TÜBİTAK

Turkish Journal of Biology

http://journals.tubitak.gov.tr/biology/

Research Article

Turk J Biol
(2014) 38: 357-364
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doi:10.3906/biy-1311-34

Investigation of potential virulence genes and antibiotic resistance characteristics of *Enterococcus faecalis* isolates from human milk and colostrum samples

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Received: 12.11.2013 • **Accepted:** 13.01.2014 • **Published Online:** 14.04.2014 • **Printed:** 12.05.2014

Abstract: Enterococci may improve the typical taste and flavor of fermented foods through their proteolytic and lipolytic activities. However, some enterococcal strains are recognized as nosocomial pathogens, which have virulence genes and resistance to certain antibiotics. Enteroccocci are also found in human milk microflora. The aim of this study was to investigate the potential virulence genes and antibiotic resistance characteristics of *Enterococcus faecalis* isolates from human milk and colostrum samples. In total, 23 *Enterococcus faecalis* strains were identified from human milk and colostrum samples. Antibiotic-resistant *E. faecalis* isolates were determined using the disk diffusion method. Vancomycin resistance genes (*vanA*, *vanB*) and some virulence genes (*agg*$_2$, *gelE*, *efaAfm*, *ccf*, *cpd*, *cad*, *cylM*, *cylB*, etc.) were investigated using polymerase chain reaction (PCR). All strains were sensitive to ampicillin, penicillin G, chloramphenicol, and vancomycin. None of the *E. faecalis* isolates contained *vanA*, *vanB*, or *efaAfm* genes. The results of this study indicated that there were no harmful enterococci strains in human milk and colostrum samples in terms of tested virulence factors and antibiotic resistance. Therefore, the *E. faecalis* isolates from human milk may have the potential to be considered as a functional culture for the food industry.

Key words: *Enterococcus faecalis*, virulence gene, antibiotic resistance, human milk, colostrum

1. Introduction

Breast milk and colostrum are the best food for the neonate because they provide all nutrients for infants and play an important role in the protection of the neonate against infectious diseases, since they contain immune system elements and different antimicrobial compounds. Breast milk is also a consistent source of commensal bacteria to the neonatal gut and, therefore, the bacterial composition of the infant fecal flora reflects the bacterial composition of breast milk (Lopez-Alarcon et al., 1997; Wright et al., 1998; Martin et al., 2004; Martin et al., 2005; Lara-Villoslada et al., 2007; Jimenez et al., 2008). The bacteria commonly isolated from breast milk include staphylococci, streptococci, lactobacilli, enterococci, and micrococci. These bacteria are considered to be components of the natural microflora of breast milk (Martin et al., 2004; Albesharat et al., 2011; Jeurink et al., 2013). Among the bacteria isolated from breast milk, species such as *Lactobacillus gasseri*, *Lactobacillus rhamnosus*, or *Enterococcus faecium* are considered to be potential probiotic bacteria (Martin et al., 2004; Martin et al., 2005; Reviriego et al., 2005). Some *Enterococcus faecium* and *E. faecalis* strains are used as starter cultures, co-cultures, or probiotics (Franz et al., 1999; De Vuyst et al., 2003; Franz et al., 2003; Hugas et al., 2003; Klein, 2003; Foulquie Moreno et al., 2006). *Enterococcus faecium* and *Enterococcus faecalis* are essential parts of the human gastrointestinal tract. Enterococci are also considered normal microflora of foods, and improve the typical taste and flavor of many foods such as cheeses and sausages through their proteolytic and lipolytic activities (Garcia et al., 2002; De Vuyst et al., 2003; Klein, 2003; Foulquie Moreno et al., 2006). Besides their beneficial characteristics, some enterococci are recognized as nosocomial pathogens, which have virulence genes and resistance to antibiotics (Franz et al., 1999; Giraffa et al., 2000; Klein, 2003; Peters et al., 2003; Foulquie Moreno et al., 2006; Poeta et al., 2006; Brede et al., 2011; Özden Tuncer et al., 2013).

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Several virulence factors such as cytolysins, serine protease, hyaluronidase, aggregation substances, extracellular surface protein and other adhesins, sex pheromone determinants, extracellular metalloendopeptidase, and hemolytic activity in enterococci, especially in *Enterecoccus faecium* and *Enterococcus faecalis* strains, have been mentioned in the literature (Franz et al., 2001; Mannu et al., 2003; Eaton and Gasson, 2005; Reviriego et al., 2005; Sanchez Valenzuela et al., 2009). Antibiotic-resistant clinical- or food-originated enterococci are widespread worldwide, and this property is transferred among bacteria by plasmids (Franz et al., 1999; Coleri et al., 2004; Oryaşın et al., 2013). Enterococci have also been described as increasingly resistant to multiple antibiotics such as erythromycin and tetracycline (Mannu et al., 2003). Therefore, it is thought that the safety of any enterococcal strain should be carefully and individually evaluated.

The aim of this study was to investigate and evaluate the potential virulence genes and antibiotic resistance characteristics of *Enterococcus faecalis* isolates from human breast milk and colostrum samples.

2. Materials and methods

2.1. Sample collection

Breast milk (n = 40) and colostrum (n = 20) samples were collected from healthy mothers in Hacettepe University Hospitals, Ankara, Turkey. The samples were collected into sterile bottles by manual expression using sanitized hands. The first 1 or 2 drops were eliminated, and then the milk samples were collected. The samples were stored in a refrigerator until analysis. The study protocol was approved by the Committee on Ethical Practice of the Faculty of Medicine, Hacettepe University, Ankara, Turkey.

2.2. Strain isolation and identification

For the isolation of enterococci from the breast milk and colostrum samples, 100 µL of the serial dilutions of each sample were inoculated on kanamycin aesculin azide agar (Fluka, Buchs, Switzerland), and then the plates were incubated at 37 °C for 48 h (Martin et al., 2003). After incubation, typical colonies on the agar medium were isolated. The typical colonies were purified twice on trypticase soy agar (Merck, Darmstadt, Germany). The pure cultures were identified to genus level by using Gram staining, catalase test, growth and blackening of bile esculin agar (Himedia, Mumbai, India), growth at 6.5% NaCl, temperatures of 10 °C and 45 °C, and pH 9.6. The pure cultures were stored at –20 °C in brain heart infusion broth (Himedia) with 30% glycerol. All isolates were identified to species level by using the API 20 STREP (bioMérieux, Marcy l'Etoile, France) biochemical test kit (Peters et al., 2003; Çıtak et al, 2004; Canzek Majhenic et al., 2005; Jurkovic et al., 2006). The results were confirmed by the 16S rDNA sequencing method using 27f (AGAGTTTGATCMTGGCTCAG) and 907r (CCGTCAATTCMTTTRAGTTT) universal primers.

2.3. Control strains

E. faecalis NCIMB 700584 (National Collection of Industrial, Marine, and Food Bacteria, UK) was used as a positive control strain for virulence genes, and *E. faecalis* ATCC 29212 was used as a reference strain.

2.4. Screening for antibiotic resistance

The strains were evaluated for resistance against some antibiotics, including ampicillin (10 µg), chloramphenicol (30 µg), erythromycin (15 µg), kanamycin (30 µg), tetracycline (30 µg), penicillin G (10 µg), gentamycin (10 µg), and vancomycin (30 µg) by using the disk diffusion method on Mueller–Hinton agar (Merck, Germany), as described by the Clinical and Laboratory Standards Institute (CLSI, 2006). All antibiotic discs were purchased from Oxoid (UK). Results were interpreted according to the cut-off levels proposed by Charteris et al. (1998) for gentamycin and kanamycin, and CLSI (2006) for the other antibiotics.

2.5. Screening for *vanA* and *vanB* genes

Genomic DNAs of *E. faecalis* strains were isolated according to the method of Miteva et al. (1991). VanA1 [5'-GGG AAA ACG ACA ATT GC-3'] and VanA2 [5'-GTA CAA TGC GGC CGT TA-3'] primers with the product size of 732 bp were used to screen the *vanA* gene in *E. faecalis* strains. VanB [5'-GTG CTG CGA GAT ACC ACA GA-3'] and VanBrev [5'-CGA ACA CCA TGC AAC ATT TC'-3'] primers with the product size of 1145 bp were used to screen the *vanB* gene in the strains (Reviriego et al., 2005). Primers were obtained from IDT (Integrated DNA Technologies, USA). PCR reactions for *vanA* and *vanB* genes were performed as an initial cycle of denaturation at 94 °C for 2 min, followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 54 °C for 1 min, elongation at 72 °C for 1 min, and a final cycle at 72 °C for 10 min (Dutka-Malen et al., 1995).

2.6. Isolation and analysis of plasmid DNA

Plasmid DNAs of the strains were isolated by the procedure described by Anderson and McKay (1983), separated by 0.8% agarose gel electrophoresis, and stained with ethidium bromide. Lambda DNA/*Eco*RI+*Hind*III marker (SM0191, Fermentas, Germany) was used as the DNA marker in agarose gel electrophoresis. *E. faecalis* NCIMB 700584 was not included in this analysis.

2.7. PCR for detection of virulence genes

The tested *E. faecalis* strains were screened for potential virulence traits such as adhesion-encoding genes (*efaAfs, efaAfm*), sex pheromones (*ccf, cpd, cad, cob*), products involved in aggregation (*agg2*), biosynthesis of an extracellular metalloendopeptidase (*gelE*), biosynthesis of

cytolysin *(cylM, cylB, cylA*), and immune evasion (*espfs*, *espfm*). PCR primers for the virulence genes (Table 1) were selected according to Reviriego et al. (2005). PCR amplifications were performed in 50-µL reaction mixtures by using 0.01 mol L^{-1} dNTP mix (Promega, Sunnyvale, CA, USA), 500 U Go Taq Flexi DNA polymerase (Promega), 50 ng of DNA, and 20 pmol of each primer obtained from IDT (Integrated DNA Technologies, Coralville, IA, USA).

Samples were subjected to an initial cycle of denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 54 °C for 30 s, and elongation at 72 °C for 1 min (Reviriego et al., 2005).

2.8. Determination of hemolytic activity

Hemolytic activity of the strains was determined on blood agar with sheep blood (Salubris, Woburn, MA, USA) as described by Çıtak et al. (2004) and Jurkovic et al. (2006).

3. Results

3.1. Distribution of the samples

The mean ages of the mothers and infants were 30.3 ± 4.9 (years) and 3.2 ± 2.2 (months), respectively. The mothers had not taken any antibiotics in the previous month in this study. Mothers with mastitis or nipple cracking were excluded from the study. Some mothers who collected the breast milk samples used breast pads (n = 18) and a cream that contains lanolin (n = 14).

3.2. Identification of isolates

In this study, 25 suspected enterococci colonies, which were isolated from 3 of the total 40 human breast milk samples and 1 of the total 20 colostrum samples, were identified to genus level as *Enterococcus* by the morphological and biochemical tests described above. In total, 23 of 25 suspected *Enterococcus* spp. isolates were identified to

Table 1. Polymerase chain reaction primers and products used for detection of virulence genes (Reviriego et al., 2005).

Genes	Primers	Sequence (5' to 3')	Product size bp
agg2	TE32 TE33	GTT GTT TTA GCA ATG GGG TAT CAC TAC TTG TAA ATT CAT AGA	1210
efaAfm	TE37 TE38	AAC AGA TCC GCA TGA ATA CAT TTC ATC ATC TGA TAG TA	735
cpd	TE51 TE52	TGG TGG GTT ATT TTT CAA TTC TAC GGC TCT GGC TTA CTA	782
cob	TE49 TE50	AAC ATT CAG CAA ACA AAG C TTG TCA TAA AGA GTG GTC AT	1405
ccf	TE53 TE54	GGG AAT TGA GTA GTG AAG AAG AGC CGC TAA AAT CGG TAA AAT	543
cad	TE42a TE43a	TGC TTT GTC ATT GAC AAT CCG ACT TTT TCC CAA CCC CTC AA	1299
efaAfs	TE5 TE6	GAC AGA CCC TCA CGA ATA AGT TCA TCA TGC TGT AGT A	705
gelE	TE9 TE10	ACC CCG TAT CAT TGG TTT ACG CAT TGC TTT TCC ATC	419
cylM	TE13 TE14	CTG ATG GAA AGA AGA TAG TAT TGA GTT GGT CTG ATT ACA TTT	742
cylB	TE15 TE16	ATT CCT ACC TAT GTT CTG TTA AAT AAA CTC TTC TTT TCC AAC	843
cylA	TE17 TE18	TGG ATG ATA GTG ATA GGA AGT TCT ACA GTA AAT CTT TCG TCA	517
espfs	TE34 TE36	TTG CTA ATG CTA GTC CAC GAC C GCG TCA ACA CTT GCA TTG CCG AA	933
espfm	TE104 TE105	TTG CTA ATG CAA GTC ACG TCC GCA TCA ACA CTT GCA TTA CCG AA	955

species level as *Enterococcus faecalis* by using the API 20 STREP with ≥90% identification level and ≥0.99 T values (Table 2). The *E. faecalis* isolates were confirmed by using the 16S rDNA sequencing method.

3.3. Screening for antibiotic resistance and *vanA* and *vanB* genes

With the exception of a single isolate, all the *E. faecalis* strains including controls were satisfactorily sensitive to ampicillin, penicillin G, chloramphenicol, and vancomycin. Most of the strains were also sensitive to gentamycin (78%) and tetracycline (78%). However, some strains were found to be intermediate-level resistant to erythromycin (97%), kanamycin (48%), and vancomycin (4%). Although there was only 1 intermediate-level vancomycin-resistant *E. faecalis* strain among the tested strains, the *vanA* and *vanB* genes were not detected in any isolate. On the other hand, 9 strains (39%) showed high-level multiple antibiotic resistance. However, most of the

Table 2. The presence of virulence genes, antibiotic resistance, and plasmid contents among *Enterococcus faecalis* isolates and the control strains.

Isolate	Source	Virulence genes	Antibiotic	Plasmid
A_{17-1}	Breast milk	*agg, cpd, cop, ccf, cad, efaAfs, gelE, cylM, cylB, cylA, espfm*	E_I, K_I	2
A_{17-2}	Breast milk	*agg, cpd, cop, ccf, cad, efaAfs, gelE, cylM, cylB, cylA, espfs, espfm*	E_I, K_I	2
A_{17-3}	Breast milk	*agg, cpd, cop, ccf, cad, efaAfs, gelE, cylM, cylB, cylA, espfm*	E_I, K_I	2
A_{17-4}	Breast milk	*agg, cpd, cop, ccf, cad, efaAfs, gelE, cylM, cylA, espfs, espfm*	E_I, K_I	2
A_{17-5}	Breast milk	*agg, cpd, cop, ccf, cad, efaAfs, gelE, cylM, cylB, cylA, espfm*	E_I, K_R	2
A_{17-6}	Breast milk	*agg, cpd, cop, ccf, cad, efaAfs, gelE, cylM, cylB, cylA, espfs, espfm*	E_I, K_I	2
A_{17-7}	Breast milk	*agg, cpd, cop, ccf, cad, efaAfs, gelE, cylM, cylB, cylA*	E_I, K_I	2
A_{17-8}	Breast milk	*agg, cpd, cop, ccf, cad, efaAfs, gelE, cylM, cylB, cylA, espfs, espfm*	E_I, K_I	2
A_{17-9}	Breast milk	*agg, cpd, cop, ccf, cad, efaAfs, gelE, cylM, cylB, cylA, espfs, espfm*	E_I, K_I	2
A_{17-10}	Breast milk	*agg, cpd, cop, ccf, cad, efaAfs, gelE, cylM, cylB, cylA, espfs, espfm*	E_I, K_I	2
A_{21-1}	Breast milk	*agg, cpd, cop, ccf, cad, efaAfs, gelE, cylM, cylA, espfs, espfm*	CN_R, E_I, VA_I, K_R	4
A_{21-2}	Breast milk	*agg, efaAfm, cpd, cop, ccf, cad, efaAfs, gelE, cylM, cylB, cylA, espfs, espfm*	CN_R, E_I, K_R	3
A_{21-3}	Breast milk	*cpd, cop, ccf, cad, efaAfs, gelE, cylM, cylB, cylA, espfs, espfm*	CN_R, E_I, K_R	3
A_{40-1}	Breast milk	*agg, cpd, cop, ccf, cad, efaAfs, gelE, cylM, cylB, cylA, espfs, espfm*	E_I, TE_R, K_R	5
A_{40-2}	Breast milk	*agg, cpd, cop, ccf, cad, efaAfs, gelE, cylM, cylB, cylA, espfs, espfm*	E_I, TE_R, K_I	5
A_{40-3}	Breast milk	*agg, cpd, cop, ccf, cad, efaAfs, gelE, cylM, cylB, cylA, espfs, espfm*	E_I, TE_R, K_R	5
A_{40-4}	Breast milk	*agg, cpd, cop, ccf, cad, efaAfs, gelE, cylM, cylB, cylA, espfs, espfm*	E_I, TE_R, K_R	5
A_{40-5}	Breast milk	*agg, cpd, cop, ccf, cad, efaAfs, gelE, cylM, cylB, cylA, espfs, espfm*	E_I, TE_R, K_I	2
K_{19-1}	Colostrum	*cpd, cop, ccf, cad, efaAfs, gelE, cylM, cylA, espfs, espfm*	CN_R, E_I, K_R	4
K_{19-2}	Colostrum	*agg, cpd, ccf, cad, efaAfs, gelE, cylM, cylB, cylA, espfs, espfm*	E_R, K_R	4
K_{19-3}	Colostrum	*cpd, cop, ccf, cad, efaAfs, gelE, cylB, cylA, espfs, espfm*	E_I, K_R	4
K_{19-4}	Colostrum	*cpd, cop, ccf, cad, efaAfs, gelE, cylM, cylB, cylA, espfs, espfm*	E_I, K_R	4
K_{19-5}	Colostrum	*cpd, cop, ccf, cad, efaAfs, gelE, cylM, cylB, cylA, espfs, espfm*	CN_R, E_I, K_R	4
Ent. faecalis ATCC 29212		*agg, cpd, cop, ccf, cad, efaAfs, gelE, cylM, cylA, espfs, espfm*	E_I, TE_R	3
Ent. faecalis NCIMB 700584		*agg, efaAfm, cpd, cop, ccf, cad, efaAfs, gelE, cylM, cylB, cylA, espfs, espfm*	E_I, K_R	NS

* CN_R; Gentamycin resistance, E_R; Erythromycin resistance, E_I; Intermediate level erythromycin resistance, K_R; Kanamycin resistance, K_I; Intermediate level kanamycin resistance, TE_R; Tetracycline resistance, VA_I; Intermediate level vancomycin resistance, NS; not studied.

multiple resistant strains were found to be intermediate-level resistant to the relevant antibiotics.

3.4. Plasmid profiles

The plasmid contents and the plasmid profiles of *E. faecalis* isolates are shown in Table 2 and Figures 1 and 2, respectively. All the *E. faecalis* strains carried a certain number of plasmids with different molecular sizes. The number of plasmids varied between 2 and 5 with a molecular size of 21,226–1584 bp or larger.

3.5. Detection of virulence genes

The presence of virulence genes among the tested strains is shown in Table 2. All the *E. faecalis* isolates (n = 23) tested in this study, including 2 control strains, contained some sex pheromone determinants (*cpd*, *ccf*, and *cad*), some cytolysin determinants (*cylM* and *cylA*), the metalloendopeptidase gene (*gelE*), and the adhesion-encoding *efaAfs* gene. Certain *E. faecalis* isolates did not contain some virulence genes such as agg_2 (22%), *cob* (4%), *cylB* (17%), *espfs* (17%), and *espfm* (4%). None of the tested *E. faecalis* isolates contained the adhesion-encoding *efaAfm* gene.

Furthermore, the tested *E. faecalis* isolates did not show phenotypic beta-hemolytic activity on blood agar with sheep blood.

4. Discussion

Enterococcus faecalis strains were predominantly isolated from the breast milk and colostrum samples in this study (Table 2). Jimenez et al. (2008) reported similar results for colostrum samples. They also noted that skin contamination was almost unavoidable during sampling of breast milk and colostrum for microbiological analysis. Therefore, they stated that there was no certainty as to the original location (internal mammary gland or skin) of the isolated bacteria. In this study, the samples were also collected by manual expression using sanitized hands and so there was no certainty about the original location of the isolated bacteria. As indicated by Jimenez et al. (2008), advanced studies will be required to explain the origin of the bacterial flora in breast milk and colostrum.

Antibiotic resistance is an important characteristic of enterococcal strains. Most of the tested *E. faecalis* strains are sensitive to ampicillin, penicillin G, chloramphenicol, vancomycin, gentamycin, and tetracycline. Similar to the results of the present study, penicillin, ampicillin, and vancomycin susceptibility and the absence of *van*A and *van*B genes were stated by Jimenez et al. (2008) in *E. faecalis* strains isolated from colostrum. In this study, 9 strains showed multiple antibiotic resistance.

Although glycopeptide antibiotics like vancomycin are frequently a last resort for treatment of nosocomial infections with multidrug-resistant pathogens, resistance to these antibiotics is a source of concern. It has also been indicated that enterococci are opportunistic pathogens. However, enterococci that are not involved in infections are generally sensitive to clinically relevant antibiotics, including vancomycin (Franz et al., 2001; Jimenez et al., 2008). The enterococci strains isolated from the breast milk and colostrum samples in this study were sensitive to the relevant antibiotics, as was the case among the enterococci of the study by Jimenez et al. (2008).

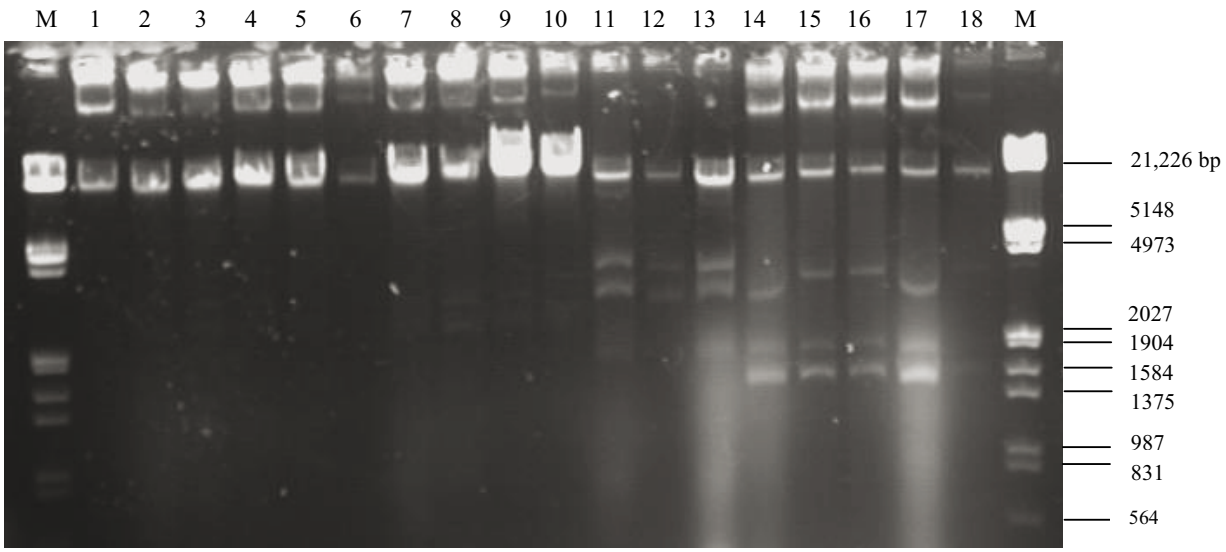

Figure 1. Plasmid profiles of *Enterococcus faecalis* isolates from human milk (1. A_{17-1}, 2. A_{17-2}, 3. A_{17-3}, 4. A_{17-4}, 5. A_{17-5}, 6. A_{17-6}, 7. A_{17-7}, 8. A_{17-8}, 9. A_{17-9}, 10. $A_{17-10,}$ 11. $A_{21-1,}$ 12. $A_{21-2,}$ 13. A_{21-3} 14. A_{40-1}, 15. A_{40-2}, 16. A_{40-3}, 17. A_{40-4}, 18. A_{40-5}, M: Lambda DNA *Eco*RI+*Hind*III marker).

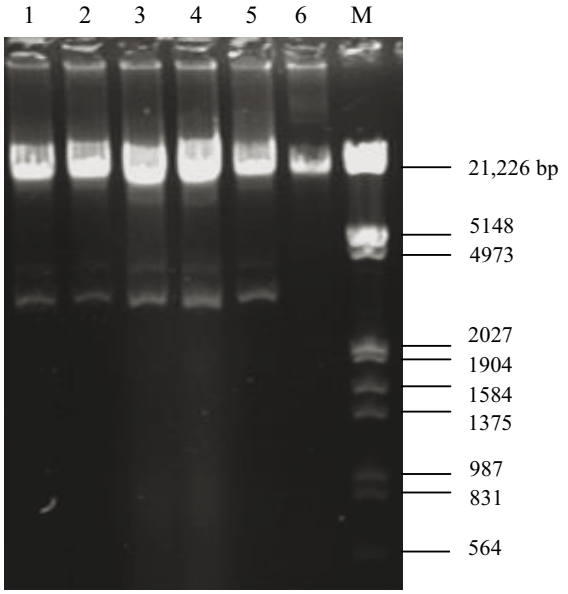

Figure 2. Plasmid profiles of *Enterococcus faecalis* isolates from human colostrum (1. K_{19-1}, 2. K_{19-2}, 3. K_{19-3}, 4. K_{19-4}, 5. K_{19-5}, 6. *E. faecalis* ATCC 29212 reference strain, M: Lambda DNA *Eco*RI+*Hind*III marker).

Multiple-antibiotic–resistant *E. faecalis* isolates contained between 2 and 5 plasmids, usually with a molecular size of 21,226–1584 bp or larger. Coleri et al. (2004) determined that clinical enterococci isolates carried between 1 and 11 plasmids, ranging in size from 2.08 to 56.15 kb. They also reported plasmid-mediated antibiotic resistance in enterococci. Abriouel et al. (2006) indicated that virulence determinants and antibiotic resistance traits of enterococci may be plasmid-borne; therefore, their potential risk in food applications needed to be carefully evaluated. Fortunately, most of the multiply resistant strains were intermediate-level resistant to the relevant antibiotics in this study.

The genes coding for enterococcal surface protein and cell wall adhesin (*espfs*, *espfm*, and *efaAfs*) and sex pheromone determinants (*cpd*, *cob*, *ccf*, and *cad*) were identified in a large number of the *E. faecalis* strains in this study. Jimenez et al. (2008) reported that all the tested *E. faecalis* strains isolated from colostrum contained the *efaAfs* gene and the sex pheromone determinants, with the exception of a single isolate in which *ccf* could not be detected. An important property that is desirable in probiotic bacteria is the adhesion of the probiotic cells onto the surface of intestinal mucosa (Ouwehand et al., 1999). The presence of surface protein and cell wall adhesin genes in the enterococci strains may also reflect, at least partially, that the enterococci strains in the breast milk or colostrum samples have this important probiotic property.

Although beta-hemolytic activity was not present in any of the tested isolates, some isolates carried hemolysin-related genes (*cylM*, *cylB*, *cylA*). It was thought that the cytolysin determinants (*cylM*, *cylB*, *cylA*) behaved as silent genes in most nonhemolytic isolates (Eaton and Gasson, 2001; Semedo et al., 2003).

It was stated that the incidence of virulence determinants, antibiotic resistance pattern, or gene transfer potential appears to be strain-specific. Therefore, the safety of any enterococcal strain of clinical or industrial interest should be carefully and individually evaluated (Eaton and Gasson, 2001; Jimenez et al., 2008). It was found that there were similar structures among some of the tested *E. faecalis* strains in terms of virulence genes, antibiotic resistance, and plasmid profiles. Therefore, molecular-based studies such as protein profile studies and RAPD-PCR studies continue to be performed with the aim of finding out whether the *E. faecalis* strains isolated from the same human milk and colostrum samples have the same phylogenetic structure or not.

In conclusion, this study indicated that *Enterococcus faecalis* is the predominant enterococcal species in breast milk and colostrum. Although all the *E. faecalis* isolates carried a certain number of plasmids with different molecular sizes, the major strains were satisfactorily susceptible to the antibiotics, including vancomycin. The *vanA* and *vanB* genes were not detected in any isolate or in the control strains. None of the *E. faecalis* isolates contained the *efaAfm* gene, and some of the tested strains were found to be free from certain virulence determinants. Although a few *E. faecalis* strains as well as the positive control strain have some virulence factors, beta-hemolytic activity, a tested phenotypic characteristic, was not detected in any of the strains. Therefore, it is thought that further investigations are needed for the determination of virulence gene expressions in the phenotype of these strains. In addition, the enterococcal isolates from human milk may have potential as a functional or probiotic culture for the food industry.

Acknowledgments

The authors would like to thank the staff at Ankara University's Biotechnology Institute Genomics Unit for 16S rDNA sequencing analysis of the isolates. We are also grateful to the staff at the Institute of Child Health of Hacettepe University and the Department of Obstetrics and Gynecology of Hacettepe University for supplying the human milk and colostrum samples.

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