academicJournals

Vol. 14(32), pp. 2517-2524, 12 August, 2015
DOI: 10.5897/AJB2015.14518
Article Number: 9FCB89154629
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Bacteriocinogenic potential and genotypic characterization of three *Enterococcus faecium* isolates from Algerian raw milk and traditional butter

Louiza Lazreg[1,3]*, Fatiha Dalache[2,3], Halima Zadi-Karam[3] and Nour-Eddine Karam[3]

[1]Department of Biotechnology, Faculty of Nature and Life Sciences, University of Sciences and Technology, Mohamed Boudiaf Oran, BP 1505 El Mnaouar, 31000, Algeria.
[2]Department of Biology, University of Mostaganem, 27000, Algeria.
[3]Laboratory of Biology of Microorganisms and Biotechnology, University of Oran 1 Ahmed Ben Bella, Algeria.

Received 22 February, 2015; Accepted 6 August, 2015

The detection of bacteriocinogenic potential of *Enterococcus sp.* isolates from Algerian raw milk coded LO4 and LO12 and from traditional butter coded BRO2 was carried on M17 buffered medium. PCR amplification of *Enterococcus sp.* DNA using specific enterococcal primers gave 733 bp fragments. The phylogenetic analysis using the neighbour joining method further supported the identification of the three strains as *Enterococcus faecium*. These bacteria were bacteriocinogenic against *Pseudomonas sp*, *Proteus mirabilis* and *E. faecium*. Lyophilisate extracts were tested for sensitivity to enzymes, heating and effect of pH. Complete inactivation in bacteriocinogenic activity was observed after treatment with proteolytic enzymes. The antibacterial activity from *E faecium* LO12 was stable (1280 AU/ml) for range pH 2 to 12. Maximal activity from BRO2 strain was at pH 7 (20480 AU/ml) and from LO4 strain was at pH 7 and 6 (2560 AU/ml). Antibacterial activities of *E. faecium* BRO2 (5120 AU/ml) and *E. faecium* LO12 (640 AU/ml) remained stable at 60°C for 30 min. The antibacterial activity of .*E faecium* LO4 was stable at 100°C for 30 min (5120 AU/ml).

Key words: Butter, milk, *Enterococcus faecium,* antibacterial activity, bacteriocinogenic.

INTRODUCTION

Traditional food manufacturing appreciated to this day by some consumers was at the origin of products from the food industry. In old times, an excess of food need to be conserved for a long time to survive in winter or in drought periods. Together with drying and salting, fermentation is one of the oldest methods of food preservation, and embedded in traditional cultures and village life (Marshall and Meijia, 2012). Today, when many secrets of fermentation are known, the nutritional and hygienic quality of food required had been mastered in the food industry. Indeed, some micro-organisms had largely been exploited in the food fermentation including lactic acid bacteria, moulds and yeast (Giraffa, 2004). They are responsible for many proprieties of fermented

*Corresponding author. E-mail: lazreglouiza@yahoo.fr. Tel: + 213 662422068.

food such as flavor, shelf life, texture and health benefits (Giraffa, 2004). The production of fermented food is based on the use of starter cultures, for instance lactic acid bacteria that initiate rapid acidification of the raw material (Leroy and DeVuyst, 2004). They acidify the food, resulting in a tangy lactic acid taste, frequently exert proteolytic and lipolytic activities, and produce aromatic compounds from, for instance, amino acids upon further bio-conversion (Williams et al., 2001; Yvon and Rijnen, 2001; Van Kranenburg et al., 2002). The group of lactic acid bacteria occupies a central role in these processes, and has a long and safe history of application and consumption in the production of fermented food and beverages (Ray and Daeschel, 1992; Wood and Holzapfel, 1995; Wood, 1997; Caplice and Fitzgerald, 1999). They increase the nutritional quality of food by increasing digestibility as in the fermentation of milk to cheese (Caplice and Fitzgerald, 1999). Their antimicrobial effect through fermentation processes has been appreciated by man and has enabled him to extend the shelf life of many foods (Savadogo et al., 2004). Among lactic acid bacteria genus *Enterococcus* is the object of many studies these last years. The advent of molecular methods has allowed distinguishing an *Enterococcus* genus, which is the largest Lactic Acid Bacteria (LAB) genus after *Lactobacillus* and *Streptococcus* (Franz et al., 2011). On the basis of 16SrRNA gene similarity, the species of *Enterococcus* fall into seven species of groups (Franz et al., 2011). *Enterococcus faecium* is widely distributed in food and their environment. Their ubiquitous nature and resistance to adverse environmental conditions account for their ability to colonize different habitats and underlie their potential to easily spread through the food chain (Fracalanzza et al., 2007).

Generally, enterococcal bacteria were found in milk and dairy product. These bacteria can also be used as starter in the food industry due to their capacity to produce lipase, protease and volatile compounds ensuring desirable organoleptic features in some specific kinds of food (Camargo et al., 2014). This genus endowed similar properties than *Lactococcus* and *Lactobacillus* such as antimicrobial activity. The last two decades had seen an intensive investigation on natural antimicrobial products synthesized by food grade lactic acid bacteria that can be used as food preservatives in place of chemical preservatives (Gautam et al., 2014). According to Šušković et al. (2010) the main antimicrobial effect of starter LAB responsible for biopreservation is the rate of acidification, but in slightly acidified products the bacteriocinogenic activity could play a crucial role to eliminate undesirable microorganisms that display acid tolerance.

Bacteriocins are ribosomally synthesized peptides produced by bacteria that are capable of killing other bacteria by forming pores in target membranes (Abee, 1995; Zacharof and Lovitt, 2012). Most of bacteriocin as is the case for lantibiotic, are initially synthesized with an N-terminal leader peptide (Parada et al., 2007). In general the peptide is modified by the action of other proteins encoded by the bacteriocin gene cluster before export (Deegan et al., 2006). Many bacteriocins from Gram positive bacteria have fairly broad spectra, and have a great potential as antimicrobial agents in food and feed production (Nigutova et al., 2005). They are frequently found as secondary metabolites produced by various microorganisms, such as the Gram-positive bacteria of the genus *Streptomyces*, lactic acid bacteria and genus *Bacillus* (Katz and Demain, 1977; Klaenhammer, 1988). Bacteriocins are widely used in food science to extend food preservation duration (Ghrairi et al., 2012). Bacteriocins inhibit pathogen infection of animal diseases (Van Heel et al., 2011). Pharmaceutical industry and medical society attempt to use bacteriocins to treatment for malignant cancers (Lancaster et al., 2007). Two bacteriocins are used in food technology: Nisin which is produced by *Lactococcus lactis* and the first antibacterial peptide found in lactic acid bacteria (Rogers, 1928), and Pediocin PA-1 marked as Alta ® 2341 which inhibits the growth of *Listeria monocytogenes* in meat product (Settanni and Corsetti, 2008).

Enterococci can produce bacteriocins, called enterocins, with inhibitory activity against strains closely related to the producer microorganism (Poeta et al., 2008). Enterocin produced by *E. faecium* has a broad-spectrum of activity towards food-borne pathogens indicating its application in food processes as a co-culture or as an additive (Leroy et al*.,* 2003). Settanni and Corsetti (2008) reported that enterocin CCM 4231 and EJ97 are used in soy milk and zucchini purée for suppression of contamination, respectively while enterocin AS-48 seems a good candidate for application in biopreservation of fruit juices (Grande Burgos et al., 2014).

In this paper, we were interested to put on evidence the bacteriocinogenic activity of three enterococcal bacteria isolated from traditional butter and raw milk towards unwanted bacteria. The isolates were identified to *E. faecium* by biochemical and molecular methods.

MATERIALS AND METHODS

Bacterial strains and growth conditions

Four *Enterococcus* sp. strains from the collection of the Laboratory of Biology of Microorganisms and Biotechnology (Oran University, Algeria) were used for this study: the strain BRO2 was isolated from a sample of traditional butter, the strains LO4 and LO12 were isolated from a sample of raw milk from a single cow and the strain H3 used as target was isolated from “hammoum” a traditionally fermented barley. They were stored at - 20°C in reconstituted skim milk (10% w/v) and before use they were propagated twice in their M17 broth media (Fluka, Switzerland) at 30°C. Several bacteria used as target strains (Table 1) were incubated at 37°C in nutritive broth (tryptone 10 g/l, meat extract 5 g/l, NaCl 5 g/l pH 7.2) or in nutritive agar.

Table 1. Inhibitory spectrum of enterococcal isolates.

Diameter of inhibitions zones (mm)	BRO2		LO4		LO12	
Target bacteria and origin	M	BM	M	BM	M	BM
Citrobacter freundi EC2	2	ND	2	ND	5	ND
E coli EC3	5	ND	2	ND	5	ND
E coli HB4	ND	ND	ND	ND	2	3
E coli ATCC 25922	ND	ND	ND	ND	ND	ND
Enterococcus faecium H3	14	12	13	15	8	6
Proteus mirabilis HB3	11	13	9	12	8	9
*Pseudomonas sp.*HB2	14	12	11	14	18	9
Pseudomonas aeruginosa HB5	ND	ND	ND	ND	7	9
Pseudomonas aeruginosa ATCC 27853	ND	ND	ND	ND	ND	ND
Staphylococcus aureus HB1	ND	ND	ND	ND	ND	ND
Staphylococcus sp. V3	2	ND	5	ND	8	ND

HB, Isolates from human biological samples; EC, isolates from water; V, isolates from meat; M, unbuffered medium; BM, buffered medium; ATCC, American Type Culture Collection; ND, not detected.

Phenotypic identification of enterococcal strains

Enterococcal strains were grown in bile esculine agar (Institut Pasteur, Algiers). Gram staining and catalase production were tested prior to inoculation of the rapid commercial system API 20 STREP gallery (Biomérieux, France) for the identification of enterococci, according to the manufacturer recommendations.

Molecular identification of enterococcal strains

Amplification of enterococcal DNA

Two colonies of each enterococcal culture on M17-lactose agar were picked up and homogenized with 1 ml of pure water in order to extract DNA, according to the boiling method described by Reischl et al. (1994). The supernatant containing the DNA was directly used for PCR reactions. Primers E1 (5'TCAACCGGGGAGGGT3') and E2 (5'ATTACTAGCGATTCCGG3') designed by Deasy et al. (2000) were used to amplify enterococcal DNA. Universal primers U1 (5'AAYATGATTACIGCIGCICARARATGGA'3) and U2 (5'-AYRTTITCICCIGGCATIACCAT-'3) were used as a control. The master mix composition for 1 reaction was 5 µl of 10X PCR buffer, 0.4 µl of 25 mM dNTP mixture, 2.5 µl of 50 mM $MgCl_2$, 1.25 µl of 10 µM downstream primers, 1.25 µl of 10 µM upstream primers, 12.2 µl pure water and 0.4 µl of *Taq* DNA polymerase (5 U/µl). PCR reaction was carried out by using thermocycler (Techne TC-412) in a final volume of 50 µl. The amplification program used was: initial denaturation step at 95°C for 5 min, 35 cycles of denaturation at 94°C for 1 min, hybridization at 60°C for 1 min, polymerization at 72°C for 1 min, final extension at 72°C for 5 min. 5 µl of each product of PCR reaction were mixed with 2 µl of loading buffer. 5 µl of each sample was electrophoresed (40 min at 135 V) on 1% agarose gel using TAE buffer containing 200 ng/ml ethidium bromide (Sigma-Aldrich). The gels were photographed on a UV transilluminator.

Phylogenetic analysis of enterococcal bacteria

The PCR products were sequenced by 96-Capillary Applied Biosystems 3730*xl* analyzer. The obtained sequences were analyzed by the BLAST software of the NCBI (http://www.ncbi.nlm.nih.gov/). The phylogenetic tree of the 16S rRNA gene was performed using Clustal X2.1 (Thompson et al., 1997) in the Mega 6.06 software (Tamura et al., 2013). The phylogenetic tree was constructed using the neighbor-joining method with bootstrap analysis for 100 replicates.

Bacteriocinogenic activity

Bacteriocinogenic activity tests were performed using a method of Spelhaug and Harlander (1989). In our study, the M17 agar medium was buffered by sodium phosphate buffer (0.1 M; pH 7.2) instead of 1% of ß-glycerophosphate. The isolates (BRO2, LO4, LO12) were grown in M17 broth (30°C, 16 h). 5 µl from each culture were spotted onto M17 agar plate (without buffer) and onto M17 buffered agar plate. The plates were incubated at 30°C for 18 h. The target strains were grown in nutritive broth up to 4 or 5 h (OD_{600nm} 0.3 to 0.5), except for *Enterococcus sp.* H3 which was grown in M17 broth. Each one of these cultures (250 µl) was used to inoculate 7 ml of soft agar in order to overlay the spotted plates. The plates were stored at 5°C for 4 h prior to be further incubated at 30°C for 24 h. Zones of inhibition of growth of target strains revealed inhibitory activity of enterococcal strains.

Sensitivity to enzymes, heat treatment and effect of different pH values on antibacterial activity produced by bacteriocinogenic strains

Culture extract preparation and bacteriocin assay

The overnight bacteriocinogenic cultures were centrifuged (12000 rpm for 15 min). The supernatants were filtered through a Millipore 0.22 µm pore size membrane, than lyophilized in lyophilizer for 6 h. Fifty µl of 200 mg made in 1 ml sodium phosphate buffer (0.1 M; pH 7.2) were tested by the well-diffusion method (Tag and McGiven, 1971).

Enzymes susceptibility

Aliquots (200 µl) of the lyophilisates were incubated at 37°C for 2 h

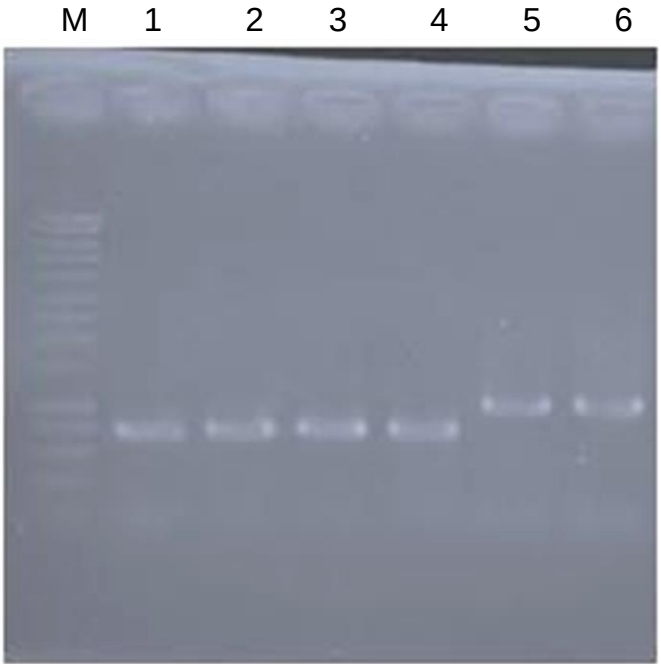

Figure 1. Electrophoresis gel of PCR products. M, Molecular marker 1000 bp; lanes 1 to 4, PCR amplification products with enterococcal primers: lane 1: BRO2; lane 2: LO12; lane 3: LO4; lane 4: H3; lanes 5 to 6, PCR amplification products with universals primers U1/U2: lane 5: LO12; lane 6: BRO2.

(Todorov et al., 2011) in the presence of 1 mg ml^{-1} of catalase, trypsin, pronase E, proteinase K, pepsin or α-chymotrypsin (Aktypis and Kalantzopoulos, 2003). All enzymatic solutions (Sigma-Aldrich) were prepared in buffer sodium phosphate (0.01 M pH 7) except the pepsin that was dissolved in HCl of 0.02 M. The susceptibility of antibacterial activity to enzymes was appreciated by the well-diffusion method (Tag and McGiven, 1971).

Heat treatment

Aliquots (200 µl) of the lyophilisates were heated at 60°C (30 and 60 min), 100°C (30 and 60 min) and at 121°C (20 min). The remaining activity was evaluated according to Graciela et al. (1995) against *E. faecium* H3. The activity was expressed as arbitrary units (AU) per milliliter.

Effect of pH values

The pH of aliquots (200 µl) of the lyophilisate were adjusted to different pH ranging from 2 to 12 with HCl (1 mole l^{-1}) or NaOH (1 mole l^{-1}). After incubation for 4 h at 30°C (Hernandez et al., 2005), and filtration through a Millipore 0.22 µm pore size membrane, the remaining activity was evaluated as described before.

RESULTS

Identification of enterococcal strains

The enterococcal strains used in this study were isolated from raw milk (LO4, LO12), traditional butter (BRO2) and fermented barley (H3) in Oran (Algeria). They are cocci shaped, Gram+, catalase negative and present a degradation of esculine on bile esculine agar medium. Based on API 20 Strep profile strains were identified as *E*. *faecium*. The identification was confirmed by PCR, using specific primers for *Enterococcus* which gave 733 base pairs fragment (Figure 1). According to the designer of primer (Deasy et al., 2000), the products of amplification and their size confirms that the four strains (BRO2, LO4, LO12, H3) belong to *Enterococcus* genus. The isolates (BRO2, LO4 and LO12) were characterized at the genotypic level by sequencing the specific amplified fragment of 16S rRNA gene to determine the species of *Enterococcus*. Nucleotide Blast analysis against the NCBI nucleotide database showed that the sequence of amplified fragment of 16S rRNA gene from three strains BRO2, LO4, LO12 were 99% identical to sequences obtained from different strains of *E. faecium*. The results of alignment were used to construct a phylogenetic tree bootstrapped for 100 times. Analysis of tree (Figure 2) showed that LO4 and LO12 strains are related with 56% of bootstrap analysis. While, BRO2 strain is closely related to cluster of LO4 and LO12 in 100% bootstrap. However, the 3 strains formed a cluster, which is closely related (100% bootstrap) to a cluster including *E. faecium* JCM 5804 and *E. faecium* NRRL B 2354.

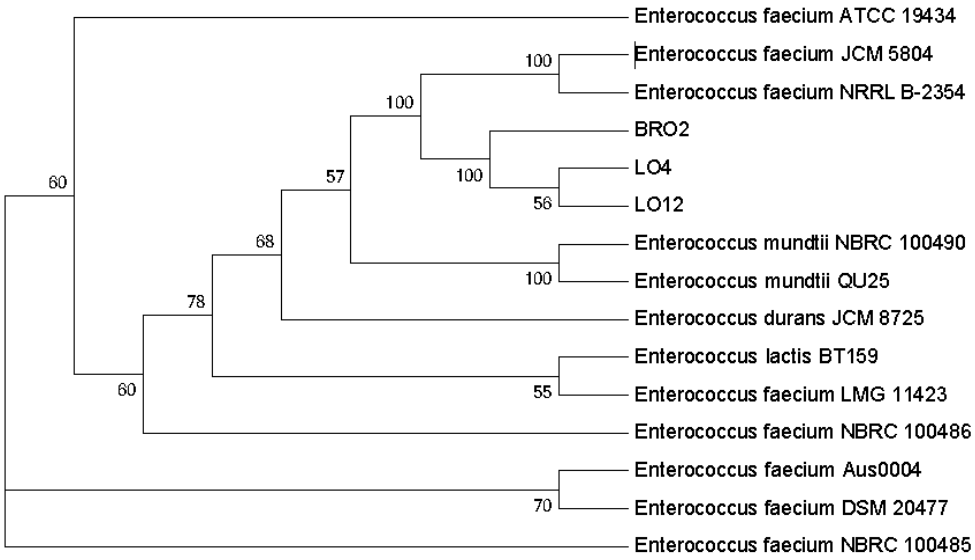

Figure 2. Neighbor-joining tree of 16SrRNA sequences from the isolates (LO4, LO12 and BRO2) and 16SrRNA sequences of 12 known *Enterococcus* species. The phylogenetic tree was bootstrapped for 100 times and clustered by using Mega 6 and Clustal X2.1.

Table 2. Effect of enzymes on inhibitory activity.

Enzymes	Inhibition zone of *E faecium* (mm)		
	BRO2	LO4	LO12
Catalase	10	10	13
α-Chymotrypsin	0	0	0
Trypsin	0	0	0
Pronase E	0	0	0
Proteinase K	0	0	0
Pepsin	10	09	11

Figure 3. Effect of enzymes on culture supernatant of *E. faecium* LO12. 1, α-Chymotrypsin; 2, catalase; 3, native supernatant; 4, trypsin; 5, Pronase E; 6, pepsin; 7, proteinase K.

Bacteriocinogenic activity

The strains *E. faecium* BRO2, LO4 and LO12 inhibited the growth of *Pseudomonas sp.* HB2, *Proteus mirabilis* HB3 and *E. faecium* H3. The strains *E. coli* HB4 and *Pseudomonas aeruginosa* HB5 were inhibited only by LO12 strain. Indeed the strains BRO2, LO4 and LO12 are bacteriocinogenic. All these inhibitions were recorded on solid buffered M17 medium and on unbuffered M17 medium (Table 1). For the target strains *Staphylococcus sp.* V3, *Citrobacter freundi* EC2 and *E. coli EC3,* no activity was recorded on buffered medium.

Effect of enzymes, heating and different pH values

The lyophilized supernatants from the culture of BRO2 LO4 and LO12 were sensitive to all tested proteolytic enzymes except pepsin. The inhibitory activity was maintained after treatment with catalase (Table 2 and Figure 3). These results suggest that inhibitory substances from strain BRO2, LO4 and LO12 are proteinaceous nature. The effect of temperature on the inhibitory activity showed that the antibacterial agent LO4 was more stable to heat treatment than the other agents. Its inhibitory activity against *E. faecium* H3 was maintained stable after heating at 60°C (for 30 and 60 min) and at 100°C for 30 min. While for antibacterial agents BRO2 and LO12, we observed that the inhibitory activity was maintained stable only at 60°C for 30 min but

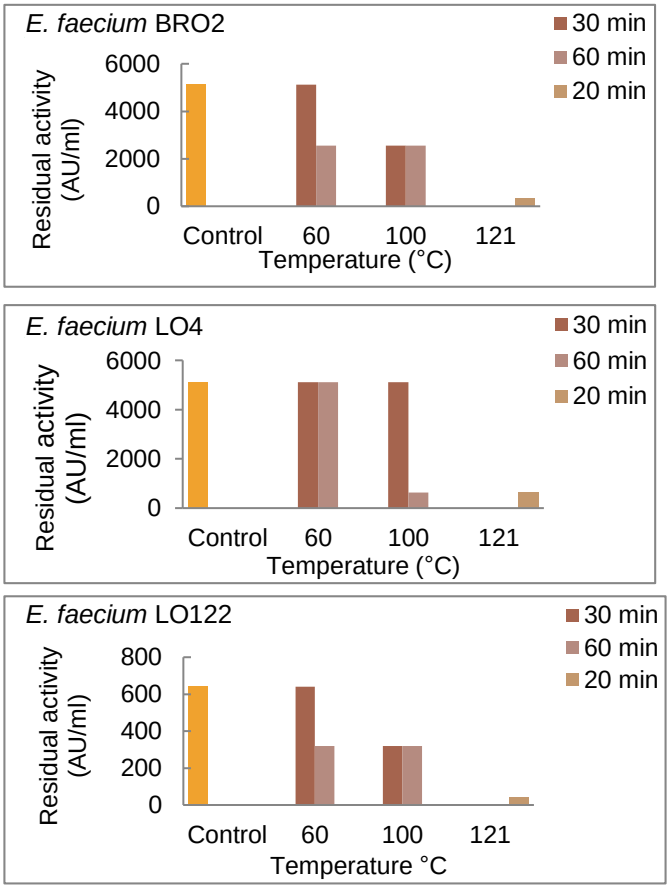

Figure 4. Residual activity of inhibitory substances from isolates BRO2, LO4 and LO12 against *E. faecium* H3.

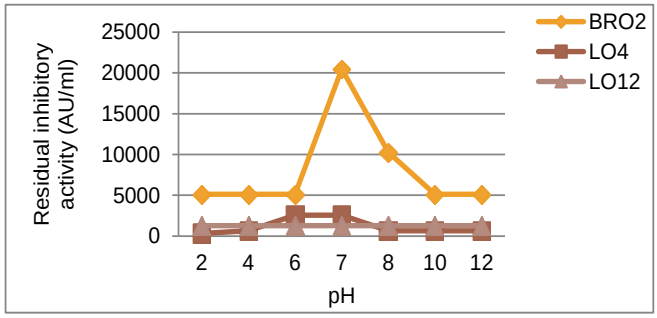

Figure 5. Effect of pH on inhibitory activity of *E. faecium* strains BRO2, LO4 and LO12 against *E. faecium* H3.

decreased to half at 60°C for 60 min and 100°C (30 and 60 min). We also observed that low activity was maintained at 121°C (20 min) for the three antibacterial agents (Figure 4). The inhibitory activity of antibacterial agent BRO2 was maximal at pH 7 (20480 AU/ml). A decrease of activity was observed for acidic and basic pH, but without a total inactivation of this agent. At pH 6 and 7, the antibacterial agent LO4 presented maximal activity (2560 AU/ml), while for acidic pH (4 and 2) and basic pH (8, 10 and 12) the inhibitory activity decrease (Figure 5). The inhibitory activity of LO12 agent was maintained stable for a large range of pH (2 to 12). Pinto et al. (2009) reported that bacteriocin produced by *E. faecium* 130 maintained total activities in the pH range from 2 to 8.

DISCUSSION

Enterococcus species are omnipresent and are alive freely in the soil, on plant and in large numbers in the dairy products where in certain cases, they prevail towards lactobacilli and lactococci (Franz et al., 1999; Giraffa, 2002). Approximately half of the *Enterococcus* species have been relatively recently described (Franz et al., 2011). The interest bearing on *Enterococcus* in recent years and their ubiquitous character encouraged to study their bacteriocinogenic potential for use in food technology. In our study, three *E. faecium* isolates were found to be bacteriocinogenic against *E. faecium* H3 and two human pathogens Gram negative bacteria. *E. faecium* H3 is more sensitive than the other target due to their close phylogenetic relationship to bacteriocinogenic strains. Growth inhibition of Gram negative bacteria was rarely reported. Some previous studies report activity of bacteriocins produced by *E. faecium* against *P. aeruginosa* (De Kwaadsteniet et al., 2005; Line et al., 2008; Gaaloul et al., 2015) and *P. mirabilis* (Line et al., 2008). In contrast, no activity was reported for *E. faecium* R111 towards *P. aeruginosa*, *Proteus vulgaris* or *E. coli* (Khay et al., 2011). The absence of sensitivity recorded for testing *Staphylococcus* strains and *E. coli* can be explained by natural variation in susceptibility and the ability to develop resistance to bacteriocins (Nascimento et al., 2010). At phylogenetic level our strains are related to *E. faecium* strain NRRL B-2354 and *E. faecium* JCM 5804. The last one produces three different types of bacteriocins, enterocin A, enterocin B, and enterocin P-like bacteriocin (Park et al., 2003).

The bacteriocins are known to be resistant to high temperature (Todorov et al., 2011). The antibacterial agent LO4 was more resistant to heat than the other studied antibacterial agents. Similar results were reported by Tulini et al. (2009) for bacteriocins from *E. faecium* 130. In our study inhibitory substances maintained a low activity at 100°C during 60 min and at 121°C during 20 min. The thermal stability of bacteriocins produced by enterococcal bacteria was also reported by Chen et al. (2007). The heat resistance can be due to the formation of small complex structures, stable cross-linkage and the generation of strongly hydrophobic portions (De Vuyst and Vandamme, 1994). It is an important characteristic for the application of these substances as natural food preservatives. The sensitivity of all antibacterial substances to proteolytic enzymes indicates that the inhibitions

are due to bacteriocins. According to Klaenhammer (1988), the bacteriocins were sensitive, at least for one proteolytic enzyme. The sensitivity to pronase E and trypsin suggest that it may be used as a biological preservative in foods and feed, as it will not affect the microbial flora of the gastrointestinal tract (Aktypis and Kalantzopoulos, 2003).

In our study inhibitory substances maintained their activity at any pH. Generally, the bacteriocins are not affected by the pH. However, pH seems to play an important role for the adsorption to target bacteria. Most of the described bacteriocins are active in a range of pH from 2 to 8 and are partially or completely inactivated at pH 10 (Tomé et al., 2009). The resistance to heat and to the large range of pH is an important characteristic for the application of these substances as natural food preservatives.

Conclusion

Three enterococcal strains identified at phylogenetic level as *E. feacium* were found to be bacteriocinogenic. The effectiveness of the bacteriocinogenic strains depends on target bacteria. Their inhibitory activity against *Pseudomonas* sp. and *P. mirabilis* are interesting. These bacteriocinogenic strains produce extracellular substances which are sensitive to proteolytic enzymes supporting strongly the proteinaceous nature. The substances from the three bacteriocinogenic strains *E. faecium* BRO2, LO4 are resisting in a large range of pH and to heat treatment of the antibacterial activity.

Conflict of interests

The authors did not declare any conflict of interest.

ACKNOWLEDGEMENTS

This work was supported by MESRS (CNEPRU F01820090065) and DGRSDT (012/2000).

REFERENCES

Abee T (1995). Pore-forming bacteriocins of Gram-positive bacteria and self-protection mechanisms of producer organisms. FEMS. Microbiol. Lett. 129:1-9.

Aktypis A, Kalantzopoulos G (2003). Purification and characterization of thermophilin ST-1, a novel bacteriocin produced by *Streptococcus thermophilus* ACA-DC 0001. Lait 83:365-378.

Camargo CH, Bruder-Nascimento A, Hwa S, Lee I, Fernandes Júnior A, Kaneno R, Mores Rall VL (2014). Prevalence and phenotypic characterization of *Enterococcus* spp. isolated from food in Brazil. Braz. J. Microbiol. 45:111-115

Caplice E, Fitzgerald GF (1999). Food fermentation: role of microorganisms in food production and preservation. Int. J. Food. Microbiol. 50: 131-149.

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.

De Kwaadsteniet M, Todorov SD, Knoetze H, Dicks LMT (2005). Characterization of a 3944 Da bacteriocin produced by *Enterococcus mundtii* ST15, with activity against Gram positive and Gram negative bacteria. Int. J. F. Microbiol. 5: 433-444.

De Vuyst L, Vandamme EJ (1994). Bacteriocins of lactic acid bacteria: Microbiology, genetics and applications. London: Blackie Academic and Professional. 1-11

Deasy BM, Rea MC, Fitzgerald GF, Cogan TM, Beresford TP (2000). A rapid PCR based method to distinguish between *Lactococcus* and *Enterococcus.* Syst. Appl. Microbiol. 23: 510-22.

Deegan LH, Cotter PD, Hill C, Ross P (2006). Bacteriocins: Biological tools for biopreservation and shelf-life extension. Int. Dairy J. 16:1058-1071.

Fracalanzza S, Scheidegger E, Santos P, Leite P, Teixeira L (2007). Antimicrobial resistance profiles of enterococci isolated from poultry meat and pasteurized milk in Rio de Janeiro, Brazil. Mem. Inst. Oswaldo Cruz. 102:853-859.

Franz CMAP, Holzapfel WH, Stiles ME (1999). Enterococci at the crossroads of food safety. Int. J. Food. Microbiol. 47:1-24.

Franz CMAP, Huch M, Abriouel H, Holzapfel W, Gálvez A (2011). Enterococci as probiotics and their implications in food safety. Int. J. Food. Microbiol. 151:125-140.

Gaaloul N, Ben Braiek O, Hani K, Volski A, Chikindas ML, Ghrairi T (2015). Isolation and characterization of large spectrum and multiple bacteriocin -producing *Enterococcus faecium* strain from raw bovine milk. J. Appl. Microbiol. 118: 343-55

Gautam N, Sharma N, Ahlawat OP (2014). Purification and characterization of bacteriocin produced by *Lactobacillus brevis* UN isolated from Dhulliachar: a traditional food product of north east India. Indian J. Microbiol. 54 (2):185-189.

Ghrairi T, Chaftar N, Hani K (2012). Bacteriocins: recent advances and opportunities in progress in food preservation, Chapter 23. eds R. Bhat, A Karim Alias and G Paliyath (Oxford: Wiley-Blackwell). pp. 485-511.

Giraffa G (2002). Enterococci from Foods. FEMS. Microbiolo. Rev. 26:163-171.

Giraffa G (2004). Studying the dynamics of microbial populations during food fermentation. FEMS. Microbiol. Rev. 28:251-260.

Graciela M, Vignolo M, de Kairuz M, Aida AP, de Ruiz H, Oilver G (1995). Influence of growth conditions on the production of lactocin 705, a bacteriocin produced by *L. casei* CRL 705. J. Appl. Bacteriol. 78:5- 10

Grande Burgos MJ, Pulido RP, del Carmen López Aguayo M, Gálvez A, Lucas R (2014). The Cyclic Antibacterial Peptide Enterocin AS-48: Isolation, Mode of Action, and Possible Food Applications Int. J. Mol. Sci. 15: 22706-22727.

Hernandez D, Cardell E, Zarate V (2005). Antimicrobial activity of lactic acid bacteria isolated from Tenerife cheese: initial characterization of plantaricin TF711, a bacteriocin-like substance produced by *Lactobacillus plantarum* TF711. J. Appl. Microbiol. 99: 77-84.

Katz E, Demain AL (1977). The peptide antibiotics of *Bacillus*: chemistry, biogenesis, and possible functions. Bacteriol. Rev. 41(2):449-474

Khay EO, Idaomar M, Castro LMP, Bernárdez PF, Senhaji NS, Abrini J (2011). Antimicrobial activities of the bacteriocin-like substances produced by lactic acid bacteria isolated from Moroccan dromedary milk. Afr. J. Biotechnol. 10(51):10447-10455.

Klaenhammer TR (1988). Bacteriocins of lactic acid bacteria. Biochimie 70 (3):337-349.

Lancaster LE, Wintermeyer W, Rodnina MV (2007). Colicins and their potential in cancer treatment. Blood Cells Mol. Dis. 38:15-18.

Leroy F, De Vuyst L (2004). Lactic acid bacteria as functional starter cultures for the food fermentation industry. Food. Sci. Tech. 15: 67-78

Leroy F, Foulquie Moreno MR, De Vuyst L (2003). *Enterococcus faecium* RZS C5, an interesting bacteriocin producer to be used as a coculture in food fermentation. Int. J. Food. Microbiol. 88:235-240

Line JE, Svetoch EA, Eruslanov BV, Perelygin VV, Mitsevich EV, Mitsevich IP, Levchuk VP, Svetoch OE, Seal BS, Siragusa GR, Stern NJ (2008). Isolation and purification of Enterocin E-760 with broad

antimicrobial activity against Gram-Positive and Gram-Negative bacteria. Antimicrob. Agents Chemother. 52(3):1094-1100.
Marshall E, Meijia D (2012). Traditional fermented food and beverages for improved livelihoods. Diversity Booklet # 21, FAO, Rome.
Nascimento MS, Moreno I, Kuaye AY (2010). Antimicrobial activity of *Enterococcus faecium* FAIR-E 198 against Gram-positive pathogens. Braz. J. Microbiol. 41: 74-81.
Nigutova K, Pristas P, Javorsky P (2005). Bacteriocin-like activity production and resistance in selected *enterococci* and *streptococci* of animal origin. Arch. Anim. Nutr. 59: 205-211.
Parada JL, Caron CR, Medeiros ABP, Soccol CR (2007). Bacteriocins from lactic acid bacteria: purification, properties and use as biopreservatives. Braz. Arch. Biol. Technol. 50(3): 521-542.
Park SH, Itoh K, Fujisawa T (2003). Characteristics and identification of enterocins produced by *Enterococcus faecium* JCM 5804T. J. Appl. Microbiol. 95:294-300.
Pinto AL, Fernandes M, Pinto C, Albano H, Castilho F, Teixeira P, Gibbs PA (2009). Characterization of anti-Listeria bacteriocins isolated from shellfish: potential antimicrobials to control non-fermented seafood. Int .J. Food Microbiol. 129(1): 50-58.
Poeta P, Igrejas G, Costa D, Sargo R, Rodrigues J, Torres C (2008). Virulence factors and bacteriocins in faecal enterococci of wild boars. J. Basic. Microb. 48:385-392
Ray B, Daeschel M (1992). Food biopreservatives of microbial origin. Boca Raton, Florida: CRC Press.
Reischl U, Pulz M, Ehret W, Wolf H (1994). PCR-based detection of mycobacteria in sputum samples using a simple and reliable DNA extraction protocol. BioTechniques 17: 844-845.
Rogers LA (1928). The inhibiting effect of *Streptococcus lactis* on *Lactobacillus bulgaricus*. J. Bacteriol. 16:321-325.
Savadogo A, Cheik Ouattara T, Imael Bassole HN, Alfred Traore S (2004). Antimicrobial activities of lactic acid bacteria strains isolated from Burkina Faso fermented milk. Pak. J. Nutr. 3:174-179.
Settanni L, Corsetti A (2008). Application of bacteriocins in vegetable food biopreservation. Int. J. Food Microbiol. 121:123–138.
Spelhaug SR, Harlander SK (1989). Inhibition of foodborne bacterial pathogens by bacteriocins from *Lactococcus lactis* and *Pediococcus pentosaceus*. J. Food. Prot. 52:856-862.
Šušković J, Kos B, Beganović J, Leboš Pavunc A, Habjanič K, Matošić S (2010). Antimicrobial activity the most important property of probiotic and starter lactic acid bacteria. Food. Technol. Biotechnol. 48:296-307.
Tag JR, McGiven AR (1971). Assay system for bacteriocins. Appl. Microbiol. 21: 943
Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30:2725-2729.
Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25:4876-4882.
Todorov SD, Prévost H, Lebois M, Dousset X, Le Blanc JGM, Franco BDGM (2011). Bacteriocinogenic *Lactobacillus plantarum* ST16Pa isolated from papaya Carica papaya from isolation to application: Characterization of a bacteriocin. Food Res. Int. 44:1351-1363.
Tomé E, Todorov SD, Gibbs PA, Teixeira PC (2009). Partial characterization of nine bacteriocins produced by lactic acid bacteria isolated from cold-smoked salmon with activity against *Listeria monocytogenes.* Food Biotechnol. 23(1): 50-73.
Tulini FL, Gomes BC, De Martinis ECP (2009). Partial purification and characterization of a bacteriocin produced by *Enterococcus faecium* 130 isolated from mozzarella cheese. Food Sci. Technol (Campinas) 31(1):155-159.
Van Heel AJ, Montalban-Lopez M, Kuipers OP (2011). Evaluating the feasibility of lantibiotics as an alternative therapy against bacterial infections in humans. Expert Opin. Drug. Metab. Toxicol. 7: 675-680.
Van Kranenburg R, Kleerebezen M, Van Hylckama Vlieg J, Ursing BM, Boekhorst J, Smit BA, Ayad EHE, Smit G, Siezen RJ (2002). Flavour formation from amino acids by lactic acid bacteria: prediction from genome sequence analysis. Int. Dairy J. 12:111-121.
Williams AG, Noble J, Banks JM (2001). Catabolism of amino acids by lactic acid bacteria isolated from cheddar cheese. Int. Dairy J. 11:203-215
Wood BJB (1997). Microbiology of fermented food. London: Blakie Academic and Professional.
Wood BJB, Holzapfel WH (1995). The genera of lactic acid bacteria. London: Blakie Academic and Professional.
Yvon M, Rijnen L (2001). Cheese flavour formation by amino acid catabolism. Int. Dairy J.11:185-201.
Zacharof MP, Lovitt RW (2012). Bacteriocins produced by lactic acid bacteria a review article. APCBEE Procedia 2:50-56.