Presence of Bacteriocin Genes among *Enterococcus faecalis* Isolated from Mastitic Bovine Milk

Turkyilmaz, S.,[1]* İnegöl, E.,[2] Öztürk, M.,[2] Uçan, N.[2] and Kaya, O.[1]

[1] Department of Microbiology, Faculty of Veterinary Medicine, Adnan Menderes University, Aydin, Turkey.
[2] Health Sciences Institute, Adnan Menderes University, Aydin, Turkey.

* **Corresponding author:** Dr. S. Turkyilmaz Department of Microbiology, Faculty of Veterinary Medicine, Adnan Menderes University, Isikli, Aydin-Turkey. E-mail: sturkyilmaz@adu.edu.tr

ABSTRACT

Enterococci produce a wide variety of antimicrobial peptides called bacteriocins, including enterocins. Strains that produce these bacteriocins appear to have an ecological advantage against others that populate the same environment. Enterococci can cause many economically important animal diseases including bovine mastitis. However little information is available about bacteriocin production of bacteria originating from mastitic bovine milk. This study was carried out to investigate the presence of the most important bacteriocin structural genes, β-hemolytic and antimicrobial activities of *Enterococcus faecalis*. Identification of *E. faecalis* among enterococci isolates and the presence of bacteriocin structural genes were investigated by polymerase chain reaction (PCR). To determine the hemolytic activity, strains were streaked onto Columbia Agar plates containing 7% blood. Presence of antimicrobial activity of *E. faecalis* isolates was tested using 7 indicator bacteria. A total of 96 enterococci were isolated from 600 mastitic milk samples. Of 96 enterococci 56 were *E. faecalis* and further studied for antimicrobial activity and presence of bacteriocin genes. Of 56 E. *faecalis* 20 (35.7%) showed β hemolysis, 23 (41.0%) and 25 (44.7%) showed antimicrobial activity against at least one of the tested indicator strains and harbored bacteriocin structural genes, respectively. To the best knowledge of the authors this is the first report converning the presence of bacteriocin structural genes among *E. faecalis* isolates from bovine mastitic milk in Turkey. The results showed the common presence of different bacteriocin encoding genes in the enterococcal isolates, indicating an elevated genetic potential of these strains to produce various bacteriocins. Further studies should be designed to investigate the distribution of these genes in other enterococcal species other than *E. faecalis*.

Keywords: Mastitis; *Enterococcus faecalis;* β hemolysis; Antimicrobial Activity; Enterocin.

INTRODUCTION

Bacteriocins are ribosomally produced peptides or proteins that generally show antimicrobial effects against isolates from the same or close species and genera of bacteriocin producers. Enterococci synthesize antimicrobial peptides including enterocins. It is thought that bacteriocin production is a special bacterial defense mechanism, which ensures the producer strain, a competitive advantage against non-producers and bacteriocin sensitive strains in the environment (1, 2).

Enterocins are classified into four groups as Group I (lantibiotic enterocins), Group II (small, non-lantibiotic peptides); Group III (cyclic enterocins); and Group IV (large proteins) (3). Group I enterocins comprise cytolysin (*cyl*L) and it is formed by two-peptide bacteriocin and both subunits have lanthionine residues (4). Group II can be divided into three subclasses: 1. enterocin of the pediocin family [enterocin A (*ent*A), enterocin P (*ent*P) and bacteriocin 31 (*bac*31)]; 2. enterocins are without a leader peptide [enterocin L50A/B (*ent*L50A/B)]; subtype 3 is linear, non-pediocin-

type enterocin [enterocin B (*ent*B)]. Group III enterocins contain cyclic antibacterial peptides like enterocin AS-48 (*ent*AS-48) (5).

Although enterococci are accepted as normal commensals they also have medical importance. Enterococci are usually cause nosocomial infections in humans (6) and have been associated with bovine mastitis in dairy cattle (7). Although enterococci can be listed among the most economically important causative agents of animal diseases such as bovine mastitis (7), little information is available on enterocins produced by enterococci isolated from mastitic bovine milk (8). There are reports which describe enterococci of environmental and human origins, producers of distinct enterocins (9, 10), but studies with *E. faecalis* isolates of animal origin and enterocin producers are very rare (11-13). The aim of the study was to determine the presence of important bacteriocin structural genes (*ent*A, *ent*B, *ent*P, *cyl*L, *bac*31, *ent*L50A/B, *ent*AS-48) and to show the β-hemolytic and antimicrobial activities of *E. faecalis* isolates obtained from mastitic bovine milk samples.

MATERIAL AND METHODS

Milk samples

Milk samples were collected from small scale dairy farms in Aydin a county of 8007 km^2 by Aegean Sea at the western part of Turkey. A total of 242 Holstein cows at 38 dairy farms (average 4-7 bovine from each farm) were included in this study. Milk was aseptically collected and 600 milk samples were obtained with mastitis (clinical or subclinical) in a recent two years period. One, 2, 3 and 4 mastitic milk samples were obtained from 55, 71, 61 and 55 cows, respectively. The age of the cows varied between 3 to 9 years.

The diagnosis of mastitis among cows with reduced milk production was carried out clinically or by California Mastitis Test. Clinical mastitis was diagnosed by changes in the udder and milk compositions by veterinary practitioners. Changes in the udder included pain, swelling, warmth and abnormal appearance (blood tinged milk, watery secretions, clots, pus) of milk. Cows that did not have clinical mastitis were subjected to further investigation for subclinical mastitis by using California Mastitis Test. The procedures and interpretations have been described previously (14). Samples were taken from cows were which had not undergone any treatment since at least two months. For collected milk samples, teat ends were cleaned using 70% alcohol moistened swabs and allowed to dry. After discarding the first few streams, 2-5 ml of the milk samples were collected into sterile 5 ml glass flasks.

Identification of *Enterococci*

Milk samples were used to culture *Enterococci*. Ten µl of milk was transferred in 5 ml Chromocult Enterococci Broth (Merck, Darmstadt, Germany) and incubated for 48 h at 37°C for selective enrichment of the enterococci. Positive cultures (a strong blue-green color of the broth indicated the presence of enterococci) were transferred to Enterococcosel Agar (Becton Dickinson, Le Pont de Claix, France) for selective isolation. Plates were incubated overnight at 37°C. One presumptive (small, translucent with brownish-black to black zones) colony was sub-cultured to blood agar. Bacteria were identified by standard methods using morphological and biochemical characteristics (14). All isolates were stored at -80°C in Brain Heart Infusion Broth (Oxoid Ltd, Hampshire, England) with 20% glycerol until use for molecular confirmation.

Hemolytic activity

E. faecalis isolates were stored overnight in enterococci broth at 37°C for growing. To determine the hemolytic activity, strains were streaked onto Columbia Agar plates (Merck, Darmstadt, Germany) containing 7% blood. Then, the plates were incubated at 37°C for 48 h under aerobic conditions. Zone formation around the colonies was interpreted as ß-hemolysis (15).

Antimicrobial activity screening

Seven strains were used as indicators (*Bacillus cereus* ATCC 11778, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 35218, *E. coli* ATCC 35150, *Listeria monocytogenes* ATCC 7644, *E. faecalis* ATCC 51299, *Micrococcus luteus* ATCC 9341). An inoculum of 0.5 McFarland from indicator bacteria was spread onto Trypticase Soy Agar (Merck, Darmstadt, Germany) plates. Plates were then incubated at room temperature for drying. Then, to be tested for antimicrobial activity an inoculum of 0.5 McFarland from our *E. faecalis* isolates was spread with a sterile swab on the indicator bacteria. Plates were incubated at 37°C for 24 h. Clear inhibition zones around the tested bacteria were interpreted as antimicrobial activity. Isolates having antimicrobial activity were considered as bacteriocin producers (16).

DNA extraction

For genomic DNA from individual pure cultures of the isolates were extracted with DNA extraction kit (InstaGene Matrix™, Bio-Rad Laboratories, Mississauga, Ontario, Canada) according to the manufacturer's instructions. DNA sample concentrations were determined by spectrophotometry (Thermo Fisher Scientific) at the wave length of 260 nm and 280 nm (17).

Identification of enterococci, *E. faecalis* and bacteriocin genes by PCR

The suspicious colonies were genetically confirmed by using genus (18) and species (19) specific PCR. Uniplex PCR was used for detection of *ent*P, *bac*31, *ent*A, *ent*B, *ent*L50A/B, AS-48 (9) and *cyl*L (4) genes. All primers used in the present study were listed in Table 1.

All the PCR reactions were carried out in a final volume of 30 µl containing 1X PCR buffer, 2 mM $MgCl_2$, 0.2 mM each of the four dNTPs, 0.5 mM of each primer and 1.25 units of Taq DNA polymerase. The cycles used were 95˚C for 5 min, 95˚C for 30 s, 53˚C(*ddlE.faecalis*), 55˚C (*tuf*, *ent*A, B and P), 57˚C (*bac*31 and AS-48), 58˚C (*cyl*L and *ent* L50A/B) for 30 s and 72˚C for 60 s for the next 35 cycles. The last cycle was 72˚C for 15 min. The amplification products were analyzed by electrophoresis on 1.5% agarose gel at 100 V for 40 min in Tris-acetate-EDTA buffer and revealed in ethidium bromide (20 µg/ml).

RESULTS

Isolation of enterococci and identification of *E. faecalis*

Among 600 milk samples investigated a total of 96 (16.0%) *Enterococcus* spp. was detected biochemically. For these, plates were incubated at 37˚C for 24 h after which one colony per sample with typical enterococci morphology was then transferred onto blood agar plates in order to obtain pure culture. These isolates were then subjected to Gram staining and catalase testing. Then, identification of the isolates was carried out by standard methods using morphological and biochemical characteristics (14). After the isolation of enterococci, identification based on genus and species were also performed using PCR (Figure 1). Of those, 56 (58.3%) *E. faecalis* were detected from the 96 isolates (Figure 2).Twenty of isolates (35.7%) showed a positive β hemolysis reaction in aerobic condition. By screening of antimicrobial activity, 23 (41.0 %) isolates inhibited the growth of at least one indicator bacterium: 13, 12 and 7 of the 56 enterococci (23.2%, 21.4%, 12.5%) showed antimicrobial activity against *M. luteus, L. monocytogenes* and *E. faecalis*, respectively (Figure 3, Table 2).

Table 1: Oligonucleotide primers used to in the study

Strain	Primer	Sequence (5'-3')	Amplicon size (bp)	Target gene	Reference
1	Ent1 Ent2	TACTGACAAACCATTCATGATG AACTTCGTCACCAACGCGAAC	112	*tuf*	18
2	DDF DDR	CACCTGAAGAAACAGGC ATGGCTACTTCAATTTCACG	475	*ddlE.faecalis*	19
3	*ent*P	GCTACGCGTTCATATGGTAAT TCCTGCAATATTCTCTTTAGC	87	Enterocin P	9
4	*bac*31	CCTACGTATTACGGAAATGGT GCCATGTTGTACCCAACCATT	130	Enterocin 31	9
5	*ent*A	GGTACCACTCATAGTGGAAA CCCTGGAATTGCTCCACCTAA	138	Enterocin A	9
6	*ent*B	CAAAATGTAAAAGAATTAAGTACG AGAGTATACATTTGCTAACCC	201	Enterocin B	9
7	*cyl*L	GGCGGTATTTTTACTGGAGT CCTACTCCTAAGCCTATGGTA	248	Cytolysin L	4
8	*ent*L50A/B	ATGGGAGCAATCGCAAAATTA TAGCCATTTTTCAATTTGATC	274	Enterocins L50 A, B	9
9	AS-48	GAGGAGTATCATGGTTAAAGA ATATTGTTAAATTACCAA	339	*ent*AS-48	9

Detection of bacteriocin genes

The *E. faecalis* isolates were investigated for the presence of seven bacteriocin genes (Figure 4, Table 2). While there were 2 isolate having no bacteriocin genes, at least one bacteriocin gene was found in 25 (44.7 %) isolates. Eight, 4 and 5 of the 56 isolates detected 1, 2, 3 and 4 bacteriocin genes respectively. However, results showed that four isolates having bacteriocin structural genes did inhibited the growth of indicator bacteria (code of the isolates: 22, 26, 27, 28) while two isolates inhibited the growth of the indicator bacteria but had no bacteriocin structural genes (code of the isolates: 5, 30). Three β-hemolytic isolates both had no bacteriocin structural genes and did not inhibited the growth of indicator bacteria (code of the isolates: 6, 11, 16)

The results showed that there were different combinations of *ent*A, *ent*B, *ent*P, *ent*L50A/B, *bac*31 and *cyl*L genes. *Ent*A and *ent*B were the most prevalent detected genes and they generally appeared together. *Ent*AS-48 gene was not found.

Table 2: The distribution of harbored bacteriocin structural genes, inhibitory spectrum and hemolytic activity of *E. faecalis* isolates

Code	Isolate Number	Hemolysis	Antimicrobial Activity			Bacteriocin Structural Genes					
			L. moncytogenes	*M. luteus*	*E. faecalis*	*ent*P	*bac*31	*ent*A	*ent*B	*cyl*L	*ent*L 50A/B
1	5	β		+				+	+		
2	6	β	+	+				+	+		
3	11	β	+	+	+		+	+	+		+
4	13	β	+	+	+			+	+		+
5	14				+						
6	17	β									
7	19	β		+	+	+					
8	20	β	+		+	+		+	+		+
9	22	β	+	+						+	
10	23			+		+					
11	24	β									
12	25			+				+	+		+
13	26	β	+			+		+	+		
14	28	β			+	+					
15	29	β	+			+		+	+		+
16	31	β									
17	32	β		+	+			+	+		
18	34		+					+	+		
19	35	β	+					+	+		
20	36	β		+		+					
21	39		+			+		+	+		
22	41							+	+		
23	42	β	+			+		+			
24	43		+			+		+	+		+
25	44	β		+		+		+	+		+
26	45							+	+		
27	46									+	
28	47					+					
29	55	β		+						+	
30	56	β		+							
Number		20	12	13	7	12	1	17	16	3	7
%		35.7	21.4	23.2	12.5	21.4	1.8	30.3	28.6	5.4	12.5
Number		20		23					25		
%		35.7		41.0					44.7		

The incidence of bacteriocin structural gene combinations was given in Figure 5.The distribution of harbored bacteriocin structural genes, inhibitory spectrum and hemolytic activity of *E. faecalis* isolates were given in Table 2.

DISCUSSION

It's acknowledged that enterococci have the ability to produce bacteriocins which inhibit especially Gram-positive foodborne bacteria and intestinal pathogens (20). It is reported that enterocin-producing isolates were able to control pathogenic bacteria in the gastrointestinal tracts of animals (21). In addition, bacteriocin producing strains showed potential to prevent bovine mastitis (22) and gingivitis (23). A new prototype formulation containing the bacteriocin, lacticin 3147 and teat seal has also been shown to be effective in controlling *Streptococcus dysgalactiae* and *Staphylococcus aureus* using experimental infection models both in non-lactating and lactating dairy cows (22). Furthermore, their anti-neoplastic activity has been reported (24). In this study, presence of β-hemolytic and antimicrobial activities as well as the occurrence of the most important bacteriocin structural genes were investigated in 56 *E. faecalis* isolated from mastitic bovine milk.

Enterococci can lead to serious economic losses caused by bovine mastitis (25) and little information is available on enterococcal pathogens isolated from milk samples. In recent studies on mastitis it was reported that 0-21.2% of the cases

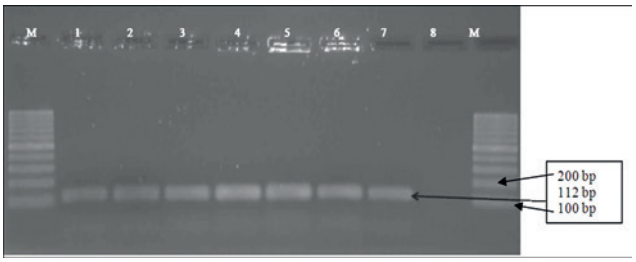

Figure 1: PCR detection of *Enterococcus* spp. isolated strains 1-6: *Enterococcus* spp. field isolates 7: Positive control (*E. faecalis* ATCC 29212) 8: Negative control (*E. coli* ATCC 25922) M: 100 bp DNA ladder.

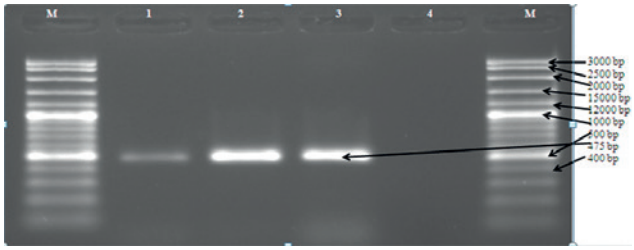

Figure 2: PCR detection of isolated *E. faecalis* strains 1-2: *Enterococcus* spp. field isolates 3: Positive control (*E. faecalis* ATCC 29212) 4: Negative control (*E. coli* ATCC 25922) M: 100 bp DNA ladder.

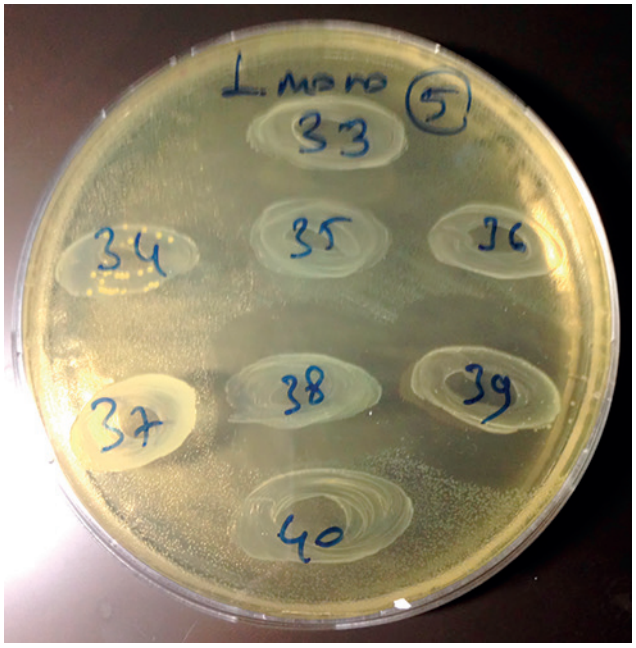

Figure 3: Examples of enterococcal isolates and their inhibition zones against *Listeria monocytogenes.*

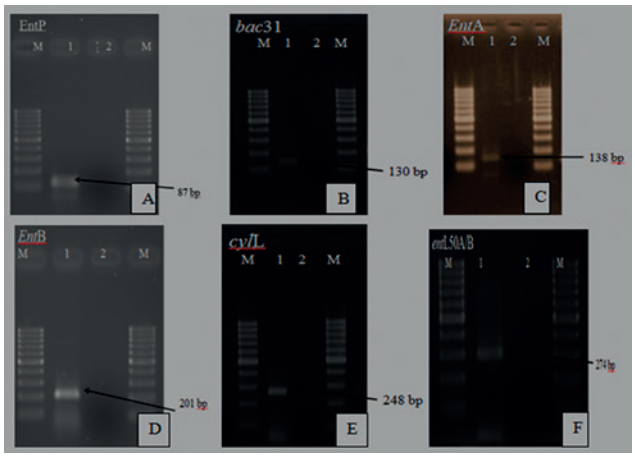

Figure 4: Agarose gel pictures of the enterocin genes of *E. faecalis* isolates. 1: Positive field isolates A: *Ent*P (87 bp) B: *bac*31 (130 bp), C: *Ent*A (138 bp), D: *Ent*B (201 bp) E: *cyl*L (248 bp) F: *Ent*L50A/B (274bp) 2. Negative control (*E. coli* ATCC 25922) M: Marker (100 bp DNA Ladder).

were caused by enterococci (26-28). The rate of *Enterococcus* spp. in the study was calculated as 16%. Analysis on species basis showed that *E. faealis* was the most common species. This finding was found similar with other studies in which *E. faecalis* has reported as the most common isolated species (26, 27).

In studies reported by Del Campo and Ike *et al*. that the rates of β-hemolysin carriage among clinical isolates of *E. faecalis* ranged from 17 to 60% (12, 29). β-hemolytic activity was detected at a rate of 35.7% for our isolates. Similarly, Coque *et al*. (30) detected β-hemolysin activity among *E. faecalis* strains of different origins (16% to 37%).

Six bacteriocin genes (*ent*A, *ent*B, *ent*P, *bac*31, *ent*LA/B, *cyl*L) were investigated by PCR. In our study 83.3% of the isolates (25 isolate gene positive) had at least one bacteriocin gene however the presence of bacteriocin gene does not mean the production of the enterocin (31). The existence of silent bacteriocin genes has been previously reported in other studies (2, 32, 33). Nes *et al.* (34) emphasized that identification of bacteriocin genes does not mean that the bacteria show antimicrobial activity. Similarly, the lack of detectable antimicrobial activity does not reflect that genes involved in bacteriocin production are defective. However, our results showed that four isolates having bacteriocin structural genes did not inhibited the growth of indicator bacteria while two isolates inhibited the growth of the indicator bacteria but have no bacteriocin structural genes (Table 2). In this context, it is essential to use susceptible indicator because some peptide bacteriocins act only on a narrow target range of bacteria and the production of peptide bacteriocins is often regulated. Problems in the production of antimicrobial activity can be attributed to a lack of functional genetic system. Therefore the antimicrobial activity may be due to the production of isolates, other than the genes tested in the present study.

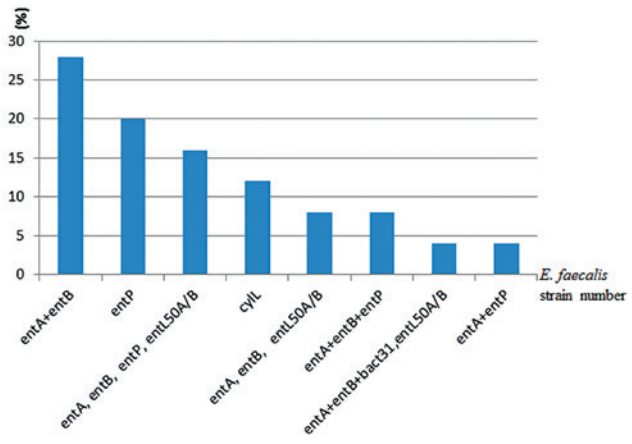

Figure 5: Incidence of bacteriocin structural genes combinations.

Parallel to our results, the structural gene of enterocin B was usually associated with the presence of enterocin A, as mentioned in former studies (9, 35). This may be since no transport genes have been found for enterocin B producers (35). It should be underlined that only one of our *E. faecalis* isolates showed wholly the *ent*A gene. Similar observations have been previously reported (9).

The enterocins A, B, P genes were found widespread among *E. faecalis* strains, while cytolysin and bacteriocin 31 structural genes were detected uncommonly in these strains. The bacteriocin 31 structural gene has been formerly reported in isolates of *E. faecium* and *E. durans* isolates recovered from different origins (9). The bacteriocin 31 structural gene was found in one *E. faecalis* strain and not occurred alone. The cytolysin structural gene has been formerly reported in isolates of *E. faecalis* isolates, recovered from food and clinical samples (32). In our study, the *cyl*L gene was identified in 3 *E. faecalis* isolates and 2 of them showed antimicrobial activity, and were not associated with any other bacteriocin genes. The β-hemolytic activities were demonstrated in 2 of these 3 *cyl*L-positive *E. faecalis* isolates. The absence of β-hemolytic activity despite the presence of all *cyl* genes in one of our *E. faecalis* isolates might be explained by low level or down-regulation of gene expression or by an inactive gene product as has been suggested by Smedo *et al*. (32).

Environmental factors are known to influence gene expression. Silent genes may also be activated by temporal factors (9, 32, 33). The production of cytolysin has been demonstrated to contribute to the severity of enterococcal disease in several animal models (36), as well as in humans (37).

There were no enterocin AS-48 genes in our *E. faecalis* isolates. Similarly, the gene encoding this bacteriocin was not found among enterococci of wild animals (37). However, AS-48 gene has been investigated in enterococcal isolates and found in *E. faecalis* (38).

In conclusion, 41.0% of the isolates included in this study showed antimicrobial activity against at least one of the tested indicator strains and the presence of bacteriocin structural genes were demonstrated in 44.7% of them. Enterocin genes were commonly distributed and they can be found in different combinations. Results showed a high incidence of strains carrying one to four genes, having a high genetic potential

of enterococcal strains to produce various bacteriocins. The presence of bacteriocin genes and the production of antimicrobial activities seem to be a common characteristic of the isolates tested. More studies should be designed to recognize the distribution of these type of genes in other enterococcal species.

ACKNOWLEDGEMENTS

This manuscript was supported by Adnan Menderes University Scientific Research Projects Unit (Project Number: VTF 14004).

REFERENCES

1. Cintas, L.M., Casaus, P., Herranz, C., Nes, I.F. and Hernández, P.E.: Bacteriocins of lactic acid bacteria. Food Sci. Technol. Int. 7: 281-305, 2001.
2. Poeta, P., Igrejas, G., Costa, D., Sargo, R., Rodrigues, J. and Torres, C.: Virulence factors and bacteriocins in faecal enterococci of wild boars. J. Basic Microbiol. 48: 385-92, 2008.
3. Franz, C.M., Van Belkum, M.J., Holzapfel, W.H., Abriouel, H. and Galvez, A.: Diversity of enterococcal bacteriocins and their grouping in a new classification scheme. FEMS Microbiol. Rev. 31: 293-310, 2007.
4. Booth, M.C., Bogie, C.P., Sahl, H.G., Siezen, R.L., Hatter, K.L. and Gilmore, M.S.: Structural analysis and proteolytic activation of *Enterococcus faecalis* cytolysin, a novel lantibiotic. Mol. Microbiol. 21: 1175-84, 1996.
5. Nilsen, T., Nes, I.F. and Holo, H.: Enterolysin A, a cell wall degrading bacteriocin from *Enterococcus faecalis* LMG 2333. Appl. Environ. Microbiol. 69: 2975-84, 2003.
6. Jett, B.D., Huycke, M.M. and Gilmore, M.S.: Virulence of enterococci. Clin. Microbiol. Rev.7: 462-478, 1994.
7. Devriese, L.A., Laurier, L., De Herdt, P. and Haesebrouck, F.: Enterococcal and streptococcal species isolated from faeces of calves, young cattle and dairy cows. J. Appl. Bacteriol. 72: 29-31, 1992.
8. Kim, S.Y., Park, Y.K., Kim, S.H., Shin, S., Koo, H.C., Youn, J.H., So, J.H., Paik, H.D., Park, Y.H. and Choi, K.Y.: Isolation of bacteriocin producing bacteria from bovine milk effective to bovine mastitis-causing and other antibiotic-resistant bacteria. The Congress of FAVA, FAVA - OIE Joint Symposium on Emerging Diseases. 27-30 October, Bangkok, Thailand, 2008.
9. De Vuyst, L., Foulquie Moreno, M.R. and Revets, H.: Screening for enterocins and detection of hemolysin and vancomycin resistance in enterococci of different origins. Int. J. Food Microbiol. 84: 299-318, 2003.
10. Özdemir, B.G., Oryaşın, E., Bıyık, H.H., Özteber, M. and Bozdoğan, B.: Phenotypic and genotypic characterization of bacteriocins in enterococcal isolates of different sources. Indian J. Microbiol. 51: 182-7, 2011.
11. Hugas, M., Garriga, M. and Aymerich, M.T.: Functionality of enterococci in meat products. Int. J. Food Microbiol. 88: 223-33, 2003.
12. Del Campo, R., Tenorio, C., Jimenez-Diaz, R., Rubio, C., Gomez-Lus, R., Baquero, F. and Torres, C.: Bacteriocin production in vancomycin-resistant and vancomycin-susceptible *Enterococcus* isolates of different origins. Antimicrob. Agents Chemother. 45: 905-12, 2001.
13. Du Toit, M., Franz, C.M., Dicks, L.M. and Holzapfel, W.H.: Preliminary characterization of bacteriocins produced by *Enterococcus faecium* and *Enterococcus faecalis* isolated from pig faeces. J. Appl. Microbiol. 88: 482-94, 2000.
14. Quinn, P.J., Carter, M.E., Markey, B.K. and Carter, G.R.: Clinical Veterinary Microbiology. Mosby-Year Book Europe Limited, Lynton House, London, England, pp. 250-350, 1994.
15. Cariolato, D., Andrighetto, C. and Lombardi, A.: Occurrence of virulence factors and antibiotic resistances in *Enterococcus faecalis* and *Enterococcus faecium* collected from dairy and human samples in North Italy. Food Control. 19: 886-92, 2008.
16. Eskin, Z., Candan, K., Tinaz, E., Beser, N., Apak, A., Guclu, O. and Bozdogan, B.: Antibacterial effects of soil bacteria isolated from beach sand at Dalyan, Turkey. 110th General Meeting of the American Society for Microbiology May 23 - 27, San Diego, California, USA, 2010.
17. Turner, P.C., Mclennan, A.G., Bates, A.D. and White, M.R.H.: Instant Notes in Molecular Biology. Nobel Publications. 613: 346, 2004.
18. Ke, D., Picard, F.J., Martineau, F., Menard, C., Roy, P.H., Ouellette, M. and Bergeron, M.G.: Development of a PCR assay for rapid detection of enterococci. J. Clin. Microbiol. 37: 3497-503, 1999.
19. Vilela, M.A., Souza, S.L., Palazzo, I.C.V., Ferreira, J.C., Morais Jr, M.A., Darini, A.L.C. and Morais, M.M.C.: Identification and molecular characterization of Van A-type vancomycin-resistant *Enterococcus faecalis* in Northeast of Brazil. Mem. Inst. Oswaldo Cruz.101: 716-19, 2006.
20. Franz, C.M.A.P., Schillinger, U. and Holzapfel, W.H.: Production and characterization of enterocin 900, a bacteriocin produced by *Enterococcus faecium* BFE 900 from black olives. Int. J. Food Microbiol. 29:255-70, 1996.
21. Laukova, A., Guba, P., Nemcova, R. and Vasilkova, Z.: Reduction of *Salmonella* in gnotobiotic Japanese quails caused by theEnterocin A-producing EK13 strain of *Enterococcus faecium.* Vet. Res. Commun. 27: 275-280, 2003.
22. Meaney, W.J., Twomey, D.P., Flynn, J., Hill, C. and Ross, R.P.: The use of a bismuth-based teat seal and the bacteriocin Lacticin 3147 to prevent dry period mastitis in dairy cows.
23. Howell, T.H., Fiorellini, J.P., Blackburn, P., Projan, S.J., dela Harpe, J. and Williams, R.C.: The effect of a mouth rinse based on nisin, a bacteriocin, on developing plaque and gingivitis in beagle dogs. J. Clin. Periodontol. 20: 335-339, 1993.
24. Hill, R.P. and Farkas-Himsley, H.: Further studies of the action of a partially purified bacteriocin against a murine fibrosarcoma. Cancer Res. 51: 1359-1365, 1991.
25. Devriese, L.A., Laurier, L., De Herdt, P. and Haesebrouck, F.: Enterococcal and streptococcal species isolated from faeces of calves, young cattle and dairy cows. J. Appl. Bacteriol. 72: 29-31, 1992.
26. Bradley, A.J., Leach, K.A., Breen, J.E., Green, L.E. and Green,

M.J.: Survey of the incidence and aetiology of mastitis on dairy farms in England and Wales. Vet. Rec. 160: 253-257, 2007.
27. Østeras, O., Sølverød, L. and Reksen, O.: Milk culture results in a large Norwegian survey – effects of season, parity, days in milk, resistance, and clustering. J. Dairy Sci. 89: 1010-1023, 2006.
28. Nam, H.M., Lim, S.K., Moon, J.S., Kang, H.M., Kim, J.M., Jang, K.C., Kim, J.M., Kang, M.I., Joo, Y.S. and Jung, S.C.: Antimicrobial resistance of enterococci isolated from mastitic bovine milk samples in Korea. Zoonoses Public Health. 57: e59-64, 2010.
29. Ike, Y., Hashimoto, H. and Clewell, D.B.: Hemolysin of *Streptococcus faecalis* subspecies zymogenes contributes to virulence in mice. Infect. Immun. 45: 528-30, 1984.
30. Coque, T.M., Patterson, J.E., Steckelberg, J.M. and Murray, B.E.: Incidence of hemolysin, gelatinase, and aggregation substance among enterococci isolated from patients with endocarditis and other infections and from feces of hospitalized and community-based persons. J. Infect. Dis. 171: 1223-9, 1995.
31. Strompfova, V., Laukova, A., Simonova, M. and Marcinakova, M.: Occurrence of the structural enterocin A, P, B, L50B genes in enterococci of different origin. Vet. Microbiol. 132: 293-301, 2008.
32. Smedo, T., Almeida Santos, M., Martins, P., Silva Lopes, M.F., Figueiredo Marques, J.J., Tenreiro, R. and Barreto Crespo, M.T.: Comparative study using type strains and clinical and food isolates to examine hemolytic activity and occurrence of the *cyl* operon in enterococci. J. Clin. Microbiol. 41: 2569-76, 2003.
33. Eaton, T.J. and Gasson, M.J. Molecular screening of *Enterococcus* virulence determinants and potential for genetic exchange between food and medical isolates. Appl. Environ. Microbiol. 67: 1628-35, 2001.
34. Nes, I.F., Diep, D.B. and Holo, H.: Bacteriocin diversity in *Streptococcus* and *Enterococcus*. J. Bacteriol. 189: 1189-98, 2007.
35. Franz, C.M., Worobo, R.W., Quadri, L.E., Schillinger, U., Holzapfel, W.H., Vederas, J.C. and Stiles, M.E.: Atypical genetic locus associated with constitutive production of enterocin B by *Enterococcus faecium* BFE 900. Appl. Environ. Microbiol. 65: 2170-8, 1999.
36. Dupont, H., Montravers, P., Mohler, J. and Carbon, C.: Disparate findings on the role of virulence factors of *E. faecalis* in mouse and rat models of peritonitis. Infect. Immun. 66: 2570-5, 1998.
37. Poeta, P., Costa, D., Rojo-Bezares, B., Zarazag, M., Klibi, N., Rodrigues, J., Torres, C.: Detection of antimicrobial activities and bacteriocin structural genes in faecal enterococci of wild animals. Microbiol. Res. 162: 257-263, 2007.
38. Joosten, H. M. L. J., Rodriguez. E., Nunez, M.: PCR detection of sequences similar to the AS-48 structural gene in bacteriocin producing enterococci. Lett. Appl. Microbiol.42: 24-40, 1997.