Food Control 47 (2015) 53–59

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Occurrence of antilisterial structural bacteriocins genes in meat borne lactic acid bacteria

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ARTICLE INFO

Article history:
Received 21 November 2013
Received in revised form
4 June 2014
Accepted 14 June 2014
Available online 21 June 2014

Keywords:
Fermented sausages
Lactobacillus
Bacteriocin
Listeria
PCR

ABSTRACT

The ability to inhibit the growth of *Listeria* cells and the presence of bacteriocin encoding genes was examined in 115 LAB strains isolated from Argentinean vacuum-packaged beef and different traditional fermented sausages. *Lactobacillus* (*L*) *sakei*, *Lactobacillus* (*L*) *curvatus* and *Enterococcus* (*E*) *faecium* showed a great inhibition of all *Listeria* strains evaluated while *Pediococcus* (*P*) *acidilactici* and *Lactobacillus* (*L*) *plantarum* demonstrated a limited or absent antilisterial activity. Both *L. curvatus* and *L. sakei* carried the *sppA*, *sppQ* and *sapA* structural genes, encoding for sakacin P, sakacin Q and curvacin A bacteriocins, respectively. Whilst *L. curvatus* exhibited a higher occurrence of these genes, *L. sakei* strains were more effective at inhibiting *Listeria* (*L*) strains, *Listeria monocytogenes* UC8159 and *Listeria innocua* 7 being the most sensitive to these bacteriocins. Among analyzed *E. faecium* strains, the wide distribution of *entA*, *entB* and *entP* genes accounted for the high antilisterial activity particularly observed against *L. monocytogenes* FBUNT. The structural gene *plantEF* was mostly present in *Lactobacillus plantarum* strains and no *pedA* gene was found in *P. acidilactici* evaluated strains. The antilisterial potential of *L. sakei* and *E. faecium* offers great possibilities for the meat industry as biopreservative cultures, although more studies are needed in order to conclude about this issue.

1. Introduction

Processing technologies as well as nutrients availability in meat are known to limit bacterial growth to those specially featured organisms, determining the type and number of bacteria present in the different meat ecosystems (Nieto-Lozano, Reguera-Useros, Pelaez-Martinez Mdel, & Hardisson de la Torre, 2006; Nychas, Skandamis, Tassou, & Koutsoumanis, 2008). Despite the progress in food biotechnology, meat industry is still under scrutiny due to the frequent outbreaks of foodborne illness. *Listeria monocytogenes* is a food-borne pathogen capable of surviving unfavorable environmental conditions, such as low pH and high sodium chloride levels, contaminating the end-products. It is also able to grow at low temperatures. Ready-to-eat foods, including deli-meats are considered as high risk products and this microorganism cannot be completely eliminated even when many risk assessment strategies have been developed for its control (Klontz et al., 2008; Ross, Rasmussen, Fazil, Paoli, & Sumner, 2009).

Biopreservation by using bacteriocinogenic LAB has gained increased attention as a means of naturally controlling the shelf life and safety of meat products (Castellano, Belfiore, Fadda, & Vignolo, 2008; Vignolo, Saavedra, Sesma, & Raya, 2012). Bacteriocins are bacterially produced antimicrobial peptides with narrow or broad host ranges (Cotter, Hill, & Ross, 2005). The production of these low-molecular-weight peptides seems to be a common phenotype among LAB, since numerous bacteriocins have been isolated (Cotter et al., 2005) (Rea et al., 2011). Based on their physico-chemical properties these small antimicrobial peptides have been classified into two major classes: class I (lantibiotics) consists of bacteriocins containing intramolecular thioether ring structures and modified amino acids resulting from post-translational modifications. Nisin is a well-known member from this family (McAuliffe, Ross, & Hill, 2001) and is currently used in many countries as food preservative (Delves-Broughton, Blackburn, Evans, & Hugenholtz, 1996). Class II includes the bacteriocins with non-modified peptides except for bacteriocins with thioester bridges and circular bacteriocins. This class has been divided into subgroups: class IIa, characterized by a conserved sequence (YGNGVXCXK/NXXC) at their N-terminus and known for their strong anti-listerial activity, IIb (two-component peptides), and IIc (thiol-activated peptides) requiring

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http://dx.doi.org/10.1016/j.foodcont.2014.06.021
0956-7135/

reduced cysteine residues for activity (Nes, Yoon, & Diep, 2007; Nishie, Nagao, & Sonomoto, 2012).

During fermentation and ripening of traditional sausages *Lactobacillus sakei*, *Lactobacillus curvatus* and to a lesser extent *Lactobacillus plantarum* and species from *Pediococcus* and *Enterococcus* genera are by far the most often isolated LAB species which must have adapted to the existing stringent conditions (Cocconcelli & Fontana, 2010; Fontana, Cocconcelli, & Vignolo, 2005; Fontana, Vignolo, & Cocconcelli, 2005; Vignolo, Fontana, & Cocconcelli, 2010). Several antilisterial bacteriocins are known to be produced by meat borne *L. sakei* strains such as sakacin A produced by *Lactobacillus sake* Lb 706 isolated from meat products (Holck, Axelsson, Birkeland, Aukrust, & Blom, 1992), sakacin P produced by *L. sake* LTH 673 and Lb674 strains isolated from fermented dry sausages (Holck, Axelsson, Hühne, & Kröckel, 1994; Tichaczek, Vogel, & Hammes, 1994); from *L. sakei* strain I151 isolated from naturally fermented Italian sausages Urso, R., Rantsiou, K., Cantoni, C., Comi, G., & Cocolin, L. (2006). and sakacin Q produced by *L. sakei* Lb674 and LTH673 (Mathiesen, Huehne, Kroeckel, Axelsson, & Eijsink, 2005) among others. On the other hand, antilisterial bacteriocins produced by *L. curvatus*, curvacin A produced by *L.curvatus* LTH1174 isolated from fermented sausages (Tichaczek, Vogel, & Hammes, 1993), curvaticin L442 produced by *L. curvatus* L442 isolated from Greek traditional fermented sausage (Xiraphi et al., 2006) and lactocin AL705 produced by *Lb. curvatus* CRL705 isolated from fermented sausages (Castellano & Vignolo, 2006) were reported. Other antilisterial compounds produced by meat borne LAB are pediocin PA-1/AcH produced by *Pediococcus acidilactici* (Pucci, Vedamuthu, Kunka, & Vandenbergh, 1988), plantaricins (A, EF and JK) produced by *L. plantarum* (Anderssen, Diep, Nes, Eijsink, & Nissen-Meyer, 1998) as well as enterocins A, B and P produced by enterococci (Aymerich et al., 2000; Cintas, Casaus, Havarstein, Hernandez, & Nes, 1997).

The aim of the present study is to analyze the occurrence of bacteriocin structural genes and various gene combinations in a target of 115 LAB isolated from different Argentinean raw meat and meat fermented products as well as its correlation with antilisterial activity.

2. Materials and methods

2.1. Bacterial strains, media and growth conditions

A total of 115 LAB strains isolated from Argentinean raw meat and meat fermented products were used in this study. The ability to produce antilisterial bacteriocins was evaluated in *L. sakei* (46 strains) and *L. curvatus* (14 strains) from pork/beef fermented sausages and vacuum-packaged beef (Castro, Palavecino, Herman, Garro, & Campos, 2011; Fontana, Cocconcelli, et al., 2005; Fontana, Vignolo, et al., 2005); *L. sakei* (25 strains) from llama fermented sausages (Lopez et al., 2012); *L. plantarum* (7 strains) from pork fermented sausages (Fontana, Cocconcelli, et al., 2005; Fontana, Vignolo, et al., 2005); *E. faecium* (16 strains) from pork/llama fermented sausages (Lopez et al., 2012), and *P. acidilactici* (7 strains) from pork/beef fermented sausages (Fontana, Cocconcelli, et al., 2005; Fontana, Vignolo, et al., 2005). *L. curvatus* CRL705, isolated from traditional fermented sausages (Vignolo, Suriani, Pesce de Ruiz Holgado, & Oliver, 1993) and *Enterococcus mundtii* CRL35 from artisanal cheeses (Saavedra, Minahk, de Ruiz Holgado, & Sesma, 2004) were used as controls for bacteriocin production. *Lactobacillus* and *Pediococcus* strains were cultured in MRS broth (Difco, BD, Buenos Aires, Argentina) and incubated at 30 °C for 18 h while *Enterococcus* strains were cultured in BHI (Brain heart infusion, Britania, Buenos Aires, Argentina) broth (Oxoid, Bioartis S.R.L., Buenos Aires, Argentina) at 37 °C for 18 h. *Listeria innocua* 7 [kindly gifted by Dr. Jean Richard del Laboratoire d' Ecologie Microbienne de la Unité de Recherches Laitieres et Gentique Appliqueé, Institut National de la Recherche Agronomique, Centre de Recherche de Jouy- en-Josas (INRA)] was grown in BHI at 30 °C; *L. monocytogenes* UC8158; UC8159; UC8160 (Università Cattolica del Sacro Cuore Culture Collection, Cremona, Italy) and FBUNT (Cátedra de Bacteriología, Facultad de Bioquímica, Química y Farmacia, UNT, Tucumán, Argentina) were grown in BHI at 37 °C for 18 h. All *Listeria* strains were used as indicator strains.

2.2. Detection of antilisterial activity

The anti-listerial activity was analyzed in all LAB strains by an agar spot test using all *L. monocytogenes* strains and *L. innocua* 7 as sensitive microorganism. Cell-free supernatants (CFS) were obtained by centrifugation at 15.600 g for 10 min; the cell-free supernatant fluid was then adjusted to pH 7.0 with 1 N NaOH. 5 μl of neutralized CFS was spotted in plates containing 10 ml of BHI 1.5% agar plus 10 ml of BHI soft agar (0.7%) inoculated with 10^7 CFU/ml^{-1} of an overnight culture of the indicator strains. Positive antimicrobial activity was evidenced after 24–48 h at 30 °C (*L. innocua* 7) or 37 °C (*L. monocytogenes*) as a clear inhibition zone on indicator organism's lawn. All experiments, unless stated otherwise, were performed in duplicate. Inhibitory activity was expressed in halo diameter as −/+ (≤0.5 mm), + (0.5–2 mm), ++ (2–4 mm), +++ (>4 mm) or − (no halos) around the spot.

2.3. PCR analysis for bacteriocin-encoding genes

All strains were tested by PCR to search the presence of different bacteriocin encoding genes. DNA was isolated from LAB colonies using Microlysis (Labogen, Milan, Italy) or following the protocol by Pospiech and Neumann (Pospiech & Neumann, 1995). Primers used and conditions for each of structural genes amplified are listed in Table 1. PCR reactions were performed using a GeneAmp® PCR Instrument System 9700 (Applied Biosystems, Italy) and My Cycler TM Thermal cycler (BIORAD, Tecnolab, Buenos Aires Argentina). The following conditions were used for PCR reactions: an initial denaturation step of 94 °C for 1 min, followed by 35 cycles of 1 min at 94 °C, 30 s at different annealing temperatures according to the primers used (Table 1) and 1 min at 72 °C, and final extension at 72 °C for 5 min. The amplified product was visualized in a 1.5% (w/v) agarose gel stained with SYBER Safe (Invitrogen, Italy) or GelRel (Biotium-Genbiotech, Argentina). All PCR runs included a blank control consisting of PCR-grade water and a non-template control (no DNA). All PCR products were purified and subject to sequencing at CERELA Sequencing Service and BMR Genomics (Padova, Italy). The resulting sequences were analyzed with the Blast program (Altschul et al., 1997).

2.4. Statistical analysis

Relations between meat borne LAB antilisterial activity and bacteriocin encoding genes presence were analyzed by means of a Multiple Correspondence Analysis (MCA) method (Abdi & Valentin, 2007). MCA is the generalization of simple correspondence analysis to several categorical variables. It is a geometric technique for displaying the rows and columns of a multi-way contingency table as points in a low-dimensional Euclidean space, such that the positions of the row and column points are consistent with their associations in the table. A matrix was created in which the rows of the data corresponded to bacteriocinogenic meat borne LAB strains and the columns to the analyzed bacteriocin genes and inhibitory activity level against the different *Listeria* strains used as indicators. The goal is to have a global view of the data that is useful for

Table 1
PCR Primers used in this study.

Primers	Sequence (5′→3′)	Amplicon size (bp)	Annealing temperature (°C)	References
curA_F	GTAAAAGAATTAAGTATGACA	180	50	Remiger, Ehrmann, & Vogel, 1996
curA_R	TTACATTCCAGCTAAACCACT			
sakP_F	ATGGAAAAGTTTATTGAATTA	200	47	Remiger et al., 1996
sakP_R	TTATTTATTCCAGCCAGCGTT			
sakQ_F	ATGCAAAATACAAAAGAACTAA	200	50	Cocolin & Rantsiou, 2007
sakQ_R	CGCTTGTTTAGAGACACCCGTT			
plnA_F	GTACAGTACTAATGGGAG	450	53	Remiger et al., 1996
plnA_R	CTTACGCCAATCTATACG			
plnEF_F	GGCATAGTTAAAATTCCCCCC	428	53	Remiger et al., 1996
plnEF_R	CAGGTTGCCGCAAAAAAAG			
entA_F	AAATATTATGGAAATGGAGTGTAT	126	48	du Toit, Franz, Dicks, & Holzapfel, 2000
entA_R	GCACTTCCCTGGAATTGCTC			
entB_F	GAAAATGATCACAGAATGCCTA	162	48	du Toit et al., 2000
entB_R	GTTGCATTTAGAGTATACATTTG			
entP_F	TATGGTAATGGTGTTTATTGTAAT	112	45	Wieckowicz, Schmidt, Sip, & Grajek, 2011
entP_R	ATGTCCCATACCTGCCAAAC			
pedA_F	AAAATATCTAACTAATACTTG	600	44	Rodriguez et al., 1997
pedA_R	TAAAAAGATATTTGACCAAAA			

interpretation. Calculations and graphics were carried out with the Infostat Professional software version 2013.

3. Results

3.1. Antilisterial activity of meat borne LAB strains

A total of 115 LAB strains isolated from Argentinean vacuum-packaged beef and different traditional fermented sausages were examined for their antilisterial activity. Four *L. monocytogenes* strains and *L. innocua* 7 used as indicators demonstrated different sensitivity to the meat borne LAB inhibitory activity displaying variable growth inhibition level (Table 2). Inhibition halos of 2–4 mm were produced by 19.1% (against *L. monocytogenes* UC8158) to 36.5% (against *L. monocytogenes* UC8160) of the meat strains. Moreover, 24.3% of assayed LAB strains were able to produce inhibition halos larger than 4 mm against *L. monocytogenes* UC8159 and when total inhibition (from 0.5 to more than 4 mm) were considered, 75.6% of LAB meat strains showed to inhibit *L. monocytogenes* UC8159. *L. monocytogenes* UC8159 was the most susceptible among this species whereas *L. innocua* 7 was the second sensitive indicator. Among 92 *Lactobacillus* strains tested, *L. sakei* exhibited greater antagonistic effects on agar medium against all *Listeria* strains, while *L. plantarum* showed a limited or absent antilisterial activity. Twelve out of 14 *L. curvatus* were able to inhibited *L. innocua 7*. All 16 *E. faecium* strains inhibited *L. innocua* 7 and 99% of them were also inhibitory against *L. monocytogenes* FBUNT and *L. monocytogenes* UC8160. On the contrary, *P. acidilactici* strains exhibited less inhibitory activity against *L. innocua* 7 than against *L. monocytogenes* strains.

Table 2
Different sensitivity of *Listeria* strains to the meat borne LAB inhibitory activity.

Diameter of inhibitory halos (mm)	*Listeria* strains				
	7	FBUNT	UC8158	UC8159	UC8160
Antilisterial activity (% of strains)					
- (no halos)	30.4	38.2	40.9	1.7	38.26
−/+ (≤0.5 mm)	5.2	11.3	8.7	2.6	6.09
+ (0.5–2 mm)	17.4	9.6	17.4	22.6	17.39
++ (2–4 mm)	31.3	32.2	19.1	28.7	36.52
+++ (>4 mm)	15.6	8.7	13.9	24.3	0.00
Total (+, ++, +++)	64.3	50.4	50.4	75.6	53.91

3.2. Detection of bacteriocin encoding genes in meat borne LAB

The presence of sakacin P (*sppA*), sakacin Q (*sppQ*), curvacin A (*sapA*), plantaricin EF (*plnEF*), plantaricin A (*plnA*), enterocins A, B and P (*entA*, *entB* and *entP*) and pediocin PA-1 (*pedA*) genes were evaluated by PCR. All PCR fragments obtained from each strain were sequenced and the sequences analyzed using Blast algorithms (data not shown). Table 3 show the distribution of bacteriocin genes among meat borne LAB strains. From 71 *L. sakei* strains only 24 strains harbor one or more bacteriocin genes. Most of the evaluated *L. curvatus* strains carried both *sppQ* and *sppA* genes while *L. curvatus* CRL1534 also contain *sapA* gene. These genes showed to be less abundant in *L. sakei*, where *sppA* and *sppQ* were detected in 8 and 7 strains, respectively. On the contrary, the structural gene for curvacin A (*sapA*) prevailed in *L. sakei* analyzed strains, 13 strains harbored this bacteriocin gene, whereas it had been detected only in one strain of *L. curvatus*. When structural genes for bacteriocins produced by *L. plantarum* were investigated, *plnEF* gene was detected in 6 out of 7 tested strains, whereas only 2 strains also carried *plnA* gene. Concerning the presence of bacteriocin genes among *E. faecium* strains from fermented sausages, all tested strains possessed one or more enterocin structural gene (s). The *entA* gene was detected in 13 out of 16 strains of *E. faecium* strains, of which it was unique for only 4 strains. On the other hand, *entP* and *entB* structural genes were present in 8 and 7 assayed strains, respectively. However, the *entP* structural gene alone was harbored in 3 of the tested strains (CRL1489, CRL1632 and CRL1634) while the enterocin B gene was always accompanied by the enterocin A structural gene. Although antilisterial activity was detected among *Pediococcus* strains, no *pedA* gene was amplify from these strains suggesting the production of a new or modified antimicrobial compound.

3.3. Multiple correspondence analyses

In order to have a global view of data, useful to interpret associations between the inhibitory activity against *Listeria* strains and the presence of bacteriocin genes in the 115 LAB strains here analyzed, a multiple correspondence analysis (MCA) was applied. A picture for the associations between the levels of a two-way contingency table was allowed and the graphical display is shown in Fig. 1. It has three sets of points, as indicated by the 3 types of point symbols (bacteriocin genes, *Listeria* strains and inhibition level). Results show that each analyzed LAB species is found in the

Table 3
Distribution of bacteriocin genes among meat borne LAB strains.

LAB specie	Strain	Bacteriocin encoding genes			Origen/Source
		sapA	*sppQ*	*sppA*	
L. sakei	CRL1464	+	−	−	Pork fermented sausages
	CRL1466	−	+	−	Pork fermented sausages
	CRL1467	+	−	−	Pork fermented sausages
	CRL1468	+	−	−	Pork fermented sausages
	CRL1469	+	−	−	Pork fermented sausages
	CRL1613	+	−	−	Vacuum-packaged beef
	CRL1862	+	−	−	Pork fermented sausages
	CRL1881	+	−	+	Pork fermented sausages
	CRL1882	−	−	+	Pork fermented sausages
	CRL1883	−	−	+	Pork fermented sausages
	CRL1884	+	−	+	Pork fermented sausages
	UC8707	−	+	+	Vacuum-packaged beef
	UC10278	−	−	+	Llama fermented sausages
	UC10283	−	−	+	Llama fermented sausages
	UC10284	+	−	−	Llama fermented sausages
	UC10285	−	−	+	Llama fermented sausages
	UC10287	−	+	−	Llama fermented sausages
	UC10294	+	−	−	Llama fermented sausages
	UC10295	+	−	−	Llama fermented sausages
	UC10298	+	−	−	Llama fermented sausages
	UC10300	−	+	−	Llama fermented sausages
	UC10302	−	+	−	Llama fermented sausages
	UC10303	−	+	−	Llama fermented sausages
	UC10304	+	+	−	Llama fermented sausages
		sapA	***sppQ***	***sppA***	
	CRL1532	−	+	+	Vacuum-packaged beef
L. curvatus	CRL1533	−	+	+	Vacuum-packaged beef
	CRL1534	+	+	+	Vacuum-packaged beef
	CRL1535	−	+	+	Vacuum-packaged beef
	CRL1536	−	+	+	Vacuum-packaged beef
	CRL1537	−	+	+	Vacuum-packaged beef
	CRL1538	−	+	+	Vacuum-packaged beef
	CRL1539	−	+	+	Vacuum-packaged beef
	CRL1652	−	+	+	Vacuum-packaged beef
	CRL1653	−	+	+	Vacuum-packaged beef
	CRL1654	−	+	+	Vacuum-packaged beef
	CRL1629	−	+	+	Pork fermented sausages
		plnA	***plntEF***		
L. plantarum	CRL1477	−	+		Pork fermented sausages
	CRL1478	+	+		Pork fermented sausages
	CRL1479	+	+		Pork fermented sausages
	CRL1480	−	+		Pork fermented sausages
	CRL1481	−	+		Pork fermented sausages
	CRL1620	−	+		Pork fermented sausages
E. faecium		***entA***	***entB***	***entP***	
	CRL1489	−	−	+	Pork fermented sausages
	CRL1491	+	+	+	Pork fermented sausages
	CRL1492	+	+	+	Pork fermented sausages
	CRL1493	+	+	−	Pork fermented sausages
	CRL1494	+	−	+	Pork fermented sausages
	CRL1632	−	−	+	Pork fermented sausages
	CRL1633	+	+	+	Pork fermented sausages
	CRL1634	−	−	+	Pork fermented sausages
	CRL1635	+	−	+	Pork fermented sausages
	CRL1636	+	+	−	Pork fermented sausages
	UC10314	+	+	−	Llama fermented sausages
	UC10315	+	−	−	Llama fermented sausages
	UC10316	+	+	−	Llama fermented sausages
	UC10317	+	−	−	Llama fermented sausages
	UC10318	+	−	−	Llama fermented sausages
	UC10319	+	−	−	Llama fermented sausages

CRL: CERELA Culture Collection; UC: Universitá Cattolica del Sacro Cuore Culture Collection; gene/s presence (+) or absence (−).

neighborhood of the genes that are present or not and the inhibition level against the indicator organisms (*Listeria* strains). LAB strains that do not harbor bacteriocin genes here analyzed are situated in the origin of the system (axes intersection). However, a relatively greater presence of bacteriocins genes may be inferred for *E. faecium* since *entA, entB* and *entP* genes are in the vicinity. The picture for *L. plantarum* indicate that more strains harbor plantaricin EF than plantaricin A genes, while the lack of *pedA* gene for *P. acidilactici* strains is clearly shown by MCA. Although most of *L. sakei* strains analyzed (66.2%) were not able to inhibit *Listeria* cells, a relatively high number of bacteriocinogenic strains carried curvacin A gene, while sakacin Q and P bacteriocins were only present in a few of them. On the contrary, more *L. curvatus* strains were able to produce sakacin P and Q and only a low percentage of them produced curvacin A. Indeed, as shown in Fig. 1, this bacteriocin was represented closer to *L. sakei* than to *L. curvatus* indicating that for these meat borne strains, *sapA* gene is more frequently present in *L. sakei* strains.

Most *E. faecium* strains were highly inhibitory against *L. monocytogenes* FBUNT, and to a lesser extent against *L. innocua* 7, being represented in the graph close and further away, respectively. Particularly for *L. sakei*, although more strains exhibited intermediate inhibition level against *L. innocua* 7 and *L. monocytogenes* UC8159, a percentage of them were also highly inhibitory to *L. monocyotgenes* UC8158 and UC8159 whereas a lower inhibition against *L. monocytogenes* UC8158, FBUNT and UC8160 was exerted by *L. sakei*. Association between inhibitory activity and *L. curvatus* strains showed absence or low inhibition against all assayed *Listeria* strains indicating a low sensitivity to sakacin Q and P mainly produced by *L. curvatus* strains. Furthermore, points corresponding to *L. sakei* and *E. faecium* are observed to lie within the parabolic configuration of intermediate to high inhibitory level against *Listeria* strains, this implying that the antilisterial profile of these two LAB strains are higher than average. On the other hand, most of *L. plantarum* strains lack of antilisterial activity which may be inferred from the graph.

4. Discussion

One important technological feature for the selection of a bioprotective culture is the production of inhibitory substances against food-borne pathogens. In this study, as a first step in selecting autochthonous strains to be consider as bioprotectives cultures, the ability to inhibit the growth of *Listeria* cells and the occurrence of bacteriocin encoding genes was examined in 115 LAB. In this study, *L. sakei*, *L. curvatus* and *E. faecium* displayed a greater antilisterial activity while was limited or absent for *P. acidilactici* and *L. plantarum*. Antimicrobial activity against *Listeria* by meat borne *L. sakei* and *L. curvatus* strains was previously reported (Alves, Martinez, Lavrador, & De Martinis, 2006; Castellano & Vignolo, 2006; Cintas et al., 1997; Nishie et al., 2012).

Since the antilisterial activity exhibited by meat borne LAB strains found in this study may be attributed to the production of bacteriocins, the presence of some known bacteriocin encoding genes were evaluated by PCR. From all tested LAB, 60 strains presenting antilisterial activity correlated with the presence of one or more bacteriocin genes, while 48 strains showed antilisterial activity but none of the screened genes suggesting the production of a different antimicrobial peptide. In addition, only one strain (*L. plantarum* CRL1620) showed no antimicrobial activity but harbored plantaricin EF genes and 6 strains revealed no antimicrobial activity and none of the tested bacteriocin genes in their genome. The presence of structural genes encoding for sakacin P and Q in meat borne *L. curvatus* found in this study agree with those previously described for *L. curvatus* LTH1174 and L442 isolated from meat fermentations (Cocolin & Rantsiou, 2007). The *plnEF* gene was detected in 6 out of 7 tested *L. plantarum* strains, whereas only 2 strains also carried *plnA* gene. It is known that this LAB specie is able to produce a wide range of antimicrobial peptides. Besides its antimicrobial activity, *plnA* also function as a peptide pheromone

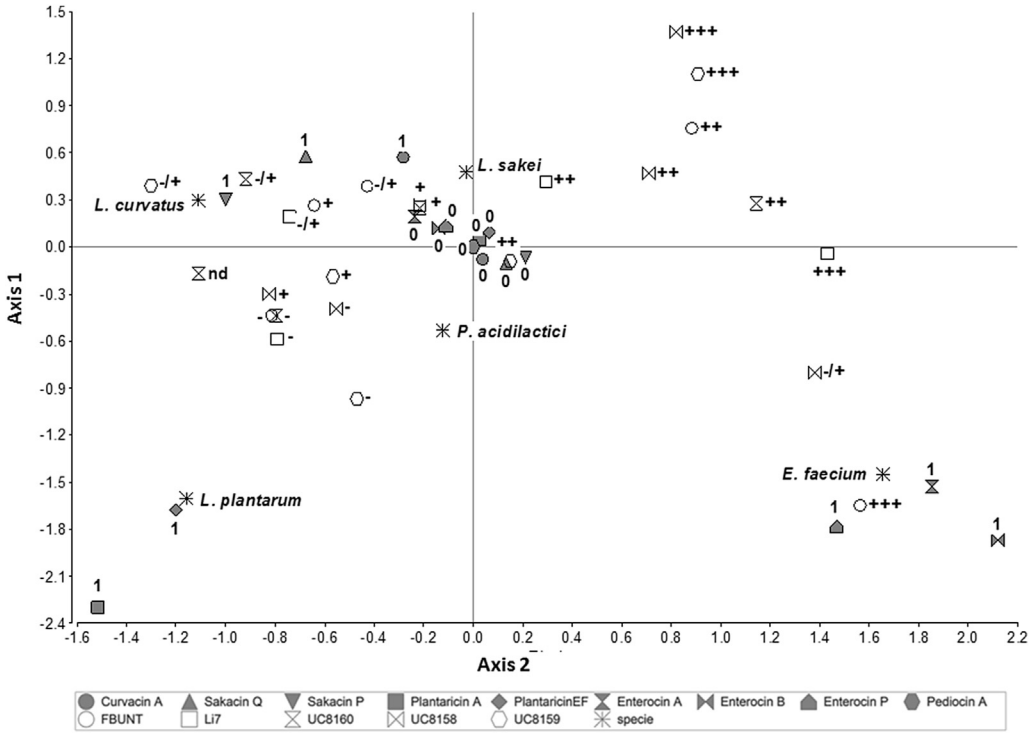

Fig. 1. Biplot obtained by Multiple Correspondence Analysis of the presence or absence of bacteriocins genes, curvacin A (●), sakacin Q (▲), sakacin P (▼), plantaricin A (■), plantaricin EF (◆), enterocin A (⧗), enterocin B (⋈), enterocin P (⬟) and pediocin PA-1 (⬣) and inhibitory activity expressed in halo diameter as −/+ (≤0.5 mm), + (0.5–2 mm), ++ (2–4 mm), +++ (>4 mm), − (no halos) or not detected (nd) around the spot against *Listeria* strains [FBUNT (○); Li 7 (▫); UC 8160 (⧖); UC8158 B (⋈); UC8159 (△)].

that induces transcription of all *pln* loci (Diep, Straume, Kjos, Torres, & Nes, 2009). Although *L. plantarum* strains from vegetable origin displaying antilisterial activity were extensively reported (Settanni & Corsetti, 2008), scarce data from those of meat origin are available with the exception of plantaricin-producers *L. plantarum* SA6 and UG1 isolated from fermented sausages which showed strong inhibitory effects against *L. monocytogenes* in salami or meat (Rekhif, Atrih, & Lefebvre, 1995). Regarding *E. faecium* all tested strains possess one or more enterocin structural gene (s). A number of reports describing bacteriocin activity in *Enterococcus* species from different origins have been appeared (Aymerich et al., 2000; Khan, Flint, & Yu, 2010). In the present study, the simultaneous presence of *entA*, *entB* and *entP* structural genes was observed in 3 out of 16 *E. faecium* strains (CRL1491, CRL1492, and CRL1633), whereas *entA* and *entB* genes were found in four strains (CRL1493, CRL1636, UC10314 and UC10316). The *entA* and *entP* genes were identified in *E. faecium* CRL1494 and CRL1635. These results are in accordance with previous observations also regarding the occurrence of multiple enterocin genes in bacteriocinogenic *Enterococcus* strains (De Vuyst, Foulquie Moreno, & Revets, 2003) (Ben Omar et al., 2004). The *entA* gene was the most frequently found among *E. faecium* strains being unique for only 25% of them, whereas *entP* as the sole enterocin gene was harbored in 18.75% of the assayed strains and finally, *entB* was always accompanied by the enterocin A structural gene. This fact may be due to the lack of transport genes found for enterocin B production. Besides the prevalence of *entA* gene (13 out of 16 strains), the high frequency of *entA* and *entP* structural genes detected among *E. faecium* here evaluated is in agreement with those previously reported for *E. faecium* strains of different origins (Ozdemir, Oryasin, Biyik, Ozteber, & Bozdogan, 2011) and from meat products (Strompfova, Laukova, Simonova, & Marcinakova, 2008). On the other hand, no *pedA* gene was detected among the analyzed *Pediococcus* strains suggesting the production of a new or modified antimicrobial compound. Different pediocins produced by *P. acidilactici* isolated from naturally fermented meat products have been previously characterized such as pediocin AcH/PA-1, SA-1 and SJ-1 (Papagianni & Anastasiadou, 2009).

MCA is a descriptive/exploratory technique used to analyze a set of observations described by a set of nominal variables. In this study, MCA was found to be a useful tool to associate the presence of bacteriocin genes with the inhibitory activity against *Listeria* cells in 115 meat borne LAB strains. The purpose of MCA is to construct a joint map of LAB strains and variable categories (presence of bacteriocin genes and *Listeria* sensitivity) in such a way that a LAB strain is relatively close to a category it is related with, and relatively far from the categories it is not related; thus MCA is linked to multidimensional scaling through the notion of distance (Hoffman & de Leeuw, 1992). Similarly, MCA was used to visualize the association between ester-synthesizing activity of dairy LAB strains and different production levels of five ethyl esters as reported by Abeijón Mukdsi et al. (Abeijón Mukdsi, Medina, Alvarez, & González, 2009).

5. Conclusions

As a conclusion, PCR screening for antilisterial bacteriocins among meat borne LAB strains allowed to establish that half of tested strains possessed at least one bacteriocin gene. Although the high occurrence of sakacin Q and P among *L. curvatus* strains here determined, antilisterial activity was found to be related to the studied *Listeria* strains. On the other hand, as a replacement for, even a low percentage of *L. sakei* strains exhibited the presence of curvacin A, sakacin Q and P, they were very effective at inhibiting *Listeria* strains, particularly *L. monocytogenes* UC8159 and *L. innocua* 7. A wide distribution of enterocin structural genes accounts for a

high antilisterial activity, particularly against *L. monocytogenes* FBUNT. The bacteriocin-producing meat borne *L. sakei* and *E. faecium* here analyzed exhibit great potential to be used as bioprotective cultures providing an additional hurdle to enhance the control of *L. monocytogenes* in meat products.

Acknowledgments

This study was carried out with the financial support from Consejo Nacional de Investigaciones Científicas y Técnicas (PIP2009 N° 0649) and FONCYT (PICT2010 N° 0655; PICT2010 N°1295) from Argentina.

Authors are grateful to Elena Bru for her collaboration with statistical analysis.

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