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Full Length Research Paper

Some technological properties of phenotypically identified enterococci strains isolated from Turkish tulum cheese

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In this study, a total of 39 enterococci strains were isolated and identified as 17 *Enterococcus faecium* (43.58%), 11 *Enterococcus faecalis* (28.21%) and 11 *Enterococcus durans* (28.21%) strains from 28 Tulum cheese samples from Isparta, Turkey. Three *E. faecium* (EYT6, EYT21 and EYT34) and 1 *E. durans* (EYT16) strains showed α-hemolytic activity on Sheep Blood Agar. None of the all strains exhibited β-hemolysis. All of the 39 enterococci strains were sensitive to vancomycin (30 µg vancomycin per disk, inhibition zone > 12 mm). Three *E. faecium* (EYT17, EYT31 and EYT39) and 1 *E. durans* (EYT19) strains were found as bacteriocin producer. *E. faecium* strains showed higher acidifying ability than *E. faecalis* and *E. durans* strains. The highest proteolytic activity value (100.5 µg tyrosine/ml) was obtained from *E. faecalis* EYT18. Proteolytic activity results showed that *E. faecalis* strains generally more active than *E. faecium* and *E. durans* strains. *E. faecalis* strains were found the most lipolytic, followed by the *E. faecium* and *E. durans* strains and *E. faecalis* EYT7 strain gave the maximum halo radius (0.53 mm). None of the 39 enterococci strains decarboxylated histidine, lysine or ornithine. However, 36 of the 39 strains (92.31%) produced tyramine from tyrosine.

Key words: Enterococci, tulum cheese, industrial properties.

INTRODUCTION

In Turkey, more than 50 varieties of cheese can be found; however, Beyaz, Kasar and Tulum cheeses are the most popular. Based on the data from Turkish Statistical Institute (Ankara, Turkey) the production of Tulum cheese was 10,000 tonnes in 2004 and also its production has increased greatly over recent years (Hayaloglu et al., 2007).

Enterococcus faecalis, *Enterococcus faecium* and *Enterococcus durans* are the species most frequently found in dairy products, where they may contribute to ripening of the cheese and production of the flavor (Cogan et al., 1997; Franz et al., 1999). Depending on the stage of ripening, they can reach numbers up to 10^4 - 10^7 cfu/g in cheeses due to their resistance to pasteurization temperatures and adaptability to different substrates and growth conditions such as low and high temperature, extreme pH and salinity (Morandi et al., 2006). Therefore, enterococci can become an important part of the fermented food microbiota, especially in cheese. Recently, specific enterococcal strains have been used as probiotic adjunct cultures in Cheddar cheese for their ability to improve the microbial balance of the intestine (Gardiner et al., 1999; Giraffa, 2003).

Experimental work suggested that enterococci may play an important role in the development of organoleptic characteristics during the ripening of cheese (Coppola et al., 1990; Litopoulou-Tzanetaki and Tzanetaki, 1992; Manolopoulou et al., 2003). The proteolytic and lipolytic activities displayed by some enterococcal strains, as well as their ability to metabolise citrate, may contribute to cheese ripening and flavour development. Because of these interesting metabolic traits, enterococci have been proposed as part of defined starter culture combinations for different European cheeses, such as Mozzarella (Villani and Coppola, 1994), Venaco (Casalta and Zennaro, 1997), Cebreiro (Centeno et al., 1999), Feta (Sarantinopoulos et al., 2002) and White pickled (Beyaz cheese) (Dagdemir and Ozdemir, 2008; Durlu-Ozkaya et al., 2001) cheeses. Enterococci have the ability to produce bacteriocins (enterocins) which are small peptides

with antimicrobial activity towards closely related Gram-positive bacteria including spoilage or pathogenic bacteria, such as *Listeria, Staphylococcus* and *Clostridium* sp. (De Vuyst and Vandamme 1994; Giraffa et al., 1995; Sarantinopoulos et al., 2002).

The aim of the study was to characterize the enterococci isolated from Turkish Tulum cheese. Industrial traits such as proteolytic, lipolytic and decarboxylase activity, bacteriocin and lactic acid production were also investigated in these strains.

MATERIALS AND METHODS

Tulum cheese samples

A total of 28 Turkish tulum cheese samples were randomly obtained from various local bazaars in Isparta, Turkey. Samples were transported under cold conditions from their place of collection to the laboratory.

Bacterial strains and growth media

Kanamycin Esculin Azide Agar (KAA) medium (Merck KgaA, Darmstadt, Germany) was used for isolation of presumptive enterococci. Enterococci strains were cultured in MRS broth (Merck KgaA, Darmstadt, Germany) at 37°C. A total of 20 indicator bacteria were used for the screening of bacteriocin production and evaluation for antimicrobial activities by enterococci strains. The 17 bacterial strains was provided by the Laboratory of Microbial Gene Technology, NLH, Ås, Norway and 3 bacterial strains (*Staphylococcus aureus* 13, *Bacillus cereus* 7 and *Listeria monocytogenes* 15) were obtained from Süleyman Demirel University, Food Engineering Department Culture Service, Isparta, Turkey among 20 indicator bacteria. All strains were kept in 15% glycerol and stored at -20°C. Agar media were prepared by adding 1.5% (w/v) granulated agar (Merck KgaA, Darmstadt, Germany) to corresponding broth media; overlay agars were prepared by the addition of 0.7% (w/v) granulated agar to the broth media.

Isolation and phenotypic identification of enterococci

For the isolation of presumptive enterococci, 10 g of each cheese sample was homogenized in 90 ml of sterile peptone water (Merck KgaA, Darmstadt, Germany). Serial 10-fold dilutions were performed and aliquots were plated on KAA medium. After 48 h incubation at 37°C, typical colonies of presumptive enterococci (which appear surrounded by a black halo) were randomly picked from plates and subcultured in order to obtain pure isolates. Presumptive enterococci isolates were cultured in MRS broth with incubation at 37°C for 18 h. Pure cultures were kept frozen at -20°C in MRS broth containing glycerol (15% v/v).

Presumptive enterococci strains were further characterized based on morphology, Gram reaction, catalase and culturel tests such as, growth in MRS broth at 10 °C, 45 °C and pH 9.6, tolerance of different salt levels (4 and 6.5% NaCl), growth in 0.1% methylen blue milk, CO_2 production from glucose, arginine and esculin hydrolysis, reduction of 0.01% tetrazolium, resistance to 0.04% tellurite and resistance to heat (60°C for 15 and 30 min) (Holt et al., 1994; Manero and Blanch, 1999; Sharpe, 1979). Sugar fermentation profiles of enterococci were determined in a modified MRS broth without glucose and beef extract, containing 0.04 g/l chlorophenol red (Merck KgaA, Darmstadt, Germany) as a pH indicator and supplemented with 1% of each specific sugar (Manero and Blanch, 1999).

Hemolytic activity and vancomycin resistance

Hemolytic activity was tested by inoculating activated cultures onto Sheep Blood Agar (Salubris A.Ş., İstanbul, Turkey) plates and incubating at 37°C for 48 h. Sheep Blood Agar plates were examined for signs of β-hemolysis (clear zones around colonies), α-hemolysis (green-hued zones around colonies) or γ-hemolysis (no zones around colonies).

Vancomycin resistance was tested by disk diffusion method on plates of MRS agar. The antibiotic was used in disks (Oxoid, Basingstoke, England), containing 30 µg of vancomycin.

Determination of antimicrobial activity and sensitivity to proteolytic enzymes

Enterococci strains were grown overnight at 37°C on MRS agar medium then these strains were transferred to MRS plates by using sterile toothpicks. After growth overnight at 37°C, an indicator lawn of 5 ml of soft agar (0.7%), containing 100 µl of an over night culture of indicator strain, was poured on the surface. After 18 h of incubation, the colonies were examined for zones of inhibition (Van Belkum et al., 1989).

The proteinaceous nature of the antimicrobial compound was verified by testing its sensitivity to proteolytic enzymes. The cell-free supernatant fluids were obtained by centrifuging enterococci cultures at 6,000*g*, 4°C for 15 min (Sigma 3K 30, rotor 12111). The cell-free supernatant adjusted to pH 7.0 with 1 N NaOH was incubated with various proteolytic enzymes at a final concentration of 1 mg/ml. Trypsin from bovine pancreas (Cat. No. 0785, pH 7.0, Amresco, Solon, Ohio, USA), pepsin from porcine gastric mucosa (Cat. No.P6887, pH 3.0, Sigma Chem Co, USA), α-chymotrypsin from bovine pancreas (Cat. No.C4129, pH 7.0, Sigma Chem Co, USA) and proteinase K from *Tritirachium album* (Cat. No. 0706, pH 7.0, Amresco, Solon, Ohio, USA) were used as proteolytic enzymes in this test. The preparations were incubated at 37°C for 2 h and then autoclaved at 80°C for 5 min to inactivate enzymes. The remaining antibacterial activity was tested by well diffusion method using *Listeria innocua* LMG2813 for EYT9, EYT19 and EYT22 and *Bacillus cereus* LMG2732 for EYT17, EYT31 and EYT39 as indicators. The supernatant adjusted to pH 7.0 without enzymes was used as a control (Franz et al., 1997).

Acidification ability

The acid production of the strains studied was tested by inoculating stationary cultures of each of them (1% v/v) into reconstitute skim milk (RSM, 10% w/v). After incubation at 37°C for 6 and 24 h, the acidity was measured by titration of the cultures at pH 8.2 with 0.1 M NaOH (Bradley et al., 1992). The data were expressed as g lactic acid per 100 ml RSM (10% w/v). Titratable acidity measures were estimated using two replicates for each bacterial strains.

Proteolytic activity

The determination of the proteolytic activity of the strains was measured according to the International Dairy Federation 149A (IDF, 1997). Stationary cultures of each strain were inoculated into RSM (10% w/v) and incubated 37°C for 24 h. Afterwards, the concentration of free aromatic amino acids liberated was measured spectrophotometrically at 650 nm (Shimadzu UV-1601 spectrofotometer). Results are expressed as µg tyrosine/ml of RSM.

Lipolytic activity

Enterococci strains were grown overnight at 37°C in MRS broth. A loopful fresh culture was placed on Tributyrin Agar (Leuschner et al., 1997). Plates were incubated at 37°C for 4 days and observed daily for halo formation around the colonies. The radius of the halo formation (in mm) at the end of incubation was measured.

Decarboxylase activity

The ability to produce biogenic amines by decarboxylation of amino acids was tested on a media designed by Bover-Cid and Holzapfel (1999), which contained either of the precursor amino acids tyrosine, histidine, ornithine or lysine. In order to induce decarboxylase activity before the actual screening test, the enterococci strains were subcultured twice in MRS broth containing 0.1% of each precursor amino acid and 0.005% pyridoxal-5-phosphate. The latter compound was previously shown to be important for inducing decarboxylase activity (Recsei et al., 1985). A 5 µl volume of each bacteria culture was spotted onto agar plates with and without amino acids and plates were incubated aerobically at 37°C for 2 - 5 days. Plates were observed for a purple colour in the producing and surrounding colonies to indicate production of biogenic amines from precursor amino acids.

RESULTS AND DISCUSSION

Isolation and phenotypic identification of enterococci

A total of 39 enterococci strains were isolated from 28 Turkish tulum cheese samples. Phenotypic characteristics of the strains were given in Table 1. All of the isolates were Gram-positive, catalase-negative, non-motile, ovoid cocci, occurring single, as pairs or in chains, resistance to heat (60°C for 15 and 30 min). All of the isolates were growth at 10 °C, 45 °C and pH 9.6; grown in presence of 4 and 6.5 % NaCl. In addition, these strains produced NH_3 from arginine and hydrolysed esculin but not produced CO_2 from glucose. 11 (28.21%) of 39 enterococci strains seemed to be *E. faecalis* (EYT7, EYT9, EYT11, EYT15, EYT18, EYT20, EYT23, EYT29, EYT33, EYT35 and EYT37), as suggested by their ability to grow in the presence of 0.04% tellurite, reduce 0.01% tetrazolium and ferment glycerol, melezitose, rhamnose and sorbitol (Devriese et al., 1993; Manero and Blanch, 1999; Sharpe, 1979). 28 enterococci strains, unable to either grow in the presence of tellurite or reduce tetrazolium, were differentiated by their ability to form acid from sugars. 17 (43.58%) of 28 enterococci strains producing acid from arabinose and melibiose were characterized as *E. faecium* (EYT1, EYT3, EYT4, EYT5, EYT6, EYT8, EYT12, EYT14, EYT17, EYT21, EYT22, EYT26, EYT27, EYT30, EYT31, EYT34 and EYT39). Other strains (28.21%) were characterized as *E. durans* (EYT2, EYT10, EYT13, EYT16, EYT19, EYT24, EYT25, EYT28, EYT32, EYT36 and EYT38) by their sugar fermentation profiles (Devriese et al., 1993; Manero and Blanch, 1999). *E. faecalis*, *E. faecium* and *E. durans* strains have also been isolated from various artisanal cheeses produced in European area by other researchers (Dagdemir and Ozdemir, 2008; Durlu-Ozkaya et al., 2001; Morandi et al., 2006). Previous studies showed that *E. faecium* strains predominates in various fermented foods, including sausage, cheese, fermented vegetables and fermented milk (Hugas et al., 2003; Klein, 2003; Oner et al., 2004) as confirmed in this study.

Hemolytic activity and vancomycin resistance

In the present paper, two of the major factors involved in a potential health risk associated with the use of enterococci were evaluated, namely cytolysin production and vancomycin resistance (Foulquíe-Moreno et al., 2003). In this study, the 3 *E. faecium* (EYT6, EYT21 and EYT34) and 1 *E. durans* (EYT16) strains exhibited α-hemolytic activity on Sheep Blood Agar. Other strains studied displayed a γ–hemolytic activity, indicating absence of hemolysis. None of the enterococci strains exhibited β-hemolysis. Hemolysis plays an important role in enterococcal virulence, as it may increase the possibility of the infection (Morandi et al., 2006).

Another important factor for the safety evaluation of enterococci is its resistance to glycopeptide vancomycin (Yoon et al., 2008). In this study, all enterococci strains were sensitive to vancomycin (30 µg vancomycin per disk, inhibition zone > 12 mm). Enterococci isolated from dairy products are known to be characterized by a higher sensitivity to most antibiotics than strains isolated from environmental and clinical sources (Batish and Ranganathan, 1986; Busani et al., 2004; Knudtson and Hartman, 1993; Messi et al., 2006).

Determination of antimicrobial activity and sensitivity to proteolytic enzymes

The growth media and incubation temperature of the bacterial strains used as indicator bacteria and inhibitory spectrum of six enterococci strains (EYT9, EYT17, EYT19, EYT22, EYT31 and EYT39) are shown in Table 2. There were several patterns of activities against the indicator bacteria. *S. aureus* LMG3022, *S. aureus* 13, *Staphylococcus carnosus* LMG2709, *L. monocytogenes* 15, *Escherichia coli* LMG3083 CFAI (ETEC) and *S. enterica* serotype Typhimurium SL1344 were not inhibited by any strain. *E. faecium* EYT17 and EYT31 strains showed the same antibacterial spectrum. These strains were found to show inhibitory activity against a broad range of bacteria including lactobacilli, lactococci and enterococci. In addition, they showed inhibitory activity against *L. innocua*, *B. cereus* (Figure 1), *Pediococcus pentosaceus* and *Pseudomonas fluorescens*. *E. durans* EYT19 and *E. faecium* EYT39 strains exhibited inhibitory activitiy towards 10 and 11 indicator bacteria among tested 20 indicator bacteria, respectively. *E. faecalis*

Table 1. Phenotypic characteristics of enterococci strains isolated from Turkish tulum cheese.

Characteristics	*Enterococcus faecalis* (n = 11)	*Enterococcus faecium* (n = 17)	*Enterococcus durans* (n = 11)
Gram reaction	+	+	+
Morphology	Ovoid cocci	Ovoid cocci	Ovoid cocci
Catalase	-	-	-
Motility	-	-	-
Growth at:			
10°C	+	+	+
45°C	+	+	+
pH 9.6	+	+	+
Growth in:			
4.0 % NaCl	+	+	+
6.5 % NaCl	+	+	+
0.1 % Methylene blue milk	+	+	+
0.04 % Tellurite	+	-	-
0.01 % Tetrazolium	+	-	-
CO_2 from glucose	-	-	-
Esculin hydrolisis	+	+	+
NH_3 from arginine	+	+	+
Survival at 60°C for:			
15 min	+	+	+
30 min	+	+	+
Acid from:			
Arabinose	-	+	-
Glycerol	+	-	-
Lactose	+	+	+
Maltose	+	+	+
Mannitol	+	+	-
Melibiose	-	+	-
Melezitose	+	-	-
Raffinose	-	-	-
Rhamnose	+	-	-
Sorbitol	+	-	-
Sucrose	+	+	-
Trehalose	+	+	+

+ = Positive reaction; - = negative reaction; n = number of enterococci strains.

EYT9 and EYT22 strains showed inhibitory effect only against *L. innocua* LMG2813.

Proteolytic enzyme assays showed that the antimicrobial substances produced by 3 *E. faecium* (EYT17, EYT31 and EYT39) and 1 *E. durans* EYT19 strains had a proteinaceous nature and were eliminated by treatment with proteinase K and partially eliminated with trypsin, α-chymotrypsin and pepsin (data not shown). However, no loss of activities of antimicrobial substances produced by *E. faecalis* EYT9 and EYT22 were observed when treated with these proteolytic enzymes (proteinase K, trypsin, α-chymotrypsin and pepsin). Previous studies showed that enterococci have the ability to produce enterocins (bacteriocins) which are small peptides with antimicrobial activity towards closely related Gram-positive bacteria including spoilage or pathogenic bacteria, such as *Staphylococcus*, *Listeria* and *Clostridium* sp. (Aymerich et al., 2000; De Vuyst and Vandamme, 1994; Giraffa, 2003; Moreno et al., 2002; Sarantinopoulos et al., 2002).

Acidification ability

The results for acid production after 6 and 24 h growth in RSM at 37°C are shown in Figure 2. After 6 h growth in 100 ml RSM at 37°C, 9 *E. faecium* (EYT1, EYT3, EYT4, EYT6, EYT14, EYT17, EYT27, EYT30 and EYT31), 9 *E.*

Table 2. Growth media and incubation temperature of indicator strains and inhibitory spectrum of six enterococci strains isolated from Turkish Tulum cheese.

Indicator strains	Media[a]	Temp (ºC)	Inhibition zone (ømm)					
			EYT9	EYT17	EYT19	EYT22	EYT31	EYT39
Enterococcus faecalis LMG 2708	M17G	30	-	18	10	-	18	18
Enterococcus faecalis LMG 2602	M17G	30	-	18	7	-	19	19
Lactococcus lactis JC17 (lacticin 481 producer)	M17G	30	-	16	10	-	16	18
Lactococcus lactis T1	M17G	30	-	18	11	-	18	19
Lactococcus lactis 731	M17G	30	-	17	10	-	19	18
Lactococcus lactis 2	M17G	30	-	12	9	-	14	14
Lactococcus lactis SIK-83 (nisin producer)	M17G	30	-	13	7	-	14	15
Lactobacillus plantarum LMG 2003	MRS	37	-	6	-	-	8	-
Lactobacillus sakei NCDO 2714	MRS	37	-	5	-	-	10	-
Staphylococcus aureus LMG 3022	TSB	37	-	-	-	-	-	-
Staphylococcus aureus 13	TSB	37	-	-	-	-	-	-
Staphylococcus carnosus LMG 2709	TSB	37	-	-	-	-	-	-
Listeria monocytogenes 15	LB	30	-	-	-	-	-	-
Listeria innocua LMG 2813	LB	30	6	13	12	7	17	15
Bacillus cereus LMG 2732	TSB	37	-	18	7	-	18	18
Bacillus cereus 7	TSB	37	-	15	-	-	17	16
Pediococcus pentosaceus LMG 2001	TSB	37	-	7	-	-	11	-
Escherichia coli LMG 3083 CFAI (ETEC)	LB	37	-	-	-	-	-	-
S. enterica serotype Typhimurium SL1344	LB	37	-	-	-	-	-	-
Pseudomonas fluorescens P1	LB	37	-	16	10	-	18	17

[a] M17G: Glucose-M17 broth (0.5% glucose); MRS: De Man, Rogosa and Sharpe Broth; LB: Luria Bertani Broth; TSB: Triyptic Soy Broth.

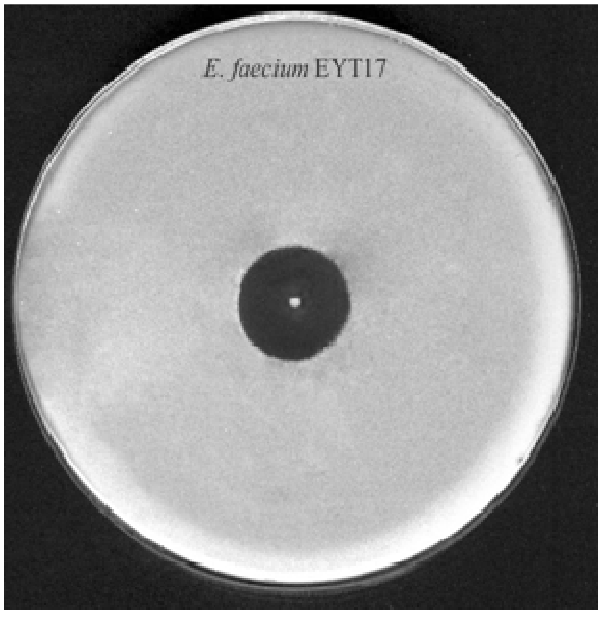

Figure 1. Antimicrobial activity of *E. faecium* EYT17 on *Bacillus cereus* LMG2732.

faecalis (EYT7, EYT9, EYT11, EYT18, EYT20, EYT23, EYT29, EYT35 and EYT37) and 7 *E. durans* (EYT10, EYT13, EYT24, EYT28, EYT32, EYT36 and EYT38) strains developed acidity of <0.30 g lactic acid. The 8 *E. faecium* (EYT5, EYT8, EYT12, EYT21, EYT22, EYT26, EYT34 and EYT39), 2 *E. faecalis* (EYT15 and EYT33) and 4 *E. durans* (EYT2, EYT16, EYT19 and EYT25) strains produced acidity of 0.30-0.40 g lactic acid. At the same time, the maximum acidity (0.36 g lactic acid/100 ml RSM) was produced by *E. faecium* EYT22 strain. After 24 h of incubation, 10 *E. faecium* (EYT3, EYT5, EYT8, EYT12, EYT14, EYT21, EYT22, EYT26, EYT34 and EYT39), 3 *E. faecalis* (EYT15, EYT29 and EYT33) and 5 *E. durans* (EYT2, EYT10, EYT16, EYT19 and EYT25) strains expressed a good acidifying activity (>0.50 g lactic acid/100 ml RSM). The 6 *E. faecium* (EYT4, EYT6, EYT17, EYT27, EYT30 and EYT31), 5 *E. faecalis* (EYT9, EYT11, EYT20, EYT23 and EYT37) and 5 *E. durans* (EYT13, EYT24, EYT28, EYT32 and EYT38) strains produced acidity of 0.40-0.50 g lactic acid/100 ml RSM. The 1 *E. faecium* (EYT1), 3 *E. faecalis* (EYT7, EYT18 and EYT35) and 1 *E. durans* (EYT36) strains expressed a low acidifying activity (0.30-0.40 g lactic acid/100 ml RSM). In this study enterococci strains examined in general, *E. faecium* showed higher acidifying ability than *E. faecalis* and *E. durans*. However, the higher acidifiying potential of *E. faecalis* strains than *E. faecium* and *E. durans* have been also confirmed by different researchers (Dagdemir and Ozdemir, 2008; Suzzi et al., 2000). Acidification activity is an important criterion for the selection of lactic starter culture strains in cheese manufacture

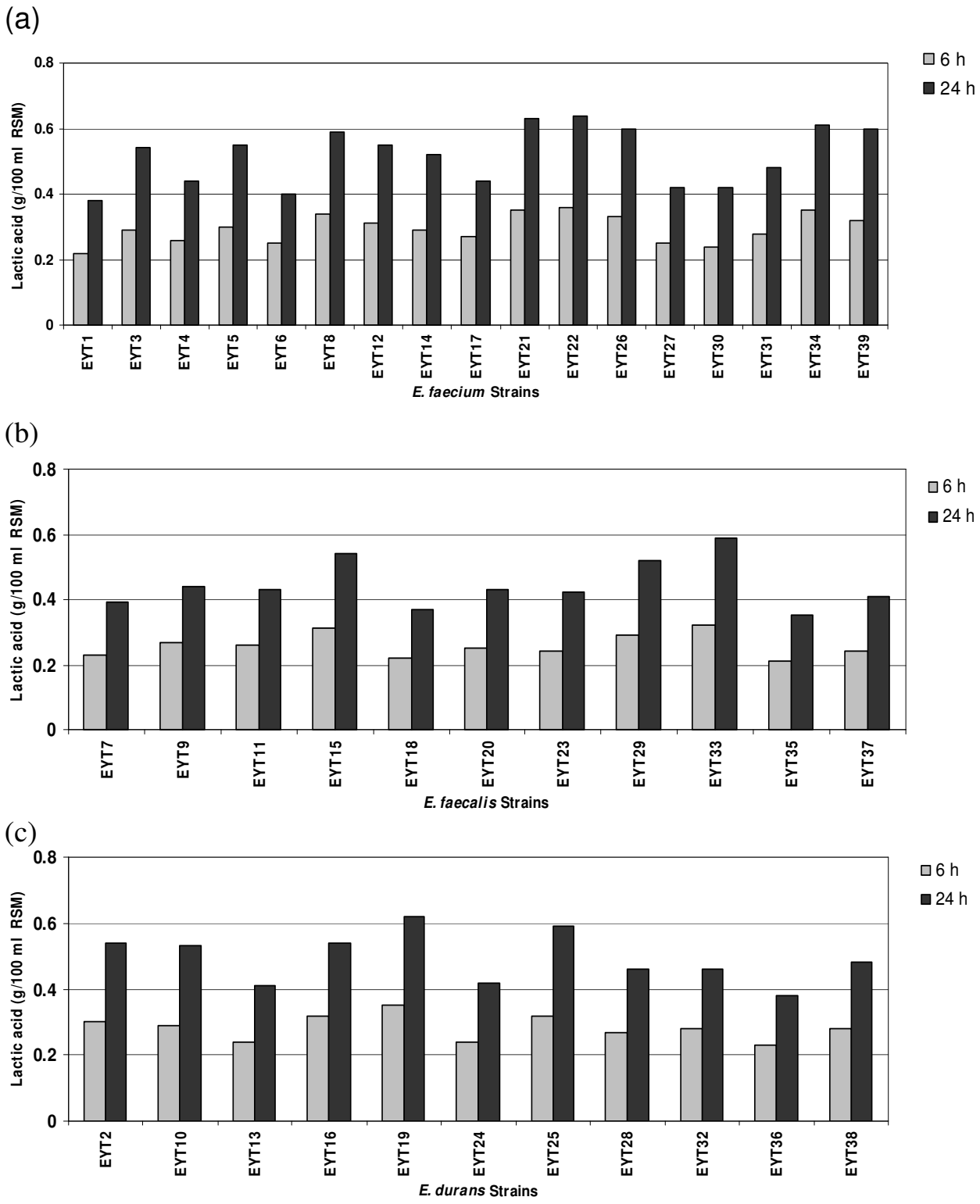

Figure 2. Lactic acid production of *E. faecium* (a), *E. faecalis* (b) and *E. durans* (c) strains after growth in RSM for 6 and 24 h at 37°C.

(Özkalp et al., 2007). Fast acid producers strains can be used as starter culture for coagulation and the prevention or reduction of the growth of adventitious microflora (Morandi et al., 2006).

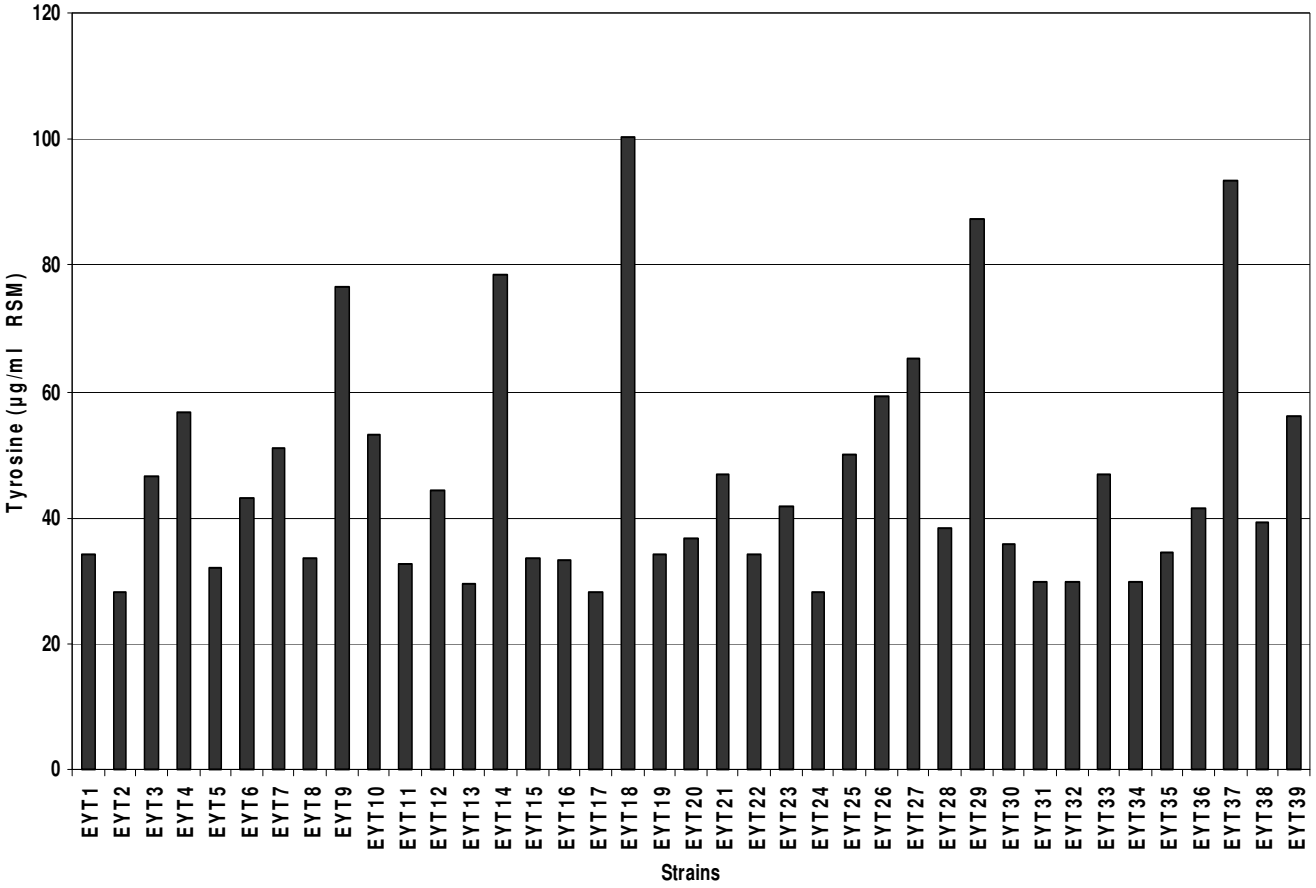

Figure 3. Proteolytic activities of *Enterococcus* strains after growth in RSM for 24 h at 37°C.

Proteolytic activity

Proteolytic activity of the enterococci strains ranged between 28.1 and 100.5 µg tyrosine/ml after growth in RSM for 24 h at 37°C (Figure 3). The highest proteolytic activity value was obtained in *E. faecalis* EYT18 strain. Proteolytic activity after 24 h growth in RSM at 37°C, 5 *E. faecalis* (EYT7, EYT9, EYT18, EYT29 and EYT37), 5 *E. faecium* (EYT4, EYT14, EYT26, EYT27 and EYT39) and 2 *E. durans* (EYT10 and EYT25) strains were measured > 50 µg tyrosine/ml. The 9 *E. faecium* (EYT1, EYT3, EYT5, EYT6, EYT8, EYT12, EYT21, EYT22 and EYT30), 6 *E. faecalis* (EYT11, EYT15, EYT20, EYT23, EYT33 and EYT35) and 5 *E. durans* (EYT16, EYT19, EYT28, EYT36 and EYT38) strains were measured 30 - 50 µg tyrosine/ml. Low proteolytic activity (< 30 µg tyrosine/ml) was detected in 3 *E. faecium* (EYT17, EYT31 and EYT34) and 4 *E. durans* (EYT2, EYT13, EYT24 and EYT32) strains. Proteolytic activity results showed that *E. faecalis* strains generally more active than *E. faecium* and *E. durans* strains as confirmed in previously reported by other studies (Andrighetto et al., 2001; Dagdemir and Ozdemir, 2008; Morandi et al., 2006; Sarantinopoulos et al., 2001).

Lipolytic activity

In this study, lipolytic activity assay confirmed that *E. faecalis* strains were the most lipolytic, followed by the *E. faecium* and *E. durans* strains (Figure 4). Two *E. faecalis* (EYT7 and EYT15) and 2 *E. faecium* (EYT17 and EYT27) strains gave a halo radius > 0.50 mm, but none of the 11 *E. durans* strains showed a halo radius > 0.50 mm. *E. faecalis* EYT7 strain gave the maximum halo radius (0.53 mm). The 5 *E. faecalis* (EYT9, EYT11, EYT18, EYT23 and EYT29), 3 *E. faecium* (EYT4, EYT6 and EYT22) and 2 *E. durans* (EYT16 and EYT28) gave a halo radius 0.30-0.50 mm and 3 *E. faecalis* (EYT20, EYT35 and EYT37), 8 *E. faecium* (EYT3, EYT5, EYT8, EYT21, EYT26, EYT31, EYT34 and EYT39) and 3 *E. durans* (EYT13, EYT19 and EYT38) strains showed a halo radius 0.10 - 0.30 mm. The 4 *E. faecium* (EYT1, EYT12, EYT14 and EYT30), 6 *E. durans* (EYT2, EYT10, EYT24, EYT25, EYT32 and EYT36) and 1 *E. faecalis* (EYT33) strains were not able to hydrolyse tributyrin (< 0.10 mm radius halo). Lipolysis plays an important role in the development of flavour and texture during cheese ripening (Sarantinopoulos et al., 2001). Lipolytic activity of enterococci has been previously reported with *E. faecalis*

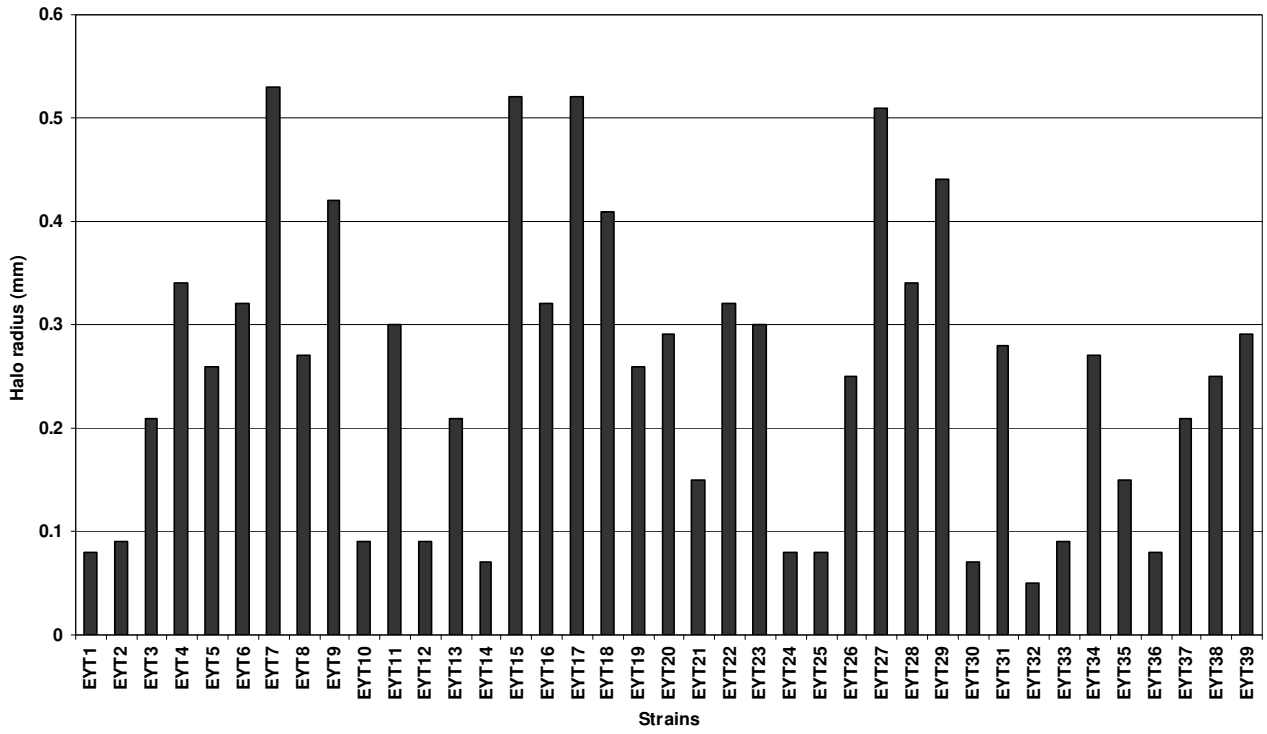

Figure 4. Lipolytic activities of *Enterococcus* strains in Tributyrin agar.

being the most lipolytic species, followed by *E. faecium* and *E. durans* (Giraffa, 2003; Sarantinopoulos et al., 2001) as confirmed in this study.

Decarboxylase activity

None of the 39 enterococci strains decarboxylated histidine, lysine or ornithine. However, 36 of the 39 strains (92.31%) produced tyramine from tyrosine. The tyrosine decarboxylase negative strains were *E. durans* EYT10, *E. durans* EYT24 and *E. faecium* EYT26. These results are in agreement with results previously reported, suggesting that tyramine was the only biogenic amine formed by enterococci (Bover-Cid and Holzapfel, 1999; Giraffa et al., 1995; Sarantinopoulos et al., 2001).

REFERENCES

Andrighetto C, Knijff E, Lombardi A, Torriani S, Vancanneyt M, Kersters K, Swings J, Dellaglio F (2001). Phenotypic and genetic diversity of enterococci isolated from Italian cheeses. J. Dairy Res. 68: 303-316.

Aymerich T, Garriga M, Ylla J, Vallier J, Monfort JM, Hugas M (2000). Application of enterocins as biopreservatives against *Listeria innocua* in meat products. J. Food Prot. 63: 721-726.

Batish VK, Ranganathan B (1986). Antibiotic susceptibility of deoxyribonuclease-positive enterococci isolated from milk and milk products and their epidemiological significance. Int. J. Food Microbiol. 3: 331-337.

Bover-Cid S, Holzapfel WH (1999). Improved screening procedure for Biogenic amine production by lactic acid bacteria. Int. J. Food Microbiol. 53: 33-41.

Bradley RL, Arnold E, Barbano DM, Semerad RG, Smith DE, Vines BK (1992). Chemical and physical methods. In Standart methods for the examination of dairy product. American Public Health Association, Washington, DC, pp. 433-531.

Busani L, Grosso MD, Paladini C, Granziani C, Pantosti A, Biavasco F, Caprioli A (2004). Antimicrobial susceptibility of vancomycin-susceptible and vancomycin-resistant enterococci isolated in Italy from raw meat products, farm animal and human infections. Int. J. Food Microbiol. 97: 17-22.

Casalta E, Zennaro R (1997). Effect of specific starters on microbiological, biochemical and sensory characteristics of Venaco, a Corsican soft cheese. Sci. Aliment. 17: 79-94.

Centeno JA, Menendez S, Hermida MA, Rodríguez-Otero JL (1999). Effects of the addition of *Enterococcus faecalis* in Cebreiro cheese manufacture. Int. J. Food Microbiol. 48: 97-111.

Cogan TM, Barbosa M, Beuvier E, Bianchi-Salvadori B, Cocconcelli PS, Fernandez I, Gomez J, Gomez R, Kalantzopoulos G, Ledda A, Medina M, Rea MC, Rodriguez E (1997). Characterization of the lactic acid bacteria in artisanal dairy products. J. Dairy Res. 64: 409-421.

Coppola S, Villani F, Coppola R, Parente E (1990). Comparison of different starter systems for water-buffalo Mozzarella cheese manufacture. Lait. 70: 411-423.

Dagdemir E, Ozdemir S (2008). Technological characterization of the natural lactic acid bacteria of artisanal Turkish White Picled cheese. Int. J. Dairy Technol. 61: 133-140.

De Vuyst L, Vandamme EJ (1994). Bacteriocins of Lactic Acid Bacteria. Microbiology, Genetics and Applications. Chapman and Hall. New York.

Devriese LA, Pot B, Collins MD (1993). Phenotypic identification of the genus *Enterococcus* and differentiation of phylogenetically dinstinct enterococcal species and species groups. J. Appl. Bacteriol. 75: 399-408.

Durlu-Ozkaya F, Xanthopoulos V, Tunail N, Litopoulou-Tzanetaki E (2001). Technologically important properties of lactic acid bacteria

isolated from Beyaz cheese made from raw ewes' milk. J. Appl. Microbiol. 91: 861-870.
Foulquíe-Moreno MR, Callewaert R, Devreese B, Van Beeumen J, De Vuyst L (2003). Isolation and biochemical characterization of enterocins produced by enterococci from different sources. J. Appl. Microbiol. 94: 214-229.
Franz CMAP, Du Toit M, von Holy A, Schillinger U, Holzapfel WH (1997). Production of nisin-like bacteriocins by *Lactococcus lactis* strains isolated from vegetables. J. Basic Microbiol. 37: 187-196.
Franz CMAP, Holzapfel WH, Stiles ME (1999). Enterococci at the cressroads of food safety? Int. J. Food Microbiol. 47: 1-24.
Gardiner GE, Stanton C, Lynch PB, Collins JK, Fitzgerald GF, Ross, RP (1999) Evaluation of Cheddar cheese as a good carrier for delivery of a probiotic strain to the gastrointestinal tract. J. Dairy Sci. 82: 379-1387.
Giraffa G (2003). Functionality of enterococci in dairy products. Int. J. Food Microbiol. 88: 215-222.
Giraffa G, Pepe G, Locci F, Neviani E, Carminati D (1995). Hemolytic activity, production of thermonuclease and biogenic amines by dairy enterococci. Italian J. Food Sci. 4: 341-349.
Hayaloglu AA, Cakmakci S, Brechany EY, Deegan KC, McSweeney PLH (2007). Microbiology, biochemistry and volatile composition of Tulum cheese ripened in goat's skin or plastic bags. J. Dairy Sci. 90: 1102-1121.
Holt GH, Krieg NR, Sneath PHA, Staley JT, Williams ST (1994). Bergey's Manual of Determinative Bacteriology. Williams and Wilkins Co., Ninth Edition.
Hugas M, Garriga M, Aymerich MT (2003). Functionality of enterococci in meat products. Int. J. Food Microbiol. 88: 223-233.
IDF, International Dairy Federation (1997). Dairy starter cultures of lactic acid bacteria (LAB). Standard of identity. 149A.
Klein G (2003). Taxonomy, ecology and antibiotic resistance of enterococci from food and gastro-intestinal tract. Int. J. Food Microbiol. 88: 123-131.
Knudtson LM, Hartman PA (1993). Antibiotic resistance among enterococcal isolates from environmental and clinical sources. J. Food Protec. 56: 489-492.
Leuschner RG, Kenneally PM, Arendt EK (1997). Method for the rapid quantitative detection of lipolytic activity among food fermenting microorganisms. Int. J. Food Microbiol. 37: 237-240.
Litopoulou-Tzanetaki E, Tzanetakis N (1992). Microbiological study of white-brined cheese made from raw goat milk. Food Microbiol. 9: 12-19.
Manero A, Blanch AR (1999). Identification of *Enterococcus* spp. with a biochemical key. Appl. Environ. Microbiol. 65: 4425-4430.
Manolopoulou E, Sarantinopoulos P, Zoidou E, Aktypis A, Moschopoulou E, Kandarakis IG, Anifantakis EM (2003). Evolution of microbial populations during traditional Feta cheese manufacture and ripening. Int. J. Food Microbiol. 82: 153-161.
Messi P, Guerrieri E, de Niederh ä usern S, Sabia C, Bondi M (2006). Vancomycin-resistance enterococci (VRE) in meat and environmental samples. Int. J. Food Microbiol. 107: 218-222.
Morandi S, Brasca M, Andrighetto C, Lombardi A, Lodi R (2006). Technological and molecular characterisation of enterococci isolated from North-west Italian dairy products. Int. Dairy J. 16: 867-875.
Moreno MRF, Leisner JJ, Tee LK, Radu S, Rusul G, Vancanneyt M, De Vuyst L (2002). Microbial analysis of Malaysian tempeh and characterization of two bacteriocins produced by isolates of *Enterococcus faecium*. J. Appl. Microbiol. 92: 147-157.
Oner Z, Sagdic O, Simsek B (2004). Lactic acid bacteria profiles and tyramine and tryptamine contents of turkish tulum cheeses. Eur. Food Res. Technol. 219: 455-459.
Özkalp B, Ozden B, Tuncer Y, Sanlıbaba P, Akçelik M (2007). Technological characterization of wild-type *Lactococcus lactis* strains isolated from raw milk and traditional fermented milk products in Turkey. Lait. 87: 521-534.
Recsei PA, Moore WM, Snell EE (1985). Pyruvolyl-dependent histidine decarboxylases from *Clostridium perfringens* and *Lactobacillus buchneri*. J. Biol. Chem. 258: 439-444.
Sarantinopoulos P andrighetto C, Georgalaki MD, Rea MC, Lombardi A, Cogan TM, Kalantzopoulos G, Tsakalidou E (2001). Biochemical properties of enterococci relevant to their technological performance. Int. Dairy J. 11: 621-647.
Sarantinopoulos P, Kalantzopoulos G, Tsakalidou E (2002). Effect of *Enterococcus faecium* on microbiological, physicochemical and sensory characteristics of Grek Feta cheese. Int. J. Food Microbiol. 76: 93-105.
Sharpe ME (1979). Identification of the lactic acid bacteria. In: Identification methods for microbiologists. Academic Press, London.
Suzzi G, Caruso M, Gadrini F, Lombardi A, Vannini L, Guerzoni ME andrighetto C, Lanorte MT (2000). A survey of the Enterococci isolated from an artisanal Italian goat's cheese (semicotto caprino). J. Appl. Microbiol. 89: 267-274.
Van Belkum MJ, Hayema BJ, Geis A, Kok J, Venema G (1989). Cloning of two bacteriocin genes from a lactococcal bacteriocin plasmid. Appl. Environ. Microbiol. 55: 1187-1191.
Villani F, Coppola S (1994). Selection of enterococcal strains for water-buffalo Mozzarella cheese manufacture. Ann. Microbiol. Enzymol. 44: 97-105.
Yoon MY, Kim YJ, Hwang HJ (2008). Properties and safety aspects of *Enterococcus faecium* strains isolated from *Chungkukjang*, a fermented soy product. LWT Food Sci. Technol. 41: 925-933.