Korean Journal for Food Science of Animal Resources

Korean J. Food Sci. An. 2018 February 38(1):172~179
DOI https://doi.org/10.5851/kosfa.2018.38.1.172

pISSN : 1225-8563 eISSN : 2234-246X
www.kosfa.or.kr

ARTICLE

Isolation and Molecular Identification of Bacteriocin-producing *Enterococci* with Broad Antibacterial Activity from Traditional Dairy Products in Kerman Province of Iran

Khodaei M[1] and Soltani Nezhad Sh[2]*

[1]Department of Microbiology, Faculty of sciences, Kerman Branch, Islamic Azad University, Kerman, Iran
[2]Department of Microbiology, Jiroft Branch, Islamic Azad University, Jiroft, Iran

OPEN ACCESS

Received September 12, 2017
Revised December 19, 2017
Accepted January 28, 2018

*Corresponding author : Soltani Nezhad, Sh
Department of Microbiology, Jiroft Branch, Islamic Azad University, Jiroft, Iran
Tel: +989133471859
E-mail: Soltanibiotech@gmail.com

Abstract One of the critical limitations to use of bacteriocins produced by lactic acid bacteria as a substitute for chemical antibiotics is the narrow spectrum of their antibacterial activity. The aim of present study was isolation and molecular identification of bacteriocin-producing enterococci with broad antibacterial spectrum. Bacteriocin-producing bacteria were isolated from native dairies in Kerman. Bacteriocins were purified by ammonium sulfate method and the effects of them were investigated on different strains of bacteria. Also, the effects of pH and heat on produced bacteriocins were investigated. High level bacteriocin-producing isolates were identified based on molecular tests. A total of 15 strains of bacteriocin-producing *Enterococcus* were isolated initially. *Enterococcus faecium* C-2 and Y-1 strains produced bacteriocins with the highest antibacterial effect. The bacteriocins were stable in pH ranges from 2 to 12 and their antibacterial activity was maintained after autoclave treatment. The maximum bactericidal effect was observed against *Listeria monocytogenes* and *Pseudomonas aeruginosa*. In conclusion, use of these bacteriocins as a substitute for chemical antibiotics is recommended.

Keywords antibacterial effect, bacteriocin, dairy products, *Enterococcus faecium*

Introduction

Antibiotic-resistant bacteria are serious threat to global public health. The overuse of antibiotics accelerates the appearance of antibiotic-resistant strains (Zhang et al., 2015). Lactobacillales are generally recognized as safe (GRAS) (Yang et al., 2012). Because lactic acid is the main metabolite produced by these bacteria, they are called lactic acid bacteria (LAB) (De Vuyst and Leroy, 2007). LAB produces antibacterial peptides including bacteriocin and bacteriocin-like inhibitory substance (BLIS) (Bowdish et al., 2005; Cintas et al., 2001; Shokri et al.,

2014). Bacteriocins produced by LAB are ribosomally synthesized antibacterial proteins or peptides that are effective against narrow or broad spectrum bacteria (Bowdish et al., 2005; Cotter et al., 2005; Yang et al., 2012). Among the LAB, enterococci are important due to high production of bacteriocin. Enterococci are gram positive, non-spore forming, catalase and oxidase negative, and aerotolerant bacteria (Rodriguez et al., 2012; Sun et al., 2014). Most of the bacteriocins are only active against Gram positive bacteria; this is a limitation for application of the bacteriocin as a biopreservative in the food industry and as substitute for chemical antibiotics in pharmaceutical industry (Abee et al., 1995; Acuna et al., 2012). Nisin A, nisin Z and pediocin PA-1 have broad antibacterial effect against Gram positive bacteria such as food-borne pathogens, but these bacteriocins are inactive or have a narrow spectrum antibacterial effect against Gram negative bacteria (Acuna et al., 2012; Settanni et al., 2005). This study reports isolation, screening and molecular identification of bacteriocin-producing enterococci from native dairy products of Kerman province with a broad antibacterial effect against Gram positive and Gram negative pathogens.

Materials and Methods

Sample collection

Milk, yogurt, and cheese samples were collected in sterile bottles from different regions of Kerman province in Iran and were immediately transported to the laboratory in an insulated ice box.

Isolation and Purification of bacteriocin

Five grams of each sample was homogenized and serially diluted six-fold in 10 ml of sterile saline phosphate buffer and were plated on de Man, Rogosa and Sharp (MRS) medium (Biolife, Italy). Plates were incubated for 48–72 h under anaerobic conditions at 37°C. Colonies were evaluated by phenotypic methods. Enterococci suspicious isolates were isolated according to growth on kanamycin aesculin azide agar (KAA) and 6.5% NaCl media. The culture media were centrifuged at 10,000 × g for 10 min at 4°C and then filtered through 0.22 µm filters to separate cell-free supernatants (CFS) (Saranya and Hemashnpagam, 2011). The supernatants were used for purification of bacteriocins. The supernatants were adjusted to pH 7.0 by adding 12 N NaOH. Ammonium sulphate (Fluka, Netherlands) was added to the supernatants (at 4°C) to obtain 60% saturation and stirred overnight. Following centrifugation at 20,000 × g for 1 h at 4°C, the pellets were dissolved in 20 mM potassium phosphate buffer (Aran et al., 2015).

Antibacterial effects

The antibacterial effects of bacteriocins from the isolated strains was investigated on indicator strains bacteria (Gram positive: *Listeria monocytogenes* ATCC 7644, *Staphylococcus aureus* PTCC 1431 and *Bacillus cereus* PTCC 1015 and Gram negative: *Escherichia coli* PTCC 1270, *Salmonella enteritidis* PTCC 1709 and *Pseudomonas aeruginosa* CZO), using agar disk diffusion assay. Muller-Hinton agar (Biolife, Italy) was seeded by 10^6 CFU/ml of each indicator strain and 25, 50 and 75µl of bacteriocins that were dissolved in 20 mM potassium phosphate buffer placed on 6.4 mm diameter blank paper discs (Padtan Teb Co., Iran) in agar plates seeded with the indicator strains. Plates were left for 1 h at room temperature prior to incubation at the optimum temperature for each indicator strains. Appearance of clear zones of inhibitions was investigated after 24 h. The best bacteriocin producers were selected based on their antibacterial effect against indicator strains (Olasupo et al., 1999; Settanni et al., 2005).

Effects of the CFS on the growth of indicator strains

The Effect of the bacteriocin-containing CFS on the growth of *L. monocytogenes* and *P. aeruginosa* was investigated. CFS from the best bacteriocin producers prepared as mentioned above. Ten millilitres of bacteriocin- containing CFS was added to 100 ml (3 h-old) culture of indicator strains and incubated at 37°C. The optical density (OD) at 600 nm was scrutinized at every 2 h-interval during 24 h (Ahmadova et al., 2013; Todorov et al., 2010).

Effects of pH, heat and trypsin on bacteriocins

The CFS was prepared as mentioned above. To test the effect of pH on antibacterial activity of the bacteriocins, pH was adjusted between 2 and 12 using HCl or NaOH, and then incubated at 37°C for 2 h. Prior to antibacterial activity assay, pH was adjusted to 7. The antibacterial effect was measured against *L. monocytogenes* ATTC 7644 by agar disk diffusion assay. To determine the effect of temperature on bacteriocin stability, the CFS was incubated at 60°C for 30 min; 100°C for 10, 20 and 45 min; and 121°C for 15 min. The antibacterial effect of CFS was assessed against *L. monocytogenes* ATTC 7644 using agar disk diffusion assay (Aran et al., 2015; Sonsa-Ard et al., 2015). The influence of trypsin (pH=7) on antibacterial activity of bacteriocins was evaluated. 20 μl of trypsin solution was added to 200 μl of each bacteriocin-containing CFS. After 2 h incubation at 37°C, trypsin activity was stopped by heating at 80°C for 5 min. The antibacterial effect of trypsinized CFS was tested against *L. monocytogenes* ATTC 7644 by agar disk diffusion assay compared to untreated supernatants (Ahmadova et al., 2013; Aran et al., 2015; Shokri et al., 2014; Sonsa-Ard et al., 2015).

Phenotypic and molecular identification

The best bacteriocin-producing enterococci were characterized using phenotypic (Gram staining, motility, catalase, oxidase and hemolysis tests) and molecular methods. Polymerase chain reaction (PCR) was used for molecular identification. Genomic DNA was extracted by DNeasy purification kit (Bioneer, Republic of Korea). The 16S rRNA gene was amplified using the universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-TACGGTTACCTTGTTACGACTT-3'). PCR was carried out in final reaction volume of 25 μl containing 10 ng of extracted DNA, 0.4 μmol/L of each primer, 0.2 μmol/L dNTP, 2 μmol/L $MgCl_2$, 2.5 μl PCR reaction buffer and 1 U of Taq DNA polymerase. The thermal reaction condition was as following: initial denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 1 min, annealing at 45°C for 30 sec, extension at 72°C for 90 sec and a final extension step at 72°C for 5 min. Amplification products were resolved on a 1% agarose gel by electrophoresis for 1 h at 80 V. The PCR products were sequenced by Bioneer Company (Republic of Korea).

Phylogenetic analysis

Phylogenetic analysis was carried out as follows: the sequences were edited using Bioedit V.5.0.9 (Hall, 1999). For identification of the nearest neighbors, a BLAST search at the NCBI genome database server (http://blast.ncbi.nlm.nih.gov/Blast.cgi) was carried (Altschul et al. 1990). Alignment, phylogenetic and molecular evolutionary analysis were conducted using MEGA version 5 (Tamura et al. 2011). To confirm the reliability of the phylogenetic tree, bootstrap test and reconstruction was performed 1,000 times (Felsenstein,. 1985). The nucleotide sequences of 16S rRNA gene of studied bacterial strains (*E. faecium* C-2 and *E. faecium* Y-1) have been deposited in GenBank under Accession No:

KU200452 and KU200451, respectively.

Results

Antibacterial effects

In this research, 15 *Enterococcus* isolates have been obtained from different native dairies in Kerman province. Ten isolates have antibacterial effects only against Gram positive indicator strains. Five isolates have antibacterial effects against both Gram positive and Gram negative indicator strains. Bacteriocin produced by C-2 (isolated from Zarand cheese) and Y-1 (isolated from Ghanateghestan yogurt) isolates have the highest antibacterial effects against all indicator strains (Table 1). The maximum antibacterial effect against the Gram positive indicator strains was observed for *L. monocytogenes* and among the Gram negative indicator strains was observed against *P. aeruginosa*. The growth of *L. monocytogenes* and *P. aeruginosa* was significantly reduced after adding bacteriocin-containing CFS from overnight culture of C-2 and Y-1 isolates compared with controls (Fig. 1).

Table 1. Antibacterial effect of bacteriocins produced by isolated strains against indicator strains by the agar diskdiffusion method

Strain	Bacteriocin	Inhibition zone (IZ) in mm		
		25 µl	50 µl	75 µl
L. monocytogenes	Y-1	18 ± 0.577	23 ± 0.288	28 ± 0.500
	C-2	17 ± 0.763	21 ± 0.577	27 ± 0.288
S. aureus	Y-1	15 ± 0.763	18 ± 0.577	21 ± 0.000
	C-2	15 ± 0.763	18 ± 0.577	21 ± 0.000
B. cereus	Y-1	14 ± 0.763	18 ± 0.577	21 ± 0.500
	C-2	16 ± 0.577	20 ± 0.288	27 ± 0.500
P. aeruginosa	Y-1	20 ± 0.577	27 ± 0.763	33 ± 0.288
	C-2	19 ± 0.000	24 ± 0.288	29 ± 0.577
S. entritidis	Y-1	10 ± 0.000	12 ± 0.577	14 ± 0.763
	C-2	11 ± 0.577	14 ± 0.763	18 ± 0.500
E. coli	Y-1	9 ± 0.500	10 ± 0.288	12 ± 0.577
	C-2	8 ± 0.763	12 ± 0.577	14 ± 0.763

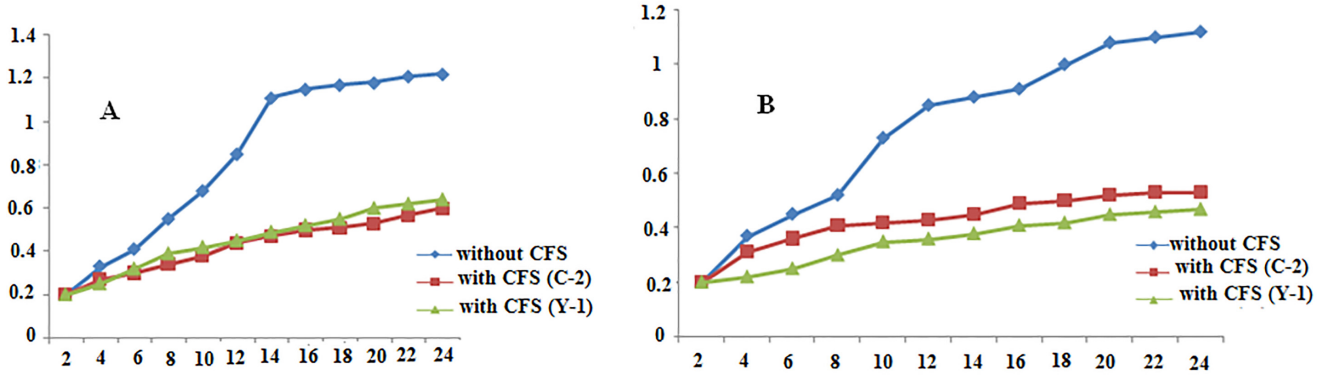

Fig. 1. Effect of the cell-free supernatants (CFS) of C-2 and Y-1 isolates on the growth of *P. aeruginosa* (a) and *L. monocytogenes* (b).

Influence of pH, heat and trypsin on the antibacterial effects

The antibacterial activity of the CFS of C-2 and Y-1 isolates were stable over a wide range of pH from 2 to 12. The CFS of Y-1 isolate was a little more resistance than C-2 isolate. The antibacterial activity of CFS of Y-1 isolates was stable at 100°C for 45 min but the bacteriocin activity of C-2 isolate was reduced by up to 25% in this condition. However, heating at 121°C for 15 min led to 25% and 50% activity loss of the bacteriocins from Y-1 and C-2 isolates, respectively. The bacteriocin activity of the both isolates was lost after treatment with trypsin (Table 2).

Table 2. Effect of different treatments (heat, pH and trypsin) on bacteriocin activity of the C-2 and Y-1 isolates

Treatments	Bacteriocin activity of C-2 (%)	Bacteriocin activity of Y-1 (%)
Heating		
60°C 30 min	100	100
100°C 10 min	75	100
100°C 20 min	75	100
100°C 45 min	75	100
121°C 15 min	50	75
pH		
2-5	80	90
5-10	100	100
10-12	70	80
Trypsin	0	0

Identification of isolates

C-2 and Y-1 isolates were Gram positive, no motion, catalyse and oxidase negative, and are alpha-hemolytic. These isolates were characterized by 16S rRNA gene analysis. Amplification of 16S rRNA gene was performed using universal primers as mentioned above. PCR amplification generated a 1500 bp fragment (Fig. 2). Fig. 3 demonstrates the infer phylogenetic relationships derived from neighbor-joining analysis of 16S rRNA gene sequences of the *E. faecium* C-2 and *E. faecium* Y-1 with highest validated described species of the genus *Enterococcus.*

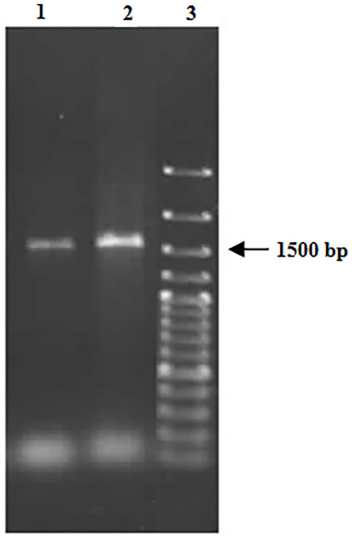

Fig. 2. Agarose gel electrophoresis of 16S rRNA gene PCR products of Y-1 isolate (1), C2 isolate (2), DNA size marker (3).

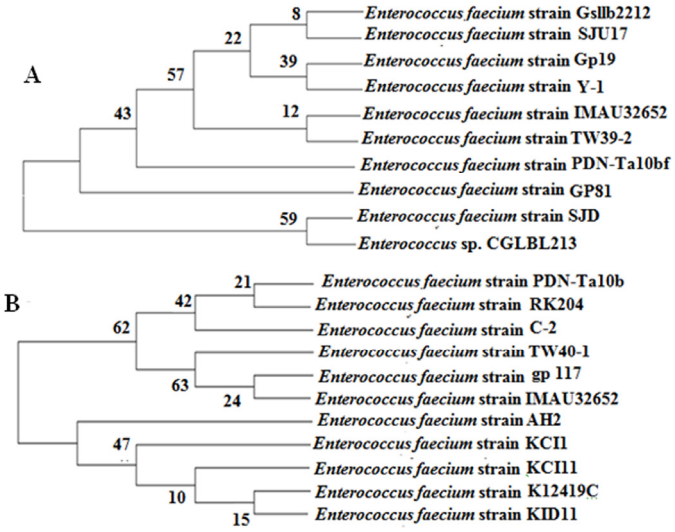

Fig. 3. Neighbor-joining tree based on 16S rRNA gene sequences, showing relationships of *E. faecium* Y-1 (A) and *E. faecium* C-2 (B) with closely related members of the genus *Enterococcus*.

Discussion

From 15 enterococci isolates, bacteriocins produced by the Y-1 isolate from Ghanateghestan yogurt and the C-2 isolate from Zarand cheese, have inhibited all indicator strains. Phylogenetic trees revealed that both isolates located in *E. faecium* species. Enterococci produce a wide group of bacteriocins (enterocins) that most of them purified and characterized from *E. faecium* and *E. faecalis* (Aran et al., 2015; Nascimento et al., 2010). For example, bacteriocin-producing strains such as *E. faecalis* LMG2333 (Nilsen et al., 2003), *E. faecalis* KT2W2G (Aran et al., 2015), *E. faecium* CN-25 (Sonsa-Ard et al., 2015) and *E. faecium* AQ71 (Ahmadova et al., 2013) have been isolated. Bacteriocins produced by enterococci are generally recognized as safe products.(Javed et al., 2011; Khan et al., 2010). They inhibit the growth of many food borne pathogens such as *L. monocytogenes*, *B. cereus* and *S. aureus* (Barbosa et al., 2014; Chen et al., 2007; Javed et al., 2011; Nilsen et al., 2003). In accordance with our results, Ahmadova et al have reported that the CFS of *E. faecium* AQ71 inhibited the growth of *L. monocytogenes* (Ahmadova et al., 2013). Bactericidal activity of bacteriocin against Gram negative bacteria is very low probably for their lipopolysaccharide (LPS) in the outer membrane (Ahmadova et al., 2013; Hammami et al., 2009; Todorov et al., 2010). However, here we reported that Bacteriocins produced by C-2 and Y-1 strains, in addition to Gram positive indicator strains, could also inhibit the growth of Gram negative indicator strains such as *P. aeruginosa, S. enterica* and *E. coli.* Similar reports have stated that class II bacteriocin , can inhibit a limited number of Gram negative bacteria, also bacteriocins produced by *E. faecium* GM-1 isolated from the faces of a newborn human infant inhibited the growth of *E. coli* and *S. typhimurium* (Kang and Lee., 2005). Enterococcal bacteriocins belong to class II bacteriocin. These bacteriocins can interact with the receptor in Gram negative bacteria, changing it to an open conformation or the receptor might act as an anchor allowing subsequent bacteriocin insertion and outer membrane disruption (Barraza et al., 2017). The antibacterial effect of the bacteriocin activity of C-2 and Y-1 isolates was lost after treatment with trypsin, indicating proteinaceous nature of antibacterial compound in the CFS. Previous studies revealed that bactericidal activity of the CFS of *E. faecium* AQ71, *E. faecium* CN-25 and *E. faecalis* KT2W2G strains was total lost following the treatment with proteolytic enzymes (Ahmadova et al., 2013; Aran et al., 2015; Sonsa-Ard et al., 2015). Bacteriocins produced by *E. faecium* C-2 and Y-1 were stable up to

121°C and remained active over a wide pH range from 2 to 12. However, some enterocins lost their antibacterial effect at 100°C and 121°C (Park et al., 2003). The pH stability of enterococci was reported variable. The thermostability and pH stability are useful characteristics for application of the bacteriocins in food and drug processing (Ahmadova et al., 2013; Hadji-sfaxi et al., 2011; Rehaiem et al., 2010).

Conclusion

Here we reported that bacteriocins produced by C-2 and Y-1 isolates have a broad antibacterial activity spectrum against both Gram positive and Gram negative bacteria, in particular pathogenic bacteria. Also, they were resistant against heat and pH ranges. As a result, use of these bacteriocins as substitutes for chemical antibiotics is recommended.

Acknowledgments

This research was supported by Department of Microbiology, Islamic Azad University, Kerman Branch, Kerman, Iran.

References

Abee T, Krockel L, Hill C. 1995. Bacteriocins: modes of action and potentials in food preservation and control of food poisoning. Int J Food Microbiol 28:169-185.

Acuña L, Picariello G, Sesma F, Morero RD, Bellomio A. 2012. A new hybrid bacteriocin, Ent35–MccV, displays antimicrobial activity against pathogenic Gram-positive and Gram-negative bacteria. FEBS open bio. 2:12-19.

Ahmadova A, Todorov SD, Choiset Y, Rabesona H, Zadi TM, Kuliyev A, De Melo Franco BDG, Chobert JM, Haertlé T. 2013. Evaluation of antimicrobial activity probiotic properties and safety of wild strain *Enterococcus faecium* AQ71 isolated from Azerbaijani Motal cheese. Food Control 30:631-641.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J Mol Biol 215:403-410.

Aran HK, Biscola V, El-Ghaish S, Jaffrès E, Dousset X, Pillot G, Haertlé T, Chobert JM, Hwanhlem N. 2015. Bacteriocin-producing *Enterococcus faecalis* KT2W2G isolated from mangrove forests in southern Thailand: purification characterization and safety evaluation. Food Control 54:126-134.

Barbosa J, Borges S, Teixeira P. 2014. Selection of potential probiotic *Enterococcus faecium* isolated from Portuguese fermented food. Int J Food Microbiol 191:144-148.

Barraza DE, Ríos Colombo NS, Galván AE, Acuña L, Minahk CJ, Bellomio A, Chalón MC. 2017. New insights into enterocin CRL35: mechanism of action and immunity revealed by heterologous expression in *Escherichia coli*. Mol Microbiol 105:922-933.

Bowdish DM, Davidson DJ, Hancock R. 2005. A re-evaluation of the role of host defence peptides in mammalian immunity. Curr Protein Pept Sci 6:35-51.

Chen YS, Yanagida F, Srionnual S. 2007. Characteristics of bacteriocin like inhibitory substances from dochi isolated *Enterococcus faecium* D081821 and D081833. Lett Appl Microbiol 44:320-325.

Cintas LM, Casaus MP, Herranz C, Nes IF, Hernández PE. 2001. Review: bacteriocins of lactic acid bacteria. Food Sci Technol Int 7:281-305.

Cotter PD, Hill C, Ross RP. 2005. Bacteriocins: developing innate immunity for food. Nat Rev Microbiol 3:777-788.

De Vuyst L, Leroy F. 2007. Bacteriocins from lactic acid bacteria: production, purification, and food applications. J Mol Microbiol Biotechnol 13:194-199.

Felsenstein J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783-791.

Hadji-Sfaxi I, El-Ghaish S, Ahmadova A, Batdorj B, Le Blay-Laliberté G, Barbier G, Haertlé T, Chobert JM. 2011.

Antimicrobial activity and safety of use of *Enterococcus faecium* PC41 isolated from Mongol yogurt. Food Control 22: 2020-207.

Hall T. 1999. Bio Edit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95-98.

Hammami I, Rhouma A, Jaouadi B, Rebai A, Nesme X. 2009. Optimization and biochemical characterization of a bacteriocin from a newly isolated *Bacillus subtilis* strain 14B for biocontrol of *Agrobacterium* spp. strains. Lett Appl Microbiol 48:253-260.

Javed A, Masud T, ul Ain Q, Imran M, Maqsood S. 2011. Enterocins of *Enterococcus faecium*, emerging natural food preservatives. Ann Microbiol 61:699-708.

Khan H, Flint S, Yu PL. 2010. Enterocins in food preservation. Int J Food Microbiol 141:1-10.

Nilsen T, Nes IF, Holo H. 2003. Enterolysin A: a cell wall-degrading bacteriocin from *Enterococcus faecalis* LMG 2333. Appl Environ Microbiol 69:2975-2984.

Kang JH, Lee MS. 2005. Characterization of a bacteriocin produced by *Enterococcus faecium* GM-1 isolated from an infant. J Appl Microbiol 98:1169-76.

Olasupo NA, Schillinger U, Narbad A, Dodd H, Holzapfel WH. 1999. Occurrence of nisin Z production in *Lactococcus lactis* BFE 1500 isolated from wara, a traditional Nigerian cheese product. Int J Food Microbiol 53:141-152.

Park SH, Itoh K, Fujisawa T. 2003. Characteristics and identification of enterocins produced by *Enterococcus faecium* JCM 5804T. J Appl Microbiol 95:294-300.

Rehaiem A, Martinez B, Manai M, Rodriguez A. 2010. Production of enterocin A by *Enterococcus faecium* MMRA isolated from 'Rayeb', a traditional Tunisian dairy beverage. J Appl Microbiol 108:1685-1693.

Rodríguez E, Arqués JL, Rodríguez R, Peirotén Á, Landete JM, Medina M. 2012. Antimicrobial properties of probiotic strains isolated from breast-fed infants. J Funct Foods 4:542-551.

Saranya S, Hemashenpagam N. 2011. Antagonistic activity and antibiotic sensitivity of lactic acid bacteria from fermented dairy products. Adv Appl Sci Res 2:528-534.

Settanni LU, Massitti O, Van Sinderen D, Corsetti A. 2005. In situ activity of a bacteriocin producing *Lactococcus lactis* strain. Influence on the interactions between lactic acid bacteria during sourdough fermentation. J Appl Microbiol 99:670-681.

Shokri D, Zaghian S, Khodabakhsh F, Fazeli H, Mobasherizadeh S, Ataei B. 2014. Antimicrobial activity of a UV-stable bacteriocin-like inhibitory substance (BLIS) produced by *Enterococcus faecium* strain DSH20 against vancomycin-resistant *Enterococcus* (VRE) strains. J Microbiol Immunol Infect 47:371-376.

Sonsa-Ard N, Rodtong S, Chikindas ML, Yongsawatdigul J. 2015. Characterization of bacteriocin produced by *Enterococcus faecium* CN-25 isolated from traditionally Thai fermented fish roe. Food Control 54:308-316.

Sun Y, Lou X, Zhu X, Jiang H, Gu Q. 2014. Isolation and characterization of lactic acid bacteria producing bacteriocin from newborn infant's feces. J Bacteriol Mycol 1:1-7.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance and Maximum Parsimony Methods. Mol Biol Evol 28: 2731-2739.

Todorov SD, Wachsman M, Tomé E, Dousset X, Destro MT, Dicks LM, Franco BD, Vaz-Velho M, Drider D. 2010. Characterisation of an antiviral pediocin-like bacteriocin produced by *Enterococcus faecium*. Food Microbiol 27: 869-879.

Yang E, Fan L, Jiang Y, Doucette C, Fillmore S. 2012. Antimicrobial activity of bacteriocin-producing lactic acid bacteria isolated from cheeses and yogurts. AMB Express 2:1-12.

Zhang QQ, Ying GG, Pan CG, Liu YS, Zhao JL. 2015. Comprehensive evaluation of antibiotics emission and fate in the river basins of China: source analysis, multimedia modeling, and linkage to bacterial resistance. Environ Sci Technol 49: 6772-6782.