Archives of Microbiology
https://doi.org/10.1007/s00203-020-02118-0

ORIGINAL PAPER

Assessment of the bioprotective potential of lactic acid bacteria against *Listeria monocytogenes* in ground beef

Johana S. Gomez[1] · Romina B. Parada[2] · Marisol Vallejo[2] · Emilio R. Marguet[2] · Augusto Bellomio[3] · Nora Perotti[1] · Kátia G. de Carvalho[1]

Received: 17 October 2019 / Revised: 13 July 2020 / Accepted: 8 November 2020

Abstract
Lactic acid bacteria can be considered as natural biopreservative and good biotechnological alternative to food safety. In this study, the antilisterial compounds produced by *Enterococcus* isolates from the Patagonian environment and their effectiveness for the control of *Listeria monocytogenes* in a food model were studied. *Enterococcus* isolates whose cell-free supernatant presented activity against *Listeria monocytogenes* were identified and evaluated for their virulence factors. The activity of the antimicrobial compounds produced by *Enterococcus* sp. against *Listeria monocytogenes* Scott A in meat gravy and ground beef during refrigerated storage was tested. The results indicated that ten *Enterococcus* isolates presented activity against *Listeria monocytogenes* and none of the selected strains presented virulence factors. *L. monocytogenes* in the food models containing the antilisterial compounds produced by *Enterococcus* sp. has decreased over the days, indicating that these compounds and cultures are an alternative to control the growth of *L. monocytogenes* in foods.

Keywords *Enterococcus mundtii* · *Listeria monocytogenes* · Antimicrobial activity · Meat model · Food safety

Introduction

Foods can be contaminated by microorganisms such as *Listeria monocytogenes*, *Pseudomonas* spp., among others and/or bacterial microbiota and the main problem in the food industry is how to prevent and eliminate the presence of pathogenic bacteria through cleaning and disinfection mechanisms, that are sometimes inefficient. Furthermore, the use of chemical agents for food preservation is not compatible with the image of minimally processed fresh products. Hence, the presence of lactic acid bacteria (LAB) is of great interest since they are Generally Recognized As Safe (GRAS) (Dal Bello et al. 2012) and they can be considered as biopreservative cultures (Stiles 1996). LAB may be used in food preservation because they compete with other microorganisms and produce compounds with antimicrobial activity such as lactic acid, acetic acid, hydrogen peroxide, diacetyl and ethanol (Leroy and De Vuyst 2004; Tomé et al. 2006). Moreover, many LAB strains are capable of producing bacteriocins in foods (Cotter et al. 2005; Panesar et al. 2007) that may inhibit the growth of pathogenic Gram-positive bacteria, as well as some yeasts and Gram-negative species (Kailasapathy 2006).

Among the LAB, *Enterococcus* is one of the genera and some strains of *Enterococcus* have health-promoting properties and a potential technological advantage in the food industry. They present interesting enzymatic activities and acidifying, reducing and proteolytic properties that improve their sensory quality, contribute to the texture of foods, aroma and flavor (Giraffa 2003; Ogier and Serror 2008). In addition, many *Enterococcus* strains produce bacteriocins that give them an advantage over pathogenic

Communicated by Erko Stackebrandt.

✉ Kátia G. de Carvalho
ka20_04@hotmail.com

[1] Planta Piloto de Procesos Industriales Microbiológicos (PROIMI), CONICET, Av. Belgrano y Pje. Caseros, San Miguel de Tucumán, 4000 Tucumán, Argentina

[2] Laboratorio de Biotecnología Bacteriana, Fac. de Cs. Naturales y Cs. de la Salud – UNPSJB, Sede Trelew, Chubut, Argentina

[3] Instituto Superior de Investigaciones Biológicas (INSIBIO, CONICET-UNT) Instituto de Química Biológica "Dr. Bernabé Bloj", Facultad de Bioquímica, Química y Farmacia – Universidad Nacional de Tucumán, Batalla de Chacabuco, 461, San Miguel de Tucuman, Tucumán 4000, Argentina

Published online: 02 January 2021

bacteria or the microbial flora of food. Therefore, *Enterococcus* could be used as natural biopreservative cultures to improve food safety.

The Patagonian marine environment has its own physical and chemical characteristics that allows the isolation of *Enterococcus* with particular physiological properties (Marguet et al. 2011). High salinity and low temperatures exert a selective pressure that results in a microbiota that has been little studied, but nevertheless presents a biotechnological potential of interest, both for the study of the production of metabolites and for the use of *Enterococcus* strains.

The presence of safety factors (hemolytic and gelatinase activities, virulence factors and antibiotic resistance) must be investigated because the safety evaluation of enterococcal strains and the use as biopreservative cultures is very important (Ben Braïek et al. 2018). The bacteriocinogenic strain should be able to grow and produce bacteriocins in situ or ex situ, to present bacteriocin diffusion through food and the parameters that influence metabolic production, and the use of LAB as bioprotective cultures should not change the sensory characteristics in foods (Dicks et al. 2004).

The objective of this work was to report the partial characterization of bacteriocins produced by Patagonian *Enterococcus* strains, to evaluate the virulence factors of the selected strains, and their potential use as biopreservative cultures/antilisterial extract) against *Listeria monocytogenes* in a food model during refrigerated storage (4 °C) for 15 days.

Material and methods

Indicator strains and culture conditions

In Table 1, the target strains used in the study are listed. LAB strain isolated from the Patagonian area was inoculated in MRS broth (Biokar Diagnostics, Beauvais, France) and

Table 1 Culture conditions and antimicrobial activity of cell-free supernatant from *Enterococcus mundtii* strains

Indicator strains	Growth media	Antimicrobial activity		
	Temperature (°C)	*E. mundtii* Tw56	*E. mundtii* Tw802	*E. mundtii* Tw807
Enterococcus faecalis ATCC 29,212	MRS, 37 °C	+ +	+ +	+ +
Enterococcus Van A	MRS, 37 °C	+ +	+ +	+ +
Enterococcus Van B	MRS, 37 °C	+ +	+ +	+ +
Enterococcus Van C	MRS, 37 °C	+ +	+ +	+ +
Enterococcus casseliflavus	MRS, 37 °C	+ +	+ + +	+ + +
Enterococcus faecium	MRS, 37 °C	+ + +	+ + +	+ +
Escherichia coli ATCC 25,922	TS, 35 °C	–	–	–
Escherichia coli ATCC 35,218	TS, 35 °C	–	–	–
Lactococcus lactis ATCC 11,454	MRS, 30 °C	–	–	–
Lactococcus garvieae 03/8460[b]	MRS, 30 °C	–	–	–
Lactococcus garvieae 03/8702[b]	MRS, 30 °C	–	–	–
Lactococcus piscium 23.3.92[b]	MRS, 30 °C	–	–	–
Lactobacillus plantarum TwLb 5	MRS, 30 °C	+	+ +	+ +
Listeria innocua Tw 67	TS, 30 °C	+ + +	+ + +	+ + +
Listeria innocua 6a	TS, 30 °C	+ + +	+ + +	+ + +
Listeria innocua ATCC 33,090	TS, 30 °C	+ +	+ + +	+ + +
Listeria monocytogenes Scott A	TS, 30 °C	+ + +	+ + +	+ + +
Listeria monocytogenes ATCC 7644	TS, 35 °C	+ + +	+ + +	+ + +
Listeria monocytogenes 1908[a]	TS, 35 °C	+ + +	+ + +	+ + +
Listeria monocytogenes 1915[a]	TS, 35 °C	+ + +	+ + +	+ + +
Listeria monocytogenes 1599[a]	TS, 35 °C	+ + +	+ + +	+ + +
Micrococcus luteus ATCC 15,307	TS, 30 °C	–	–	–
Streptococcus iniae MT 2376[b]	MRS, 37 °C	–	–	–
Pseudomona aeruginosa ATCC 27,853	TS, 30 °C	–	–	–
Yersinia ruckerii 02/1607/C[b]	TS, 25 °C	–	–	–

Diameter of clear zone: +, ≥ 10 mm; + +, ≥ 15 mm; + + +, ≥ 20 mm; –, no inhibition

[a]Strains provided by Lic. Ledesma (Fac. de Cs. Naturales y Cs. de la Salud, Universidad Nacional de la Patagonia San Juan Bosco)

[b]Strains provided by Dr. Fernandez-Garayzabal (Escuela de Medicina Veterinaria, Universidad Complutense de Madrid)

was grown at 25 °C for 18 h, and the other bacteria were inoculated in BHI broth (Difco, Le Pont de Claix, France) and were grown at 37 °C for 24 h. Cell-free supernatants (CFS) were separated to evaluate the antimicrobial activity. LAB strain was grown in MRS broth at 30 °C for 18 h and was centrifuged at 8000 rpm (for 15 min at 4 °C) in a high-speed centrifuge (Hettich Zentrifugen, model Mikro 22R, Germany). The CFS obtained was treated at 100 °C for 5 min, was filtered through a 0.20 µm pore sterilizing syringe filter (Sartorius, Stedim Biotech, Germany) and was stored at − 20 °C. Antimicrobial activities of the LAB strains isolated were evaluated by the double-layer diffusion test, according to Farías et al. (1994), using *Listeria innocua* ATCC 33,090 and *Listeria monocytogenes* Scott A as indicators, and the results were expressed as arbitrary units per milliliter (AU mL^{-1})., Nisin (1000 UI mL^{-1}, Nisaplin, Danisco, Denmark) and distilled water were used as positive and negative controls, respectively.

Isolation, phenotypic and genetic identification of LAB strains

The LAB strains were isolated from the provinces of Chubut and Santa Cruz (between − 42.00 latitude and − 52.00 latitude) of the VIRCH-Valdes and Río Senguer-Golfo San Jorge regions (Patagonia, Argentina). The isolates, as well as their phenotypic identification, were made in MRS agar medium (Biokar) as described by Vallejo et al. (2009). Three isolates were selected to be identified by 16S rDNA amplification and sequencing as described by Delcarlo et al. (2019).

Determination of virulence factors

Evaluation of strain safety and PCR for the detection of virulence factors

The production of gelatinase, hemolysin and exopolysaccharides was carried out as described by Delcarlo et al. (2019). The susceptibility of *Enterococcus* strains to penicillin, ampicillin, amoxicillin/clavulanic, erythromycin, rifampin, vancomycin trimethoprim/sulfamethoxazole, and teicoplanin was evaluated according to the National Committee for Clinical Laboratory Standards (NCCLS 2015) guidelines. Extracted DNA was used as a template for the amplification of virulence genes and the general PCR conditions were those described by the authors in Table 2.

Characterization of antimicrobial activity

Determination of the active principle causing the inhibitory activity

The CFSs were fractionated into three aliquots. The first CFS was called "crude". The second CFS was neutralized to pH 6.5 with 0.5 M NaOH (called "neutralized CFS") and the third CFS "heated" was neutralized and heated at 100 °C for 5 min. Then, the above preparations were evaluated as Vallejo et al. (2009).

The CFSs were incubated at 37 °C for 1 h with catalase (2 mg mL^{-1}), trypsin, proteinase K, lipase or lysozyme (Sigma, USA) in a final concentration of 1.0 mg mL^{-1} (Vallejo et al. 2009). Enzyme solutions were diluted with sterile water and used as negative and positive controls, respectively. The residual antilisterial activity was determined according to Farías et al. (1994).

Table 2 PCR amplification of potential enterococcal virulence factors

Virulence Factor	Sequence (5`–3`)	Size (pb)	Reference
Agg	f: AAGAAAAAGAAGTAGACCAAC r: AAACGGCAAGACAAGTAAATA	1553	Eaton and Gasson (2001)
gelE	f: ACCCCGTATCATTGGTTT r: ACGCATTGCTTTTCCATC	419	Eaton and Gasson (2001)
Esp	f: TTGCTAATGCTAGTCCACGACC r: GCGTCAACACTTGCATTGCCGAA	933	Eaton and Gasson (2001)
hylEfm	f: GAGTAGAGGAATATCTTAGC r: AGGCTCCAATTCTGT	661	Rice et al.(2003)
IS16	f: CATGTTCCACGAACCAGAG r: TCAAAAAGTGGGCTTGGC	547	Werner et al. (2011)
$CylL_l$	f: GATGGAGGGTAAGAATTATGG r: GCTTCACCTCACTAAGTTTTATAG	253	Semedo et al. (2003)
$CylL_s$	f: GAAGCACAGTGCTAAATA AGG r: GTATAAGAGGGCTAGTTTCAC	240	Semedo et al. (2003)

Stability test for antimicrobial compounds produced by *Enterococcus* sp.

The influence of temperature on CFSs activity was evaluated by keeping the CFSs at 40–100 °C for 30 min and then the activity against *L. monocytogenes* Scott A was observed. Different pH treatments ranging from 2 to 10 were used to evaluate the residual antilisterial activity. The mixture was incubated for 2 h at 25 °C and residual antilisterial activity was evaluated.

PCR screening for enterocin structural genes

The presence of structural genes of bacteriocins present in the genome of the bacteria studied was carried out by PCR and the primers used for the amplification of enterocin A, B, P, L50A, L50B, mundticin KS, bacteriocina 96, bact 31, 1071 A/B, enterocin Q and HirJM 79 and corresponding references are listed in Table 3. General PCR conditions were described by Schelegueda et al. (2015).

Bacteriocin production from LAB in different culture media and incubation conditions

The culture media employed for bacteriocin production were: MRSs broth (Biokar), MRSc (Biokar), LAPTgs (Raibaud et al. 1963) and LAPTgc. Subscripts *s* and *c* denote without or with cysteine (Sigma, USA) (0.5 mg mL^{-1}), respectively. Incubation temperatures were 25, 30 and 37 °C, without or with agitation (100 rpm). The CFSs were neutralized (pH 6.5) and the antilisterial activity quantification was performed by the critical dilution technique (Rosa et al. 2002).

Determination of antimicrobial spectrum and quantification of antimicrobial activity

Since *E. mundtii* Tw56, Tw802 and Tw807 had the highest antimicrobial activity against *L. monocytogenes,* they were selected to determine their spectrum of activity against other strains that cause food spoilage (Table 1). The antimicrobial activity of neutralized CFSs against the indicator microorganism was performed according to Farías et al. (1994). The formation of inhibition halos in the plates was evaluated after 18 h at 30 °C.

Evaluation of bacteriocinogenic strains against *Listeria monocytogenes* Scott A in food models

The protective effect of *E. mundtii* Tw56, Tw802 and Tw807 against *L. monocytogenes* Scott A was evaluated either by

Table 3 Primer sequences for PCR amplification of enterocin genes in *Enterococcus mundtii* Tw56, Tw802 and Tw807

Enterocin	Sequence (5´–3´)	Size (pb)	Reference
Ent A	f: GGTACCACTCATAGTGGAAA	138	De Vuyst et al. (2003)
	r: CCCTGGAATTGCTCCACCTAA		
Ent B	f: CAAAATGTAAAAGAATTAAGTACG	201	De Vuyst et al. (2003)
	r: AGAGTATACATTTGCTAACCC		
Ent P	f: GCTACGCGTTCATATGGTAAT	87	De Vuyst et al. (2003)
	r: TCCTGCAATATTCTCTTTAGC		
Ent LB50A	f: ATGGGAGCAATCGCAAAATTA	274	De Vuyst et al. (2003)
	r: TTTGTTAATTGCCCATCCTTC		
Ent LB50B	f ATGGGAGCAATCGCAAAATTA	274	De Vuyst et al. (2003)
	r: TAGCCATTTTTCAATTTGATC		
Bact 96	f GTGGAGAGGACGAAAGGAGA	291	Henning et al.(2015)
	r: TTGATTAGTGGAGAGGACGGATTA		
Bact 31	f: CCTACGTATTACGGAAATGGT	130	Özdemir et al.(2011)
	r: GCCATGTTGTACCCAACCATT		
1071 A/B	f: GGGGAGAGTCGGTTTTTAG	273	Martín et al.(2006)
	r: ATCATATGCGGGTTGTAGCC		
EntQ	f: ATGAATTTTCTTCTTAAAAATGGTATCGCA	105	Belgacem et al. (2010)
	r: TTAACAAGAAATTTTTTCCCATGGCAA		
mun KS	f: TGAGAGAAGGTTTAAGTTTTGAAGAA	379	Zendo et al. (2005)
	r: TCCACTGAAATCCATGAATGA		
HirJM 79	f: ATGAAAAAGAAAGTATTAAAACATTGTGTTATTCTAGG	408	Almeida et al. (2011)
	r ATAAGTTAAGCTTGTACTACCTTCTAGGTGCCCATGGACC		

direct application or incorporation of the neutralized antilisterial extract (Yang et al. 1992) in meat gravy and ground beef models. The food models were divided into 14 fractions. Each one (A–N), received the following treatment: A) control, *L. monocytogenes* Scott A (10^4 CFU mL^{-1} or g^{-1}); B, C and D) control, *E. mundtii* Tw56, Tw802 or Tw807 (10^7 CFU mL^{-1} or g^{-1}), respectively; F, G and H) control, antilisterial extract (10^4 AU mL^{-1} or g^{-1}) produced by *E. mundtii* Tw56, Tw802 or Tw807, respectively; I, J and K) *L. monocytogenes* Scott A (10^4 CFU mL^{-1} or g^{-1}) and *E. mundtii* Tw56, Tw802 or Tw807 (10^7 CFU mL^{-1} or g^{-1}), respectively and L, M and N) *L. monocytogenes* Scott A (10^4 CFU mL^{-1} or g^{-1}) and antilisterial extract (10^4 AU mL^{-1} or g^{-1}) produced by *E. mundtii* Tw56, Tw802 or Tw807, respectively.

Meat gravy model

A simulated meat gravy model was prepared mixing proteose peptone (1.8% w v^{-1}), meat extract (1.2% w v^{-1}), yeast extract (0.6% w v^{-1}) and corn starch (2.0% w v^{-1}) and the experiments were performed as described by Carvalho et al. (2018). The inoculated gravy portions were refrigerated at 4 °C for 15 days. For counting, the gravy portions were submitted to serial decimal dilutions in 0.85% NaCl (w v^{-1}) solution and 100 µL of each dilution were spread plated onto two plates of Oxford agar (Biokar) for the enumeration of *L. monocytogenes* Scott A and two plates of MRS agar (Biokar) for enumeration of *Enterococcus* strains. Plates were incubated at 37 °C for 48 h, colonies were counted and results expressed as log CFU·mL^{-1}. The enumeration of *L. monocytogenes* Scott A and *Enterococcus* strains were evaluated at times 0, 5, 10 and 15 days. All experiments were done three times.

Ground beef model

Ground beef burgers from the muscles of the quadriceps femoris obtained from a local butcher's shop were prepared as described by Acuña et al. (2015). They were refrigerated at 4 °C in sterile plates for 15 days. Samples of each condition were evaluated on days 0, 1, 3, 5, 7, 10 and 15.

Food model samples were diluted using sterile saline (0.85%) as diluent and 100 µL of each dilution were surface-plated onto Oxford agar (Biokar) to count *L. monocytogenes* Scott A and onto MRS agar (pH 5.5) (Biokar) to enumerate *Enterococcus* strains. All plates were incubated at 37 °C for 48 h, the average number of colonies was calculated, and the results were expressed as the log of CFU mL^{-1} or g^{-1}. In addition, a total mesophilic control was performed in the ground beef, using Agar Palte Count (Biokar) at 30 °C for 48 h. All experiments were done three times. In addition, the production of bacteriocins was evaluated by the methodology before mentioned (Farías et al. 1994).

Data analysis

Variance analysis (ANOVA) was applied to the results of microbial enumeration in simulated food models (Carvalho et al. 2018).

Results and discussion

Strain selection and safety evaluation

The present study evaluated 9 LAB strains (*E. mundtii* Tw56, *E. mundtii* TW222, *E. mundtii* Tw278, *E. faecium* Tw452, *E. faecium* Tw465, *E. faecalis* Tw471, *E. mundtii* Tw492, *E. mundtii* Tw802 and *E. mundtii* Tw807), isolated from marine invertebrates (sea cucumber, mussel, marine snail) and *E. faecium* Tw6, isolated from sheep's milk. Their CFSs presented a potent inhibitory activity on *L. innocua* ATCC 33,090, *L. monocytogenes* Scott A and other indicator strains (Table 1). Three strains produced inhibition halos larger than 20 mm in diameter, identified as *E. mundtii* Tw56, *E. mundtii* Tw 802 and *E. mundtii* Tw 807, were selected for use as a biopreservative potential in food model. The results obtained in this study indicated that the selected *Enteroccocus* strains do not present potential virulence factors, gelatinase activity (no clear zones) in agar plates and resistance to antibiotic.

The demand for minimally processed and safe food products means highlighting the search for new preservation methods. In this study, characterized LAB strains and heir CFSs presented a potent inhibitory activity on *L. innocua* ATCC 33,090, *L. monocytogenes* Scott A and other indicator strains. The bacteriocins produced by *Enterococcus* sp. were evaluated because of their antagonistic activity against pathogenic microorganisms (De Kwaadsteniet et al. 2005). These microorganisms have been removed from FAO's GRAS list because they are associated with food-borne diseases (Khan et al. 2010), but *Enterococcus* strains do not generally have virulence factors and may be a suitable LAB to be applied in food preservation (Franz et al. 2003). Giraffa 2003; Schelegueda et al. 2016). Gelatinase is an important virulence factor present in isolates from food, environmental and clinical specimens (Eaton and Gasson 2001). Another enterococcus virulence factor is the presence of hemolysin, because it is the cause of enterococcal disease in humans and animals (Semedo et al. 2003). Hemolytic activity in human blood agar and exopolysaccharide production were not observed in all *Enterococcus* strains studied in this work. There were no halos of complete hemolysis around the colonies and no black colonies of the selected strains and the absence of virulence genes was observed by

PCR techniques. Since the genus *Enterococcus* has caused great concern due to its increasing resistance to antimicrobial agents, that of the different strains to vancomycin and other antibiotics was evaluated. None of the strains showed resistance to penicillin, ampicillin, amoxicillin/clavulanic, erythromycin, trimethoprim/sulfamethoxazole, teicoplanin or vancomycin and only the strain *E. mundtii* Tw807 exhibited resistance to rifampicin. Before using enterococci in foods their safety, as well as their functional and beneficial traits, should be carefully evaluated. The strains should be phenotypically and genotypically characterized and ought to be free from any pathogenicity and virulence factors (Braïek and Smaoui 2019).

Characterization of antimicrobial activity

The characterization of antilisterial compounds secreted by *Enterococcus* strains confirmed they were sensitive to proteolytic enzymes, evidencing the proteinaceous nature. The CFSs were thermostable after heating at 100 °C for 30 min and active in a broad pH range (between 2 and 10). Antimicrobial activity was not changed by the treatment with lipase or catalase, demonstrating that neither lipids nor hydrogen peroxide were involved in the inhibitory activity. Bacteriocin sensitivity to different enzymes is used for the identification of potential cultures in the control of foodborne pathogens, as observed in Barbosa et al. (2014), Carvalho et al. (2018) and Decarlo et al. (2019). The selected strains of *Enterococcus* sp in this work were described as bacteriocin or bacteriocin-like substance producers and their possible applicability as biopreservative in the food industry. The antagonistic activity was not lost after treatment with trypsin, showing a protein composition of the antimicrobial substances and neither after treatment with lysozyme that acts on glycosidic bonds (Delcarlo et al. 2019).

PCR amplifications were performed on the genomic DNA of *E. mundtii* Tw56, *E. mundtii* Tw802 and *E. mundtii* Tw807 to find out the presence of structural bacteriocin genes in the genome of the selected strains. The structural gene of enterocin KS was amplified in the genome of the three strains. The structural gene of enterocin B was present in the strains *E mundtii* Tw802 and Tw807 (Fig. 1). In contrast, none of the structural genes of enterocin A, P, L50A, Q, Bact 96, 31, 1071 A/B and HirJM 79 could be amplified.

Molecular methodologies are important in antagonism studies because they allow the identification of bacteriocin codifying genes and sequences of genes that codify antimicrobial metabolites. Genetic sequencing with the enzymatic profile of the bacteriocins in this study demonstrated the presence of bacteriocin structural genes which coded for mundticin KS, according to what was observed by other authors (Ogaki et al. 2016; Decarlo et al. 2019).

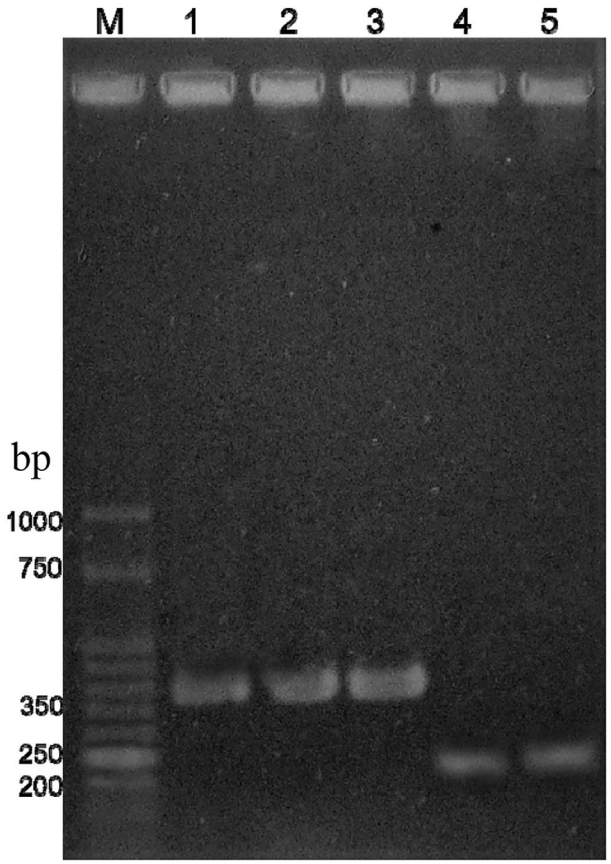

Fig. 1 Amplification results for genes encoding enterocins. Line 1, 2, and 3: enterocin KS (*E. mundtii* Tw56, *E. mundtii* Tw802 and *E. mundtii* Tw807). Line 4 and 5: enterocin B (*E. mundtii* Tw802 and *E. mundtii* Tw807). M: molecular weight marker (kbp)

Bacteriocin production in different culture media and incubation conditions

For the ten strains studied, different culture media and conditions were tested to obtain the highest production of bacteriocins. In Table 4 observe the antimicrobial activity of the CFSs obtained in the different media with and without the addition of cysteine and incubating at 25, 30 and 37 °C. The higher anti-listeria activity of strains *E. mundtii* Tw56, *E. mundtii* Tw802 and *E. mundtii* Tw807 was also confirmed in the conditions tested. Bacteriocin production ranged from 200 to 819,200 AU mL^{-1} depending on the strain, temperature, and culture media used. The lowest production of bacteriocins was obtained at 37 °C, except for that of strains *E. mundtii* Tw802 and *E. mundtii* Tw807, where the lowest production was observed at 25 °C, using LAPTgc as a culture medium. The maximum antimicrobial activity produced by the ten strains studied occurred between 25 and 30 °C, using LAPTg, except for *E. faecium* Tw6 and *E. mundtii* Tw492.

Table 4 Bacteriocin production (AU mL^{-1}) by *Enterococcus* strains., at different incubation temperatures and culture media

Enterococcus strains	Incubation Temperatures											
	25 °C				30 °C				37 °C			
	$LAPTg_s$	$LAPTg_c$	MRS_s	MRS_c	$LAPTg_s$	$LAPTg_c$	MRS_s	MRS_c	$LAPTg_s$	$LAPTg_c$	MRS_s	MRS_c
E. faecium Tw6	400	800	800	1600	400	800	800	1600	200	400	400	800
E. mundtii Tw56	409,600	204,800	204,800	409,600	102,400	819,200	6400	102,400	3200	12,800	6400	12,800
E. mundtii Tw222	12,800	102,400	12,800	102,400	204,800	204,800	6400	102,400	3200	12,800	6400	6400
*E. mundtii Tw*278	51,200	51,200	12,800	102,400	204,800	25,600	12,800	102,400	3200	6400	6400	12,800
E. faecium Tw452	51,200	20,800	3,200	12,800	12,800	6,400	3200	12,800	3200	12,800	6400	6400
E. faecium Tw465	12,800	25,600	6,400	6,400	102,400	25,600	3200	6,400	3200	6400	6400	6400
E. faecalis Tw471	204.800	25,600	6,400	51,200	25,600	51,200	6400	6,400	3200	3200	6400	1600
E. mundtii Tw492	25,600	51,200	25,600	409,600	204,800	102,400	102,400	102,400	3200	6400	51,200	12,800
E. mundtii Tw802	12,800	800	204,800	6,400	819.,00	819,200	102,400	12,800	3200	1600	25,800	6400
E. mundtii Tw807	25,600	800	102,400	1,600	409,600	204,800	25,600	51,200	3200	1600	51,200	12,800

The culture media are indicated on each column. Where *s* no cysteine, *c* with cysteine (0.5 $mg.mL^{-1}$)

For these strains, MRSc was the best medium for bacteriocin production. Agitation (100 rpm) did not increase bacteriocin production and cysteine addition increased the antimicrobial activity of some strains (Table 4).

The results in this study indicate that the best bacteriocin production by the selected LAB occurs at temperatures between 25 and 30 °C, which do not coincide with the optimum bacterial growth temperature (37 °C). This behavior may be related to the change of the redox potential of the culture broth (Vázquez et al. 2004), and, therefore, corroborates that under conditions of low oxygen level the production of bacteriocin can be improved by LAB. Bacteriocin levels depend on the temperature, pH and nutrients, as observed in this study.

The production of bacteriocin by LAB in food depends on optimal biosynthesis but does not parallel bacterial growth, besides the interaction with components of the food matrix. (Malheiroset al. 2015; Engelhardtet al. 2018). Several studies demonstrated that LAB growth is dependent on temperature, pH and nutrient availability because LAB are microorganisms that require rich media, appropriate conditions for growth and competitive flora (Van den Berghe et al. 2006).

The results obtained in this work are in agreement with those published by other authors. Barbosa et al. (2014) determined that *Lactobacillus sakei* MBSa1 was grown in MRS broth at different temperatures (25, 30 and 37 °C) but the optimum temperature of bacteriocin production was 25 °C. Souza et al. (2017) determined that the production of bacteriocins by *Lactococcus lactis* CECT-4434 depended on the temperature of incubation, obtaining the best results in a range of 30–37 °C with or without pH control.

Determination of the antimicrobial spectrum of CFSs produced by selected LAB

The antibacterial spectra of the antimicrobial peptides were similar and lactic cultures and Gram-negative strains were resistant to all these compounds (Table 1). *E. mundtii* Tw56, Tw802 and Tw807 presented the higher antimicrobial activity against *L. monocytogenes* Scott A. The inhibitory activity of CFSs was tested on a wide range of microorganisms and they were active against *L. monocytogenes*, *L. innocua*, *Enterococcus* spp. and *Lactobacillus plantarum* TwLb5 (Table 1). Antimicrobial activity for indicator strains showed small differences, where some strains had a lower bacteriocin production than others, which is consistent with previous studies (Aspri et al. 2017; Delcarlo et al. 2019).

Evaluation of bacteriocinogenic strains against *Listeria monocytogenes* Scott A in food models

Figure 2 shows the effect of extracts of bacteriocinogenic cultures or co-inoculation of bacteriocin-producing *Enterococcus* against *L. monocytogenes* Scott A in meat gravy and ground beef incubated at 4 ºC. In simulated meat gravy, the *Listeria* control was increased ~ 3 log CFU mL^{-1} units with respect to day 0 (Fig. 2a). A remarkable log CFU mL^{-1} unit decrease of the viable cell count of *L. monocytogenes* Scott A was observed in presence of the antilisterial extracts produced by *E. mundtii* (Tw56, Tw802) until disappearing completely on day 10 of treatment. In presence of the antilisterial extract produced by *E. mundtii* Tw807, the counts of *L. monocytogenes* Scott A showed no significant differences with respect to day 0

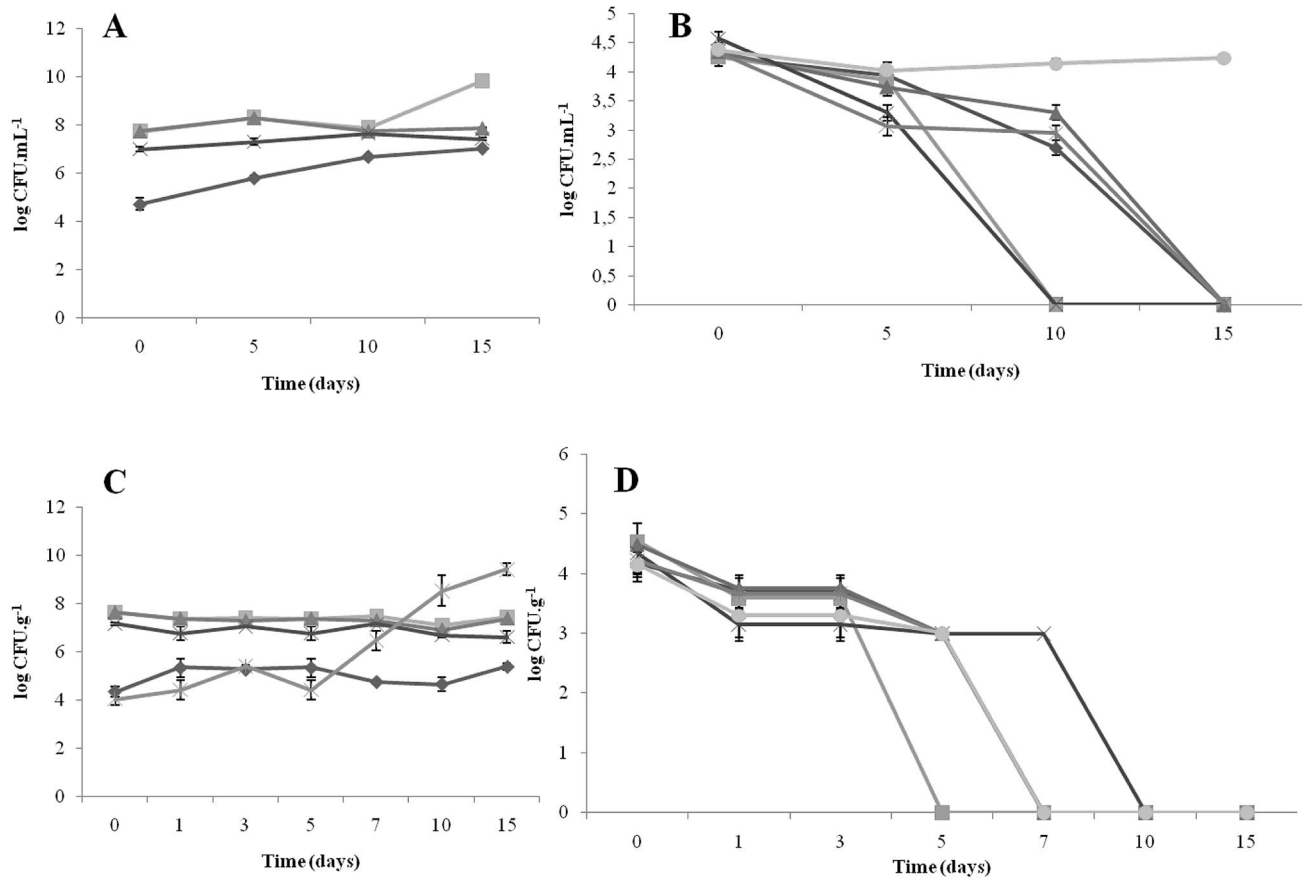

Fig. 2 Counts of *Listeria monocytogenes* Scott A (log CFU mL^{-1} or g^{-1}) in the meat gravy (**a** and **b**)/ground beef models (**c** and **d**) were **a** and **c** (respectively): (diamond) *Listeria monocytogenes* Scott A. (Square) *E. mundtii* Tw56 (triangle) *E. mundtii* Tw802. (-x-) *E. mundtii* Tw807. (astrick) Total mesophiles. **b** and **d** (respectively): (diamond) *L. monocytogenes* Scott A + *E. mundtii* Tw56. (square) *L. monocytogenes* Scott A + antilisterial extract *E. mundtii* Tw56. (triangle) *L. monocytogenes* Scott A + *E. mundtii* Tw802. (-x-) *L. monocytogenes* Scott A + antilisterial extract *E. mundtii* Tw802. (astrick) *L. monocytogenes* Scott A + *E. mundtii* Tw807. (astrick) + *L. monocytogenes* Scott A + antilisterial extract *E. mundtii* Tw807

(Fig. 2b). When *L. monocytogenes* Scott A was inoculated in presence of 10^7 (CFU mL^{-1}) of *E. mundtii* Tw56, Tw802 or Tw807 the counts of *Listeria* progressively decreased until disappearing completely after 15 days (Fig. 2b).

The count of *L. monocytogenes* Scott A in the control ground beef stored at 4 °C was 4.0 log CFU g^{-1} and remained the same until the 15th day. In presence of the antilisterial extract produced by *E. mundtii* Tw56, no growth of *Listeria* was observed since day 5 of storage under refrigeration while a decrease occurred in the presence of the antilisterial extract produced by *E. mundtii* Tw807 until *Listeria* completely disappeared on day 7. In the ground beef with antilisterial extract produced by *E. mundtii* Tw802, no count was observed on day 10. On the other hand, it was found that the bacteriocinogenic strains were able to produce bacteriocin (1000 UA mL^{-1}) under these refrigeration conditions. The sensory evaluation is necessary and further studies will be carried out.

L. monocytogenes is commonly found in meats, vegetables and milk and is as an important foodborne pathogen causing various clinical syndromes. Listeriosis has been associated with consumption of a variety of foods, including soft cheeses, meat and vegetable products (Schlech and Acheson 2000) and the ability of *Listeria* to grow in a wide temperature range (1–45 °C), low pH and high salt tolerance, make it difficult to control in food (Aspri et al. 2017). Hence, the use of bacteriocins to control *Listeria* sp. in food is a promising means, whether produced in situ or added in the different food matrixes.

Bacteriocins can be used for food biopreservation (addition of bacteriocin producing strains or purified or semi-purified bacteriocins) and the strategy of their use to food depends on the components present in the food matrix so that they do not affect the antimicrobial activity, due to the amphiphilic characteristic and high content of hydrophobic amino acids present in the bacteriocin, and their proteolytic degradation by enzymes

in the food matrix (Favaro et al. 2015; O'Connor et al. 2015; Ahmad et al. 2017; Favaro and Todorov 2017).

In this work, it was observed that the antilisterial extracts of *E. mundtii* strains showed a similar and even better inhibitory activity against *L. monocytogenes* Scott A than the treatment with the respective bacteriocinogenic strains (*E. mundtii* Tw56, Tw802 and Tw807) in food models stored and refrigerated for 15 days. These results coincide with what was observed by Carvalho et al. (2018), who attribute this greater capacity of the antimicrobial extracts to inhibit and eliminate *Listeria*, because no adaptation time is required by the bacteriocinogenic strains to begin the production of antimicrobial compounds even though bacteriocin in situ production could be inhibited by food components and storage conditions (Urso et al. 2006). Other studies have assessed the bacteriocin production by LAB directly in different food matrixes or the effect in the control of pathogenic bacteria when adding purified or semi-purified bacteriocins to food (Aspri et al. 2017; Lianou et al. 2017; Delcarlo et al. 2019).

In conclusion, *E. mundtii* Tw56, *E. mundtii* Tw802 and *E. mundtii* Tw807 were characterized as showing no presence of virulence factors, broad spectrum of antimicrobial activity, stability in a wide temperature and pH range, sensitivity to digestive proteases. In addition, they reduced the growth of *L. monocytogenes* Scott A in meat food models at 4 °C both when they were applied as cell-free extract or co-inoculated in foods. The features of *E. mundtii* Tw56, *E. mundtii* Tw802 and *E. mundtii* Tw807 point to them as biopreservative candidates to be used in the future in foods.

Acknowledgements We acknowledge the financial support from the Universidad Nacional de la Patagonia San Juan Bosco and Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina (PIP 11220130100272).

Author contributions JG: responsible for the experiments of the production of antimicrobial substances, food model. RP: responsible for the experiments of the biology molecular. MV: responsible for the experiments of the identification of LAB and writing of the paper. EM: responsible for the experiments of the identification of LAB. AB: responsible for the characterization of antimicrobial substances and writing of the paper. NP: responsible for the experiments of antimicrobial substances and writing of the paper. KGC: responsible for the experiments of production of antimicrobial substances, food model and writing of the paper.

Compliance with ethical standards

Conflict of interest The authors declare that they have No conflict of interest.

References

Acuña L, Corbalan NS, Fernandez IC, Morero RD, Barros-Velazquez J, Bellomio A (2015) Inhibitory Effect of the Hybrid Bacteriocin Ent35-MccV on the Growth of *Escherichia coli* and *Listeria monocytogenes* in Model Food. Syst Food Bioprocess Technol 8:1063–1075. https://doi.org/10.1007/s11947-015-1469-0

Ahmad V, Khan MS, Jamal QMS, Alzohairy MA, Karaawi MA, Siddiqui MU (2017) Antimicrobial potential of bacteriocins: in therapy, agriculture and food preservation. Int J Antimicrob Agents 49:1–11. https://doi.org/10.1016/j.ijantimicag.2016.08.016

Almeida T, Brandao A, Muñoz-Atienza E, Gonc A, Carmen Torres A, 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 Protect 74:1252–1260. https://doi.org/10.4315/0362-028X.JFP-11-016

Aspri M, O'Connor PM, Field D, Cotter PD, Ross P, Hill C, Papademas P (2017) Application of bacteriocin-producing *Enterococcus faecium* isolated from donkey milk, in the bio-control of *Listeria monocytogenes* in fresh whey cheese. Int Dairy J 79:1–9. https://doi.org/10.1016/j.idairyj.2017.04.008

Barbosa MS, Todorov SD, Belguesmia Y, Choiset Y, Rabesona H, Ivanova IV, Chobert JM, Haertlé T, Franco BDGM (2014) Purification and characterization of the bacteriocina produced by *Lactobacillus sakei* MBSa1 isolated from Brazilian salami. J Appl Microbiol 116:1195–1208. https://doi.org/10.1111/jam.12438

Barbosa MS, Todorov SD, Ivanova I, Chobert JM, Haertlé T, Franco BDGM (2015) Improving safety of salami by application of bacteriocins produced by an autochthonous *Lactobacillus curvatus* isolate. Food Microbiol 46:254–262. https://doi.org/10.1016/j.fm.2014.08.004

Belgacem ZB, Abriouel H, Omar NB, Lucas R, Martínez-Canamero M, Gálvez A, Manai M (2010) Antimicrobial activity, safety aspects, and some technological properties of bacteriocinogenic *Enterococcus faecium* from artisanal Tunisian fermented meat. Food Control 21:462–470. https://doi.org/10.1016/j.foodcont.2009.07.007

Ben Braïek O, Morandi S, Cremonesi P, Smaoui S, Hani K, Ghrairi T (2018) Safety, potential biotechnological and probiotic properties of bacteriocinogenic *Enterococcus lactis* strains isolated from raw shrimps. Microbial Pathog 117:109–117. https://doi.org/10.1016/j.micpath.2018.02.021

Ben Braïek O, Smaoui S (2019) Enterococci: Between emerging pathogens and potencial probiotics. Biomed Res Int 2019:1–13. https://doi.org/10.1155/2019/5938210

Carvalho KG, Kruger MF, Nader Furtado D, Todorov SD, Franco BDGM (2009) Evaluation of the role of environmental factors in the human gastrointestinal tract on the behaviour of probiotic cultures of *Lactobacillus casei* Shirota and *Lactobacillus casei* LC01 by the use of a semi-dynamic in vitro model. Annals Microbiol 59:439–445. https://doi.org/10.1007/BF03175128

Carvalho KG, Bambirra FHS, Nicoli JR, Oliveira JS, Santos AMC, Bemquerer MP, Miranda A, Franco BDGM (2018) Characterization of multiple antilisterial peptides produced by sakacin P-producing *Lactobacillus sakei* subsp. sakei 2a. Arch Microbiol 200:635–644. https://doi.org/10.1007/s00203-018-1477-3

Cotter PD, Hill C, Ross P (2005) Bacteriocins: developing innate immunity for food. Nat Rev Microbiol 3:777–788. https://doi.org/10.1038/nrmicro1273

Dal Bello B, Cocolin L, Zeppa G, Field D, Cotter PD, Hill C (2012) Technological characterization of bacteriocin producing *Lactococcus lactis* strains employed to control *Listeria monocytogenes* in Cottage cheese. Int J Food Microbiol 153:58–65. https://doi.org/10.1016/j.ijfoodmicro.2011.10.016

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 Food Microbiol 105:433–444. https://doi.org/10.1016/j.ijfoodmicro.2005.03.021

De Vuyst L, Foulquie Moreno MR, Revets H (2003) Screening for enterocins and detection of hemolysin and vancomycin resistance in enterococci of different origins. Int J Food Microbiol 84:299–318. https://doi.org/10.1016/S0168-1605(02)00425-7

Delcarlo SB, Parada R, Schelegueda LI, Vallejo M, Marguet ER, Campos CA (2019) From the isolation of bacteriocinogenic LAB strains to the application for fish paste biopreservation. LWT Food Sci Technol 110:239–246. https://doi.org/10.1016/j.lwt.2019.04.079

Dicks LMT, Mellett FD, Hoffman LC (2004) Use of bacteriocin-producing starter cultures of *Lactobacillus plantarum* and *Lactobacillus curvatus* in production of ostrich meat salami. Meat Sci 66:703–708. https://doi.org/10.1016/j.meatsci.2003.07.002

Eaton TJ, Gasson MJ (2001) Molecular screening of *Enterococcus* virulence determinants and potential for genetic exchange between food and medical isolates. Appl Environ Microbiol 67:1628–1635. https://doi.org/10.1128/AEM.67.4.1628-1635.2001

Engelhardt T, Szakmár K, Kiskó G, Mohácsi-Farkas C, Reichart O (2018) Combined effect of NaCl and low temperature on antilisterial bacteriocin production of *Lactobacillus plantarum* ST202Ch. LWT Food Sci Technol 8:104–109. https://doi.org/10.1016/j.lwt.2017.10.027

FAO/WHO (2002) Fao/who working group report on drafting guidelines for the evaluation of probiotics in food. World Health Organization, Geneva

Farías LM, Totola AH, Miranda CMS, Carvalho MAR, Damasceno CAV (1994) Extration, partial purification and characterization of a bacteriocin (fragilicin) produced by a strain of *Bacteroides fragilis* isolated from *Callithix penicillata*. Res Microbiol 145:9–16. https://doi.org/10.1016/0923-2508(94)90062-0

Favaro L, PennaPennaTodorov ALBSD (2015) Bacteriocinogenic lactic acid bacteria from cheeses—application in biopreservation? Trends Food SciTechnol 41:37–48. https://doi.org/10.1016/j.tifs.2014.09.001

Favaro L, Todorov SD (2017) Bacteriocinogenic lactic acid bacteria strains for fermented meat preservation: perspectives, challenges, and limitations. Probiotics Antimicro Prot 9:444–458. https://doi.org/10.1007/s12602-017-9330-6

Franz CMAP, Stiles ME, Schleifer KH, Holzapfel WH (2003) Enterococci in foods—a conundrum for food safety. Int J Food Microbiol 88:105–122. https://doi.org/10.1016/S0168-1605(03)00174-0

Giraffa G (2003) Functionality of enterococci in dairy products. Int J Food Microbiol 88:215–222. https://doi.org/10.1016/S0168-1605(03)00183-1

Henning C, Vijayakumar P, Adhikari R, Jagannathan B, Gautam D, Peter M, Muriana P (2015) Isolation and taxonomic identity of bacteriocin-producing lactic acid bacteria from retail foods and animal sources. Microorganisms 3:80–93. https://doi.org/10.3390/microorganisms3010080

Kailasapathy K (2006) Survival of free and encapsulated probiotic bacteria and their effect on the sensory properties of yoghurt. LWT Food Sci Technol 39:1221–1227. https://doi.org/10.1016/j.lwt.2005.07.013

Khan H, Flint S, Yu PL (2010) Enterocins in food preservation. Int J Food Microbiol 141:1–10. https://doi.org/10.1016/j.ijfoodmicro.2010.03.005

Leroy F, De Vuyst L (2004) Lactic acid bacteria as functional starter cultures for the food fermentation industry. Trends Food Sci Technol 15:67–78. https://doi.org/10.1016/j.tifs.2003.09.004

Lianou A, Kakouri A, Pappa EC, Samelis J (2017) Growth interactions and antilisterial effects of the bacteriocinogenic *Lactococcus lactis* subsp. cremoris M104 and *Enterococcus faecium* KE82 strains in thermized milk in the presence or absence of a commercial starter culture. Food Microbiol 64:145–154. https://doi.org/10.1016/j.fm.2016.12.019

Malheiros PS, Sant'Anna V, Todorov SD, Franco BDGM (2015) Optimization of growth and bacteriocin production by *Lactobacillus sakei* subsp *sakei* 2a. Brazilian J Microbiol 46:825–834. https://doi.org/10.1590/S1517-838246320140279

Marguet ER, Vallejo M, Sierralta Chichisola V, Quispe JL (2011) Actividad antagonista de bacterias lácticas aisladas del medio marino contra cepas de *Listeria*. Acta Bioquím Clín Latinoam 45:305–310

Martín M, Gutiérrez J, Criado R, Herranz C, Cintas LM, Hernández PE (2006) Genes encoding bacteriocins and their expression and potential virulence factors of *Enterococci isolated* from wood pigeons (*Columba palumbus*). J Food Protect 69:520–531. https://doi.org/10.4315/0362-028X-69.3.520

National Committee for Clinical Laboratory Standards (2015) Performance standards for antimicrobial susceptibility testing. Approved standard M2–A7, 7th edn. NCCLS, Wayne

O'Connor PM, Ross RP, Hill C, Cotter PD (2015) Antimicrobial antagonists against food pathogens: a bacteriocin perspective. Curr Opi Food Sci 2:51–57. https://doi.org/10.1016/j.cofs.2015.01.004

Ogaki MB, Rocha KR, Terra MR, Furlaneto MC, Furlaneto-Maia L (2016) Screening of the enterocin-encoding genes and antimicrobial activity in *Enterococcus* species. J Microbiol Biotechnol 26:1026–1034. https://doi.org/10.4014/jmb.1509.09020

Ogier JC, Serror P (2008) Safety assessment of dairy microorganisms: the *Enterococcus* genus. Int J Food Microbiol 126:291–301. https://doi.org/10.1016/j.ijfoodmicro.2007.08.017

Özdemir GB, Oryaşın E, Bıyık HH, Özteber M, Bozdoğan B (2011) Phenotypic and genotypic characterization of bacteriocins in enterococcal isolates of different sources. Indian J Med Microbiol 51:182–187. https://doi.org/10.1007/s12088-011-0143-0

Panesar PS, Kennedy JF, Gandhi DN, Bunko K (2007) Bioutilisation of whey for lactic acid production a review. Food Chem 105:1–14. https://doi.org/10.1016/j.foodchem.2007.03.035

Raibaud P, Galpin JV, Duclezeau R, Mocquot G, Oliver G (1963) Le Genre Lactobacillus dans le tube digestif du rat. II. Caracters de souches heterofermentaires isolates de rats. 'holo' et 'gnotoxeniques.' Annales de Microbiologie (Annales de L'Institute Pasteur) 124:2223–2235

Rice LB, Carias L, Rudin S, Vael C, Goossens H, Konstabel C, Klare I, Nallapareddy SR, Huang W, Murray BE (2003) A potential virulence gene, hylEfm, predominates in *Enterococcus faecium* of clinical origin. J Infect Dis 187(3):508–512. https://doi.org/10.1086/367711

Rosa CM, Franco BDGM, Montville TJ, Chikindas ML (2002) Purification and mechanistic action of a bacteriocin produced by a brazilian sausage isolated, *Lactobacillus sakei* 2a. Food Saf 22:39–54. https://doi.org/10.1111/j.1745-4565.2002.tb00329.x

Schelech WF, Acheson D (2000) Foodborne listeriosis. Clin Infec Disease 31:770–775. https://doi.org/10.1086/314008

Schelegueda LI, Vallejo M, Gliemmo MF, Marguet ER, Campos CA (2015) Synergistic antimicrobial action and potential application for fish preservation of a bacteriocina produced by *Enterococcus mundtii* isolated from Odontesthes platensis. LWT Food Sci Technol 64:794–801. https://doi.org/10.1016/j.lwt.2015.06.017

Schelegueda LI, Zalazar AL, Gliemmo MF, Campos CA (2016) Inhibitory effect and cell damage on bacterial flora of fish caused by chitosan, nisin and sodium lactate. Int J Biol Macro 83:396–402. https://doi.org/10.1016/j.ijbiomac.2015.11.033

Semedo T, Almeida Santos M, Martins P, Silva Lopes MF, Figueirede Marques JJ, Teneiro R (2003) Comparative study using type strains and clinical and food isolated to examine hemolytic activity and ocurrence of the cyl operon in enterococci. J Clin Microbiol 41:2569–2576. https://doi.org/10.1128/JCM.41.6.2569-2576.2003

Souza EC, de Souza O, de Azevedo P, Domínguez JM, Converti A, de Souza P, Oliveira R (2017) Influence of temperature and pH on the production of biosurfactant, bacteriocin and lactic acid

by *Lactococcus lactis* CECT-4434. CyTA J Food 15(4):525–530. https://doi.org/10.1080/19476337.2017.1306806

Stiles ME (1996) Biopreservation by lactic acid bacteria. Antonie Van Leeuwenhoek 70:331–345. https://doi.org/10.1007/BF00395940

Tomé E, Teixeira P, Gibbs PA (2006) Antilisterial inhibitory lactic acid bacteria isolated from commercial cold smoked salmon. Food Microbiol 23:399–405. https://doi.org/10.1016/j.fm.2005.05.004

Urso R, Rantsiou K, Cantoni C, Comi G, Cocolin L (2006) Technological characterization of a bacteriocin-producing *Lactobacillus sakei* and its use in fermented sausages production. Int J Food Microbiol 110:232–239. https://doi.org/10.1016/j.ijfoodmicro.2006.04.015

Vallejo M, Olivera NL, Sequeiros C, Marguet ER (2009) Actividad antilisteria de bacterias ácido lácticas aisladas de peces marinos. Analecta Veterinaria 29:19–23

Van den Berghe E, de Winter T, de Vuyst L (2006) Enterocin A production by *Enterococcus faecium* FAIR-E 406 is characterized by a temperature- and pH-dependent switch-off mechanism when growth is limited due to nutrient depletion. Int J Food Microbiol 107:159–170. https://doi.org/10.1016/j.ijfoodmicro.2005.08.027

Vázquez A, Cabo ML, González MP, Murado MA (2004) The role of amino acids in nisin and pediocin production by two lactic acid bacteria. A factorial study. Enzyme Microbial Technol 34:319–325. https://doi.org/10.1016/j.enzmictec.2003.11.005

Werner G, Fleige C, Geringer U, van Schaik W, Klare I, Witte W (2011) IS element IS16 as a molecular screening tool to identify hospital-associated strains of *Enterococcus faecium*. BMC Infect Dis 11:80–88. https://doi.org/10.1186/1471-2334-11-80

Xin B, Zheng J, Xu Z, Li C, Ruan L, Peng D (2015) Three novel lantibiotics, ticins A1, A3, and A4, have extremely stable properties and are promising food biopreservatives. Appl Environ Microbiol 81:6964–6972. https://doi.org/10.1128/AEM.01851-15

Yang R, Johnson MC, Ray B (1992) Novel method to extract large amounts of bacteriocins from lactic acid bacteria. Appl Environ Microbiol 58:3355–3359

Zendo T, Eungruttanagorn N, Fujioka S, Tshiro Y, Namura K, Sera Y (2005) Identification and production of a bacteriocin from *Enterococcus mundtii* QU 2 isolated from soybean. J Appl Microbiol 99:1181–1190. https://doi.org/10.1111/j.1365-2672.2005.02704.x