. durans*, *S. liquefaciens*, *S. diasetylactis*, *S. faecalis*, *S. faecalis* spp., *S. faecium*, *Lactobacillus casei*, *L. rafinolactis*, *L. plantarum*, *L. helveticus*, *L. citvorum*, *L. curvatus*, *L. brevis*, *L. fermenti*, *L. salivarus*, *L. corineformis*, *L. acidophylus*, *L. leischmanii*, *L. bucheri*, *L. kefir*, *L. bulgaricus*, *Leuconostoc dextranicum*, *L. brevis*, Pediococci and moulds. In about 85% of Tulum cheeses and depending on the area of sampling, number of microbes ranged from 3.0×10^4 to 10^{14} cfu/g (Kamber 2007, 2008). In other research, yeasts and moulds numbered as 7 $\log_{10}$ cfu/g. In the same study, researchers report that total coliform bacteria have not obtained *Micrococcus* spp. and *Streptococcus* spp. could not be distinguished on agar plates. It is mentioned that coliform bacteria were identified, but their numbers decreased during the ripening and total numbers of coliform bacteria decreased to < 1 log cfu/g at the end of the ripening. It could be said that the coliform bacteria may be affected with low pH, a_w, high salt concentration and also, presence of higher amount of moulds and mesophilic bacteria (Kalit et al. 2010).

From these findings, *Lactococcus lactis* subs. *lactis* and *Enterococcus faecalis* are being used as ones of the starter cultures of Tulum cheese (Hayaloglu et al. 2007). Presence of these bacterial groups is typically found in semi-hard artisanal cheeses (Serhan et al. 2009). In a study, it was mentioned that *Lactococcus garviae* is a well recognized fish pathogen and isolated from subclinical mastitis in water buffalos. Also, *L. garvieae* was reported as a member of autochthonous microbiota of dairy products from raw

milk. But, *L. garvieae* was known to be harmless to human health. *L. garvieae* dairy strains have desirable manufactural properties and some authors propose that are this bacterium be a part of starter culture. It is known that the *Enterococcus* genera are typically found in raw milk cheeses. Some of enteric bacteria such as coliform, enterococci, and related bacteria are involved in fecal contamination and evaluated as fecal indicator in milk product. Presence of these bacteria gives some information about poor manufacturing practice and sanitation (Alegria et al. 2009). Tulum cheese has various stages of coliform bacteria during its production. It has been shown that they found at higher levels in the beginning of ripening. However, with the aging of the cheese, the number of coliform bacteria decreases (Hayaloglu et al. 2007). Hence, coliform bacteria were not detected with culture based analyses, but found in DGGE.

It was reported that the lactic acid bacteria such as *Enterococcus* and *Lactobacillus* genera are predominantly found in Tulum cheeses. Unripened cheeses generally included 16 strains which have members of *Enterococcus*, *Lactobacillus*, *Streptococcus*, *Lactococcus* and *Pediococcus* genera. Some of them decrease when cheese was ripened. Contrarly to general LAB, *Enterococcus* genera increase due to their tolerance of high salt and low pH levels. *Lactobacillus* and *Lactococcus* genera are predominant in unripened cheeses, but *Lactococcus* genus disappear after 3 months of ripening, *Lactobacillus* and *Enterococcus* genera become dominant in late ripening (Cakmakci et al. 2008). According to the bacterial numbers of cheeses samples, it could be said that *Enterococcus* spp., *Lactococcus* spp. and *Lactobacillus* spp. were not decreased while cheeses were ripening (Table 3). Gurses and Erdogan(2006) studied isolation and identification of LAB of Tulum cheeses by culturel methods in Erzurum, Turkey. According to their results, *Lactobacillus* was determined as dominant genus, and the others were also found in low proportions in Erzurum Tulum cheese.

Enterococcus genus played a significant role during ripening. Thus, some cheeses had unique taste and odor. In a study, it was shown that *E. faecium* and *E. faecalis* were isolated from Tulum cheeses ripened both in plastic containers and goat skin bag (Cakmakci et al. 2008). In the present study, we have detected these genera in C2 samples by both culture based and DGGE analyses. It may indicate that *E. faecalis* or *E. faecium* is a member of starter culture combination of Tulum cheese. *Lactobacillus* genus which is known as main group of LAB and found in many cheeses (Cakmakci et al. 2008). *Lactobacillus gallinarum* was only detected predominantly in C2 sample. Kara and Akkaya (2015) studied Lactic acid bacteria of Afyon Tulum cheese in Afyonkarahisar, Turkey. Lactic acid bacteria 53.01%, *Lactobacillus* spp., 12.04%, *Lactococcus* spp., 8.43% *Leuconostoc* spp., 1.20% *Pediococcus* spp. and 25.30% *Enterococcus* spp. were isolated in Afyon Tulum cheese samples. *Lactobacillus paracasei* ssp. paracasei (15.66%) was identified the highest percentage.

Streptococcus genus is naturally found in raw milk and can be encountered in every step of cheese ripening. In a study, Tulum cheeses produced without starter culture, the streptococcal bacteria was about 4.23×10^6 cfu/g, while cheeses ripened with starter culture it was 6.17×10^6 cfu/g (Kamber 2007). In our study, Streptococcal bacteria were not seen on agar plates, but in the DGGE results *Streptococcus thermophilus*, *Streptococcus infantarius* subs. *infantarius* (in C1, C2 and C3 samples) and *Streptococcus equinus* (C1 sample) were found.

Conclusion

In this study, we described the dynamics of microbial composition of unique Izmir Tulum cheeses after production process by traditional culture method and 16S rRNA gene analysis such as DGGE and Q-PCR methods. Totally, eight different microorganisms were identified with DGGE.

İzmir Tulum cheese is made from milk obtained from cows, sheep and goats fed by natural vegetation and water sources of İzmir and its counties. The use of coarse sea salt in the production of Izmir Tulum cheese prevents cheese from easily spreading. At the same time, it provides a suitable environment for the development of microflora that specific to İzmir Tulum cheese. DGGE technique was useful for the determination and profiling of microbial community found in cheese samples.

In a future, it would be interesting to determine the effects of biochemical characteristics of milk (pH, nutrients, a_W, etc.) and the influence of changes in conditions on this microbial community succession.

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