academicJournals

Vol. 8(3), pp. 14-21, April 2016
DOI: 10.5897/JMA2015.0360
Article Number: 2CBA28D58373
ISSN 2141-2308

http://www.academicjournals.org/JMA

Journal of Microbiology and Antimicrobials

Full Length Research Paper

Antimicrobial compounds produced by *Enterococcus* spp. isolates from fecal samples of wild South American fur seals

Carolina Baldisserotto Comerlato[1], Júlia Roberta Buboltz[2], Naiara Aguiar Santestevan[1], Amanda de Souza da Motta[1] and Ana Paula Guedes Frazzon[1,2]*

[1]Programa de Pós-Graduação em Microbiologia Agrícola e do Ambiente, Departamento de Microbiologia,Instituto de Ciências Básicas da Saúde (ICBS), Universidade Federal do Rio Grande do Sul (UFRGS), Av. Sarmento Leite 500, Porto Alegre, CEP 90050-170, Rio Grande do Sul (RS), Brasil.
[2]Laboratório de Bacteriologia, Departamento de Microbiologia, ICBS, UFRGS. Av. Sarmento Leite 500, Porto Alegre, CEP 90050-170, Rio Grande do Sul (RS), Brasil.

Received 31 December, 2015; Accepted 14 April, 2016

The aims of this study were to identify bacteriocinogenic activity in 13 enterococci isolated from fecal samples of wild South American (*Arctocephalus australis*) and Subantarctic fur seals (*Arctocephalus tropicalis*); to determine the physicochemical characteristics and antimicrobial spectrum of antimicrobial compounds against Gram-positive and Gram-negative bacteria; and to evaluate the presence of bacteriocin structural genes by PCR. Out of 13 enterococci screened for antimicrobial activity, five enterococci showed activity against *Listeria monocytogenes* ATCC 35152, an important pathogen linked to food. Of these, only the *E. mundtii* strain J5 maintained the activity after the pH was adjusted (pH 6.5). The activity of antimicrobial compounds from the *E. mundtii* strain J5 (ACs-J5) was lost after proteolytic enzyme treatment; however, the activity was maintained after heat, pH (acidic and basic conditions) and chemical treatment. ACs-J5 showed narrow spectrum activity. Only the *mundticin* KS gene was detected in the J5 strain and no plasmid was present. In conclusion, the properties presented by ACs-J5 make it a valuable biopreservative in food industries in avoiding pathogenic microorganisms such as *L. monocytogenes* and it should be a good candidate for probiotic application.

Key words: Antimicrobial compounds, *Enterococcus mundtii*, wild fur seal, antilisterial activity.

INTRODUCTION

In recent years, natural antimicrobials, such as bacteriocins, have received a good deal of attention over a number of microorganism control issues (Maldonado-Barragán et al., 2016; Gao et al., 2016), ribosomally synthesized antimicrobial peptides and proteins that usually only show inhibitory activity to closely related bacterial species. A number of bacteriocins produced by LAB have potential as novel antimicrobial agents in

*Corresponding author. E-mail: ana.frazzon@ufrgs.br.

various practical applications from food to medicine (Nes et al., 2014; Gao et al, 2016). The interest of the food industry in bacteriocin compounds has increased in recent years, as they can be used as natural preservatives, replacing some chemicals, and increasing food shelf life (Yang et al., 2008). However, identifying new substances that have a broad spectrum of action, stable in the food, innocuous, while retaining the food's color properties, texture, taste and aroma, is difficult (de la Fuente-Salcido et al., 2013).

Several classification systems used to group bacteriocins has been developed, and most of this information is based on findings from LAB. Two major classes, the Class I constitutes the lantibiotics and Class II constitutes the unmodified non-lantibiotics, are well defined (Nes et al., 2014). Class II bacteriocins are commonly found and characterized in enterococci. According to Nes et al. (2014), Class II can be further divided into four subgroups: Class IIa (the pediocin-like and strong antilisterial enterocins); Class IIb (the two-peptide bacteriocins); the circular bacteriocins; and the leaderless bacteriocins (synthesized without a leader peptide) Bacteriocinogenic enterococci strains have been found in foods, such as fresh milk, and fecal samples from humans and animals. The bacteriocinogenic strains have been characterized, especially in *Enterococcus faecalis* and *E. faecium*, however these peptides have also been isolated from *E. mundtii*, *E. avium*, *E. hirae* and *E. durans.* The enterococcal bacteriocins proved to be active against gram-positive foodborne pathogens, such as *Listeria monocytogenes*, *Staphylococcus aureus* and *Clostridium* spp. (Nes et al., 2014).

Enterococcus mundtii has been found to produce class IIa bacteriocins. Mundticin ATO6 was purified from *E. mundtii* isolated from chicory endive (Bennik et al., 1998), mundticin KS was identified in *E. mundtii* NFRI 7393 isolated from grass silage in Thailand (Kawamoto et al., 2002) and *E. mundtii* CRL35 from regional Argentinian cheese produced enterocin CRL35 (Saavedra et al., 2004). On the other hand, the structures of bacteriocins produced by *E. mundtii* have not yet been determined (De Vuyst et al., 2003).

The intestinal microbiota is considered a rich source of bacteriocin-producing strains. Though there has been considerable research on LAB bacteriocins to date, there are few studies on the screening for enterococci bacteriocin-producing strains isolated from fecal samples of wild animals (Poeta et al., 2007; Poeta et al., 2008; Brandão et al., 2010; Almeida et al., 2011). The aims of this study were to identify bacteriocinogenic enterococci strains isolated from fecal samples of wild South American (Arctocephalus australis) and Subantarctic fur seals (Arctocephalus tropicalis), to determine the physicochemical characteristics and the spectrum of antimicrobial compounds and to evaluate the presence of enterocin genes by PCR.

MATERIALS AND METHODS

Enterococci strains

Thirteen enterococci strains (three *E. faecalis*, one *E. faecium*, four *E. hirae*, two *E. casseliflavus*, two *E. gallinarum* and one *E. mundtii*) identified by biochemical and molecular methods in a previous study from fecal samples of wild young South American and Subantarctic fur seals were selected (Table 1) (Santestevan et al., 2015). The colonies were preserved in a 10% (w/v) skimmed milk Molico® (Nestlé) solution supplemented with 10% (v/v) glycerol (LabSynth®) and frozen at -20°C. Prior to each experiment, an aliquot of frozen bacterial cells was recovered on Brain Heart Infusion Agar (BHIA, Oxoid) and incubated at 37°C for 24 h.

Screening for antilisterial activity by the double-agar layer test

Antibacterial activity of the strains was detected by the double-agar layer test (Lewus and Montville, 1991). To determine the inhibitory spectrum of the isolates against *L. monocytogenes* ATCC 35152, all enterococci strains were inoculated with a sterile toothpick on Trypticase Soy Agar (TSA, Himedia, Mumbai, India) and incubated for 18 h at 37°C. Simultaneously, an indicator organism was grown at 37°C for 18 h and 100 µL of this culture with 10^6 CFU mL^{-1} was inoculated in 10 mL Trypticase Soy Broth (TSB, Himedia, Mumbai, India) containing 0.7% agar. This culture was equilibrated at 45°C, mixed and then poured as an overlay onto the plate with growth of enterococci. The plates were incubated for 18 h at 37°C. Antilisterial activity was visually detected by observing clear inhibition zones around the producer strain and those strains in which antimicrobial activity was observed.

Antimicrobial activity of crude extraction from *Enterococcus* spp.

The crude extract bioassay of five strains, which showed antimicrobial activity in the double-agar layer test against *L. monocytogenes*, was performed according to Motta and Brandelli (2002). The strains were grown in 100 mL TSB at 37°C for 18 h, and the cells were harvested by centrifugation at 3,680 x g for 15 min at 4°C. The pH of the cell-free supernatants was adjusted to 6.5 with sterile 1 M NaOH to exclude acid production as the inhibitory mechanism, heated at 90°C for 10 min to inactivate remaining cells, and then filtered through a 0.22 µm pore-size nylon syringe filter (Chromafil). The antimicrobial substance was kept at 4°C for further characterization.

In the agar spot test, 20 µL of crude extract were dripped onto TSA plates previously inoculated with 100 µL of *L. monocytogenes* ATCC 35152 at 10^6 CFU mL^{-1}. The plates were incubated at 37°C for 18 h, and examined for clearance zones of the bacterial lawn in the agar overlay. The assays were performed in triplicate.

Determination of growth curve of selected strain and analysis of antimicrobial activity

The selected producer strain was submitted to growth curve to evaluate the maximum production of the antimicrobial substance. The strain was grown in TSB, and 1 mL of the adjusted bacterial inoculum (approximately 10^7 CFU mL^{-1}) was added into 100 mL of TSB and incubated at 35°C under shaking (180 rev min^{-1}). Aliquot samples were taken at specific time intervals(1, 4, 8, 16, 32, 40 and 48 h) to determine the number of viable cells (CFU mL^{-1}), the

Table 1. *Enterococcus* spp. isolated from fecal samples of wild young South American fur seals and Subantarctic fur seals. The table displays the occurrence of virulence genes and antimicrobial resistance against *Listeria monocytogenes* ATCC 35152

Origin	Isolate	Species	Antimicrobial resistance[1*]					Virulence genes[1**]				IZ[2]
			CIP	ERY	NIT	NOR	TET	*gelE*	*cylA*	*ace*	*asa*	(mm)
South American	B3	*E. faecalis*	S	S	S	S	S	+	-	+	-	0
	C18	*E. hirae*	S	S	S	S	**R**	**+**	-	**+**	**+**	0
	C19	*E. hirae*	S	S	S	S	S	-	-	-	-	0
	E10	*E. faecalis*	S	S	S	S	S	+	-	+	+	0
	E16	*E. faecalis*	S	S	S	S	S	+	-	+	-	0
	J2	*E. casseliflavus*	S	**R**	S	S	S	**+**	-	**+**	**+**	0
	J3	*E. casseliflavus*	S	**R**	S	S	S	**+**	-	**+**	-	0
	J5	*E. mundtii*	S	S	S	S	S	**+**	-	-	-	25
Subantarctic fur	F1	*E. gallinarum*	S	S	**R**	S	S	-	-	-	-	15
	F2	*E. hirae*	S	S	**R**	S	S	-	-	-	-	14
	F3	*E. gallinarum*	S	S	**R**	S	S	+	-	+	-	12
	F5	*E. hirae*	S	S	S	S	S	-	-	-	-	7
	F12	*E. faecium*	**R**	**R**	**R**	S	S	-	-	-	-	0

Santestvan et al., 2015. *Antibiotics: ciprofloxacin (CIP), erythromycin (ERI), nitrofurantoin (NIT), norfloxacin (NOR) and tetracycline (TET). ** Sensitive (S): resistance (R): positive (+); and negative (-). 2. Inhibition zone (mm).

the pH and antimicrobial activity in millimeters (mm) these parameters were determined as described elsewhere (Motta and Brandelli, 2002). The cell-free supernatant pH was adjusted (pH 6.5) to verify the antimicrobial activity of *L. monocytogenes* ATCC 35152. Growth curve was performed in triplicate.

Arbitrary units assay

Antimicrobial activity was quantified by arbitrary units per mL at the time interval in which the highest antimicrobial activity (greatest halo) was observed in mm. The activity unit of the antimicrobial substance was determined in appropriate twofold serial dilutions on microplates. The dilutions were tested against *L. monocytogenes* ATCC 35152 with 10^6 CFU mL^{-1}, using the agar spot test. The arbitrary units per milliliter (AU mL^{-1}) were defined as a reciprocal of the highest dilution factors generating an area of inhibition indicating the potency of the antimicrobial activity (Bigwood et al., 2012).

Effects of proteolytic enzymes, pH, temperature and chemicals on antimicrobial compounds stability

To gain insight into the nature of the antimicrobial compounds (ACs) of the selected strain, the following treatments were used: (a) the sensitivity to proteolytic enzymes was evaluated by addition of enzymes trypsin (Sigma), papain (Merck) and proteinase K (Merck) at a final concentration of 2 mg ml^{-1}} to the antimicrobial substance. Incubations were performed at 35°C for trypsin and papain and 37°C for proteinase K for 1 h. To inactivate enzymes, the treatments were subjected to heating at 100°C for 3 min. The AC was used as untreated control together with the enzyme controls; (b) The effect of pH on the antimicrobial activity of the ACs was investigated, and aliquots of ACs were subjected to different conditions of pH 1.0 to 12.0 (pH increases by 1.0 unit), adjusted with 1 M HCl or 1 N NaOH and incubated at 37°C for 2 h; (c) The effect of different temperatures was determined by incubating the AC at 30 to 100°C (in 10°C increments) for 30 min, and 121°C for 15 min; (d) The effects of chemicals on the ACs were also evaluated by treatments with 50% butanol (Vetec), 95% ethanol (Vetec), acetone (Dynamic), chloroform (Vetec), methanol (Vetec) and DMSO (Nuclear) at a proportion of 1:1, and 10% Tween 80 (Vetec) at a proportion of 9:1 with incubations at 37°C for 1 h. After the different treatments the antimicrobial activities were evaluated by the agar spot method. The indicator microorganism used was *L. monocytogenes* culture ATCC 35152.

Determination of antimicrobial spectrum of antimicrobial compounds

The following cultures were selected to determine the antimicrobial spectrum of antimicrobial compounds: *Bacillus cereus* (ATCC 14579), *Staphylococcus aureus* (ATCC 190506), *Enterococcus faecalis* (ATCC 29212), *Streptococcus agalactiae* (ATCC 13813), *Klebsiella pneumoniae* (ATCC 700603), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853). Four different serotypes of *L. monocytogenes* isolated from cheese (Mello 2007) were also evaluated by agar spot test with 20 µL of the ACs. The antimicrobial activity was measured in millimeter and the cultures were standardized with 10^6 CFU mL^{-1}.

Detection of enterocin genes by PCR

Genomic DNA was extracted by the physicochemical method of Donato (2007). To identify the genes encoding the enterocins, PCR amplification was performed using specific primers to well-known structural enterocin genes: enterocin A (*entA*), enterocin B (*entB*), enterocin P (*entP*) and mundticin KS (*mun*KS) (Park et al., 2003; Foulquié et al., 2003; Gutierrez et al., 2005; Zendo et al., 2005). Amplification was performed in a 25 µL mixture, containing 100 ng of DNA, 1.5 mM of $MgCl_2$, 200 µM of each dNTP, 1X reaction buffer, 1 U of *Taq* polymerase and 0.5 µM of each primer. Amplification was carried out in an Eppendorf Mastercycler Personal 5332 Thermocycler (Eppendorf®) according to the following program: 1 cycle at 94°C for 5 min; 35 cycles at 94°C for 1

min, at 52°C for *entA*, or 47°C for *entB*, or 55°C for *ent P* and *mun KS* for 1 min, at 72°C for 2 min; 1 cycle at 72°C for 15 min. Finally, PCR products were resolved using electrophoresis on 1.5% (w/v) agarose gel, visualized on a UV transilluminator and photographed.

Detection of plasmid in selected producer strain

Plasmid DNA extraction was performed according to Sambrook and Russel (2001). The presence of plasmid DNA was observed using electrophoresis on 0.7% agarose gel. The *Escherichia coli* strain TOP10 DH10 BETA (Invitrogen®), containing a plasmid, was used as positive control. This assay was performed in duplicate.

Statistical analysis

Statistical analysis was performed with the data obtained in millimeters in the antimicrobial activity tests at different pH and temperatures. ANOVA followed by Tukey with a 95% confidence level was performed on the data and considered significant where the P-value is equals to or less than 0.05. Testing was completed with Statistica software version 12.

RESULTS

Antimicrobial activity-producing strains

Among the 13 enterococci strains selected, five (36.46%) showed activity against *L. monocytogenes* ATCC 35152 in the double-agar layer test (Table 1). Of these five strains selected, only the crude extract bioassay of the *E. mundtii* J5 strain showed an antilisterial activity and was therefore chosen for subsequent tests.

Antimicrobial compounds produced by *E. mundtii* J5 and quantification of antimicrobial activity

The growth curves of the *E. mundtii* J5 strain related to antimicrobial compounds (ACs-J5) activity and pH are presented in Figure 1. The strain reached the mid exponential phase after 6 h of incubation and the highest (16 to 18 mm) inhibition zones were observed in the stationary phase (12 to 40 h). The initial pH of the culture was 6.5; after 12 h it passed to 5.0, reaching 4.0 at 48 h growth. The antimicrobial activity of ACs-J5 was 400 AU mL^{-1} against *L. monocytogenes* ATCC 35152.

Stability of ACs-J5

The effect of different treatments on the antimicrobial activity of ACs-J5 is presented in Table 2. The activity of ACs-J5 was lost after proteolytic enzyme treatment, suggesting its proteinaceous nature. However, ACs-J5 tolerance to pH treatments (1 to 12). The greater antimicrobial activity of AC-J5 was observed at pH 4.0 to 7.0, with the highest antilisterial activity at pH 6.0 ($p < 0.05$). At Ph 1.0 to 3.0, the AC-J5 activity remained, in media, at 63% ($p < 0.05$); and at pH 8.0 to 12.0 the activity was around 66%, compared to the controls ($p < 0.05$). As shown in Table 2, ACs-J5 was resistant towards the heat treatments. The only significant reduction in activity was seen at 121°C for 15 min where the activity decreased to 75% ($p < 0.05$). There was a significant difference at 121°C ($p = 0.03$). Methanol, DMSO, acetone and ethanol did not inactivate the antilisterial activity of ACs-J5, while butanol and chloroform did. On the other hand, the antilisterial activity was increased by 75% after the treatment of ACs-J5 with Tween 80 (Table 2).

Determination of antimicrobial activity spectrum of ACs-J5

All *L. monocytogenes* strains tested showed sensitivity to AC-J5, but *B. cereus*, *S. aureus*, *E. faecalis*, *S. agalactiae*, *K. pneumoniae*, *E. coli* and *P. aeruginosa* strains were not affected by the ACs-J5 (Table 3). Interestingly, *L. monocytogenes* serotype 1c presents the largest halo (45 mm) and serotype 4b the smallest (15.8 mm).

Identification of the enterocin genes and plasmid in *E. mundtti* J5 strain

The mundticin KS gene was detected in the *E. mundtii* J5 strain. The other enterocin genes tested were negative. No plasmids were observed in the *E. mundtii* J5 strain.

DISCUSSION

L. monocytogenes has been recognized as one of the most relevant foodborne bacteria pathogens (Scallan et al., 2011). During this study, five (38.5%) enterococci strains isolated from fecal samples of wild fur seals showed antilisterial activity. Bacteriocinogenic enterococcal strains isolated from fecal samples of wild animals were previously identified by Almeida et al. (2011), Brandão et al. (2010), and Poeta et al. (2008, 2007). Poeta et al. (2008) found bacteriocinogenic isolates in 17.85% of the enterococci isolated from fecal samples of wild animals. Brandão et al. (2010) also found a similar number (45.6%) of bacteriocinogenic enterococcal isolates of fecal origin from humans, pets, wild animals and birds against *L. monocytogenes* CECT4032. Only E. mundtii J5 produced antilisterial activity after the pH was adjusted. Other studies evaluating the bacteriocin-like substances from enterococci showed similar data to this (Birri et al., 2010; Adeniyi et al., 2015). Birri et al. (2010) worked with the supernatant of 104 LAB study from the gastrointestinal tracts of children, and only one isolate of *E. avium* (0.96%) was a producer of an antagonist

Table 2. Effect of proteolytic enzymes, pH, heat, and chemical treatments on the ACs-J5 activty against *L. monocytogenes* ATCC 35152.

Treatments		Concentration	Percentage of residual activity
Enzymes	Proteinase K	2 mg ml^{-1}	0
	Trypsin	2 mg ml^{-1}	0
	Papain	2 mg ml^{-1}	0
pH	1	-	55*
	2	-	60*
	3	-	75*
	4	-	95
	5	-	95
	6	-	100
	7	-	100
	8	-	75*
	9	-	70*
	10	-	65*
	11	-	60*
	12	-	60*
Heat	30 to 90° C for 30 min	-	100
	100° C for 30 min	-	90
	121° C for 15min	-	75*
Chemicals	Methanol	50% (v/v)	80
	Butanol	50% (v/v)	43
	DMSO	50% (v/v)	80
	Acetone	50% (v/v)	90
	Chloroform	50% (v/v)	6
	Ethanol	50% (v/v)	80
	Tween 80	10% (v/v)	175
Control		-	100

*Significantly difference ($p<0.05$).

Table 3. Antimicrobial activity spectrum of ACs-J5.

Indicator microorganism	Inhibition zone (mm)
Listeria monocytogenes ATCC 7644 (serotype 1/2c)	30
*L. monocytogenes** (serotype 1/2a)	25.5
*L. monocytogenes** (serotype 1/2b)	24.3
*L. monocytogenes** (serotype 4b)	15.8
*L. monocytogenes** (serotype 1c)	45
Enterococcus faecalis ATCC 29212	0
Streptococcus agalactiae ATCC 13813	0
Staphylococcus aureus ATCC 190506	0
Klebsiella pneumoniae ATCC 700603	0
Escherichia coli ATCC 25922	0
Pseudomonas aeruginosa ATCC 27853	0

*isolate from raw cow milk.

substance. Many substances produced by microorganisms have been identified as inhibitors of pathogenic bacteria. However, the inhibition caused by enterococci may not be related to bacteriocins, but other inhibitory substances, such as organic acids resulting from their metabolism (Gaamouche et al., 2014).

In the present study, the greatest antilisterial activity was observed in the stationary phase. This is in partial

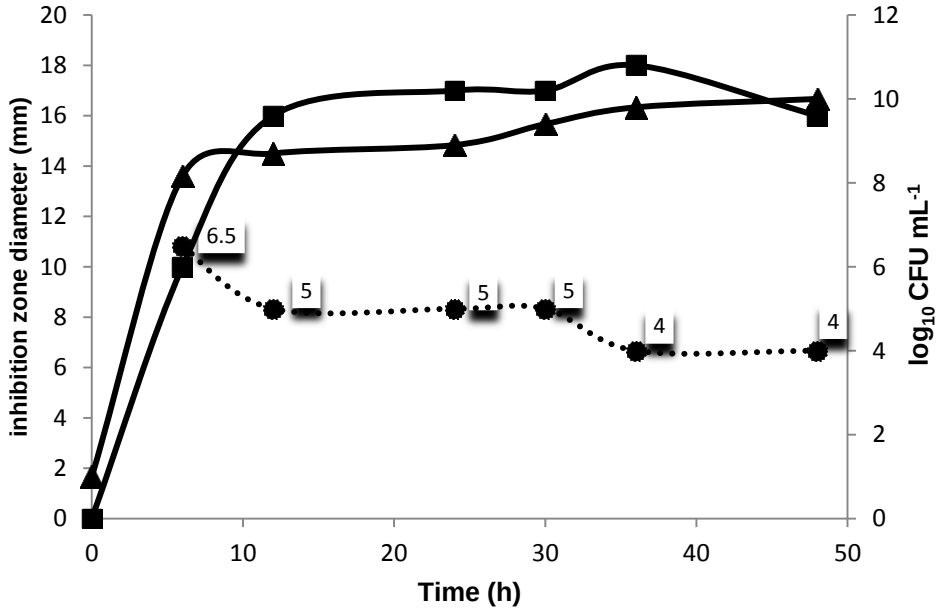

Figure 1. Growth curve (triangles) indicating phase of antimicrobial substance production (squares) of *Enterococcus mundtii* J5 strain and pH of the culture (dots) monitored for 48 h in TSB medium at 35°C

agreement with the results obtained by Leroy and De Vuyst (2002), in which production of enterocin RZC5 (from *E. faecium*) occurred in the early growth phase. The production of enterocin HJ35 by *E. faecium* HJ35 also occurred in the mid-log growth phase, reaching maximum production during the late stationary phase (Yoon et al., 2005). Moshood and Tengku Haziyamin (2012) showed that maximum bacteriocin production of *E. faecium* B3L3 occurs at the end of the exponential phase.

Bacteriocins produced by LAB generally have a low antimicrobial activity in arbitrary units per milliliter (AU mL-1). However, the low values of ACs-J5 (400 AU mL-1) found in this study could be related to the technique used to determine the activity. Some studies carried out to show the enterocin production occurring in fermenters controlled conditions such as pH, for example, which is an important factor in the production of peptides. This can interfere with the antimicrobial activity obtained in arbitrary units per milliliter. Under these conditions, generally, the antimicrobial activity values obtained are much higher than those found in this work. Mundticin KS showed an antimicrobial activity of 6,400 to 12,800 AU/mL-1 when produced in a fermenter (Kawamoto et al., 2002).

Xiaoyuan et al. (2014) showed the highest antilisteria activity reaching 51,200 AU/mL-1 from enterocin A after 24 h of induction in a 5 L fermenter. The ACs-J5 were stable within a wide range of pH and heat treatment and sensitive to all proteolytic enzymes tested, suggesting their proteinaceous nature. These features were typical of class IIa bacteriocins (Nes et al., 2014). The thermal and pH stability of ACs-J5 may constitute an advantage for potential use as a biopreservative in combination with thermal processing used to preserve food products.The stability of enterocins against treatment with chemicals has great importance from the technological point of view, since many organic and inorganic compounds are used for food processing, by incorporating the ingredients. In this study, the ACs-J5 were sensitive to chloroform and to a lesser extent butanol. Motta et al (2007) also observed that the activity of bacteriocin produced by Bacillus spp. was affected by butanol and to a lesser extent by acetone and methanol. Using the treatment with Tween 80, an increase in antimicrobial activity was observed. Similar results were observed for the mundticin CRL1656 (Espeche et al., 2014). The antimicrobial compounds produced by E. mundtii J5 did not inhibit the growth of other gram-positive and gram-negative strains. Paschoalin et al. (2011) reported antimicrobial activity of *Enterococcus* spp. for the production of enterocins capable of inhibiting the development of *L. monocytogenes*, besides inhibiting gram-negative bacteria, such as *E. coli* and *S. paratyphi*.

Only the *mundticin* KS gene was detected in the J5 strain. Mundticin KS is a positively charged, hydrophobic, 43-amino-acid peptide that contains the highly conserved YGNGV motif found in the N-terminal part of many class IIa bacteriocins in the classification described by Klaenhammer (1993).

In the present study, we did not detect plasmid in *E. mundtii* J5 strains. This is in agreement with Criado et al.

(2008), who observed the presence of enterocin P located in a chromosomal fragment. In a similar development, Moshood et al. (2015) were able to detect the enterocin gene in the chromosomal DNA of *Enterococcus mundtii* C4L10.

Conclusion

In this study a bacteriocinogenic enterococci strain (*E. mundtii* J5 isolated from the fecal samples of wild South American fur seal) was identified. The Antimicrobial compounds produced by *E. mundtii* J5 produced should belong to the class IIa, since were heat-stable and strongly inhibits the growth of *L. monocytogenes* strains. These properties make ACs-J5 a valuable biopreservative in food industries for avoiding pathogenic microorganisms and should be a good candidate for probiotic application. However, further studies on the purification and characterization of the intended antibiotic would be necessary.

Conflict of Interests

The authors have not declared any conflict of interest.

ACKNOWLEDGEMENTS

The authors thank the government agencies Conselho Nacional de Desenvolvimento Científico e Tecnológico do Brasil (CNPq - #444335/2014-5 and #303251/2014-0) and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) of the Brazilian government. They are grateful to biologist Mauricio Tavares from Grupo de Estudos de Mamíferos Aquáticos do Rio Grande do Sul and all staff from Ceclimar- UFRGS.

REFERENCES

Adeniyi BA, Adetoye A, Ayeni FA (2015). Antibacterial activities of lactic acid bacteria isolated from cow faeces against potential enteric pathogens. Afr. Health Sci. 15(3):888-895. http://dx.doi.org/10.4314/ahs.v15i3.24.

Almeida T, Brandão A, Muñoz-Atienza E, Gonçalves A, Torres C, Igrejas G, Hernández PE, Herranz C, Cintas LM, Poeta P (2011). Identification of bacteriocin genes in enterococci isolated from game animals and saltwater fish. J. Food Prot. 74(8):1252-1260. http://dx.doi.org/10.4315/0362-028X.JFP-11-016

Bennik MH, Vanloo B, Brasseur R, Gorris LG, Smid RJ (1998). A novel bacteriocina with a YGNGV motif from vegetable-associated *Enterococcus mundtii*: Full characterization and interaction with target organisms. Biochim. Biophys. Acta. 1373(1):47-58. http://dx.doi.org/10.1016/S0005-2736(98)00086-8

Bigwood T, Hudson JA, Cooney J, McIntyre L, Billington C, Heinemann JA, Wall F (2012). Inhibition of *Listeria monocytogenes* by *Enterococcus mundtii* isolated from soil. Food Microbiol. 32(2):354-360. http://dx.doi.org/10.1016/j.fm.2012.07.015

Birri DJ, Brede DA, Forberg T, Holo H, Nes IF (2010). Molecular and genetic characterization of a novel bacteriocin locus in *Enterococcus avium* isolates from infants. Appl. Environ. Microbiol. 76(2):483-492. http://dx.doi.org/10.1128/AEM.01597-09.

Brandão A, Almeida T, Muñoz-Atienza E, Torres C, Igrejas G, Hernández PE, Cintas LM, Poeta P, Herranz C (2010). Antimicrobial activity and occurrence of bacteriocin structural genes in *Enterococcus* spp. of human and animal origin isolated in Portugal. Arch. Microbiol. 192(11):927-936. http://dx.doi.org/10.1007/s00203-010-0619-z 123

Criado R, Gutiérrez J, Budin-Verneuil A, Hernández PE, Hartke A, Cintas LM (2008). Molecular analysis of the replication region of the pClZ2 plasmid from the multiple bacteriocin producer strain *Enterococcus faecium* L50. Plasmid 60(3):18-189. http://dx.doi: 10.1016/j.plasmid.2008.07.002.

de la Fuente-Salcido NM, Casados-Vázquez LE, Barboza-Corona JE (2013). Bacteriocins of *Bacillus thuringiensis* can expand the potential of this bacterium to other areas rather than limit its use only as microbial insecticide. Can. J. Microbiol. 59(8):515-522. http://dx.doi.org/10.1139/cjm-2013-0284.

De Vuyst L, Foulquié Moreno MR, Revets H (2003). Screening for enterocins and detection of hemolysin and vancomycin resistance in enterococci of different origins. Int. J. Food. Microbiol. 84(3):299-318.

Donato ST (2007) Comparação de métodos convencionais e semi-automatizados para identificação de *Enterococcus* spp. frente a Biologia Molecular em identificações discrepantes. Fortaleza, Brasil, 86 p. (M. Sc. Dissertation. Faculdade de Medicina. UFC).

Espeche MC, Juárez Tomás MS, Wiese B, Bru E, Nader-Macías ME (2014). Physicochemical factors differentially affect the biomass and bacteriocin production by bovine *Enterococcus mundtii* CRL1656. J. Dairy. Sci. 97(2):789-797. http://dx.doi.org/10.3168/jds.2013-7070.

Foulquié MMR, Callewaert R, Devreese B, Van Beeumen J, De Vuyst L (2003) Isolation and biochemical characterization of enterocin produced by enterococci from different sources. J. Appl. Microbiol. 94(2):214-229. http://dx.doi.org/10.1046/j.1365-2672.2003.01823.x

Gaamouche S, Arakrak A, Bakkali M, Laglaoui A (2014). Antimicrobial activity of lactic acid bacteria and bacteriocins isolated from a traditional brine table olives against pathogenic bacteria. Int. J. Curr. Microbiol. App. Sci. 3(11):657-666.

Gao Y, Li B, Li D, Zhang L (2016). Purification and characteristics of a novel bacteriocin produced by *Enterococcus faecalis* L11 isolated from Chinese traditional fermented cucumber. Biotechnol. Lett. 67(5):1-6. http://dx.doi.org/10.1007/s10529-016-2055-x

Gutierrez J, Criado R, Citti R, Martín M, Herranz C, Nes I, Cintas L, Hernández P (2005). Cloning, production and functional expression of enterocin P, a sec-dependent bacteriocin produced by *Enterococcus faecium* P13, in *Escherichia coli*. Int. J. Food Microbiol. 103(3):239-250. http://dx.doi.org/10.1016/j.ijfoodmicro.2004.11.035

Kawamoto S, Shima J, Sato R, Eguchi T, Ohmomo S, Shibato J, Horikoshi N, Takeshita K (2002). Biochemical and genetic characterization of *mundticin* KS, an antilisterial peptide produced by *Enterococcus mundtii* NFRI 7393. Appl. Environ. Microbiol. 68(8):3830–3840. http://dx.doi.org/10.1128/AEM.68.8.3830-3840.2002

Klaenhammer TR (1993). Genetics of bacteriocins produced by lactic acid bacteria. FEMS Microbiol. Rev. 12:39-85. http://dx.doi.org/10.1111/j.1574-6976.1993.tb00012.x

Leroy F, De Vuyst L (2002). Bacteriocin production by *E. faecium* RZSC5 is cell density limited and occurs in the very early growth phase. J. Food. Microbiol. 72(1-2):155-164. http://dx.doi.org/10.1016/S0168-1605(01)00635-3

Lewus CB, Montville TJ (1991). Detection of bacteriocins produced by lactic-acid bacteria. J. Microbiol. Meth. 13(2):145-150. http://dx.doi.org/10.1016/0167-7012(91)90014-H

Maldonado-Barragán A, Caballero-Guerrero B, Martín V, Ruiz-Barba JL, Miguel Rodríguez J (2016). Purification and genetic characterization of gassericin E, a novel co-culture inducible bacteriocin from *Lactobacillus gasseri* EV1461 isolated from the vagina of a healthy woman. BCM Microbiol. 16:37. http://dx.doi.org/10.1186/s12866-016-0663-1

Mello JF (2007). Caracterização molecular do gene *iap* de *Listeria monocytogenes* isoladas de alimentos no estado do Rio Grande do Sul. Rio Grande do Sul, Brasil, 156 p. (M. Sc. Dissertation. Instituto de Ciência e Tecnologia de Alimentos (UFRGS).

Moshood AY, TengkuHaziyamin ATAH (2012) Optimization of temperature and pH for the growth and bacteriocin production of

Enterococcus faecium B3L3. IOSR J. Pharm. 2(6):49-59.
Moshood AY, Tengku Haziyamin ATAH, Abdul H (2015). Detection and characterization of enterocin encoding genes in *Enterococcus mundtii* strain C4l10 from the cecum of non-broiler chicken. Int. J. Biol. Pharm. Allied. Sci. 7(4):131-136.
Motta AS, Brandelli A (2002). Characterization of an antibacterial peptide produced by *Brevibacterium linens*. J. Appl. Microbiol. 92(1):63-70. http://dx.doi.org/10.1046/j.1365-2672.2002.01490.x
Motta AS, Lorenzini DM, Brandelli A (2007). Purification and partial characterization of an antimicrobial peptide produced by a novel *Bacillus* sp. isolated from the Amazon Basin. Curr. Microbiol. 54(4):282–286. http://dx.doi.org/10.1007/s00284-006-0414-x
Nes IF, Diep DB, Ike Y (2014). Enterococcal bacteriocins and antimicrobial proteins that contribute to niche control. In: Gilmore MS, Clewell DB, Ike Y, et al., editors. Enterococci: From Commensals to Leading Causes of Drug Resistant Infection [Internet]. Boston: Massachusetts Eye and Ear Infirmary. Available at: http://www.ncbi.nlm.nih.gov/books/NBK190428/
Park S, Itoh K, Fujisawa T (2003). Characteristics and identification of enterocins produced by *Enterococcus faecium* JCM 5804T. J. Appl. Microbiol. 95(2):294-300. http://dx.doi.org/10.1046/j.1365-2672.2003.01975.x
Paschoalin VMF, Bellei B, Miguel M, Mere Del Aguila EM, Silva JT (2011). Purification of a bacteriocin produced by *Enterococcus faecium* and its effectiveness for preservation of fresh-cut lettuce. J. Microbiol. Antimicrob. 3(5):119-125.
Poeta P, Costa D, Rojo-Bezares B, Zarazaga M, Klibi N, Rodrigues J, Torres C (2007).Detection of antimicrobial activities and bacteriocin structural genes in faecal enterococci of wild animals. Microbiol. Res. 162(3):257-263.
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. Microbiol. 48(5):385-392.
Saavedra L, Minahk C, de Ruiz Holgado AP, Sesma F (2004). Enhancement of the enterocin CRL35 activity by a synthetic peptide derived from the NH2-terminal sequence. Antimicrob. Agents Chemother. 48(7):2778-2781. http://dx.doi.org/10.1128/AAC.48.7.2778-2781.2004.
Sambrook J, Russel DW (2001). Molecular cloning: A laboratory manual. Cold Spring Harb Protoc, New York, NY.
Santestevan NA, de Angelis Zvoboda D, Prichula J, Pereira RI, Wachholz GR, Cardoso LA, de Moura TM, Medeiros AW, de Amorin DB,Tavares M, d'Azevedo PA, Franco AC, Frazzon J, Frazzon AP (2015). Antimicrobial resistance and virulence factor gene profiles of *Enterococcus* spp. isolates from wild *Arctocephalus australis* (South American fur seal) and *Arctocephalus tropicalis* (Subantarctic fur seal). World J. Microbiol. Biotechnol. 31(12):1935-1946. http://dx.doi.org/10.1007/s11274-015-1938-7.
Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, Jones JL, Griffin PM (2011). Foodborne illness acquired in the United States–Major pathogens. Emerg. Infect. Dis. 17(1):7-15.
Xiaoyuan H, Ruoyu M, Yong Z, Da T, Xiumin W, Di X, Jianzhong H, Jianhua W (2014). Biotechnical paving of recombinant enterocin A as the candidate of anti-*Listeria* agent. BMC Microbiol. 14:220. http://dx.doi.org/10.1186/s12866-014-0220-8
Yang Y, Tao WY, Liu YJ, Zhu F (2008). Inhibition of *Bacillus cereus* by lactic acid bacteria starter cultures in rice fermentation. Food Control. 19(2):159-161.
Yoon YC, Park HJ, Lee NK, Paik HD (2005). Characterization and enhanced production of enterocin HJ35 by *Enterococcus faecium* HJ35 isolated from human skin. Biotechnol. Bioproc. E10:296-303. http://dx.doi.org/10.1007/BF02931845.
Zendo T, Eungruttanagorn N, Fujioka S, Tashiro Y, Nomura K, Sera Y, Kobayashi G, Nakayama J, Ishizaki A, Sonomoto K (2005). Identification and production of a bacteriocin from *Enterococcus mundtii* QU 2 isolated from soybean. J. Appl. Microbiol. 99:1181-1190. http://dx.doi.org/10.1111/j.1365-2672.2005.02704.x