Characterization of bacPPK34 a bacteriocin produced by *Pediococcus pentosaceus* strain K34 isolated from "Alheira"

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A B S T R A C T

Different lactic acid bacteria were isolated during different stages in the production of "Alheiras", a traditionally fermented sausage produced in the north of Portugal, between 2005 and 2007, in a total of 484 isolates. One of 484 isolates (K34) produced a bacteriocin, designated as bacPPK34, and was identified as a strain of *Pediococcus pentosaceus* by 16S rRNA sequencing. The highest bacteriocin production was noted at late log/early stationary phase after 15–18 h of growth in MRS broth at 37 °C (3200 AU/ml) against *Enterococcus faecalis* ATCC 29212 and 12800 AU/ml against *Listeria monocytogenes* (L1, L2, L3). bacPPK34 was between 2.5 kDa and 6.2 kDa in size, as determined by tricine-SDS-PAGE. Complete inactivation or significant reduction in antimicrobial activity was observed after treatment of cell-free supernatants with proteinase K, pepsin and trypsin. No change in activity was recorded when treated with catalase. The bacteriocin was resistant to treatments with lipase and detergents Triton X-100, Tween 20, SDS, NaCl, urea and EDTA. Furthermore, the bacteriocin remained active after 2 h at pH 2–12 and temperature treatments at 60, 80, 100 °C, 1 month of storage at −20 and 4 °C and 20 min at 121 °C. Addition of bacPPK34 to a mid-log culture of *L. monocytogenes* and *E. faecalis* ATCC 29212 inhibited growth. The bacteriocin did not adhere to the surface of the producer cells.

1. Introduction

Fermented meat products are part of the daily diet in rural areas of Portugal and have become very popular in urban centers. "Alheira" is a traditional fermented meat product typical in the northern regions of Portugal (Trás-os-Montes), with its origin being dated in the end of the fifteenth century. "Alheira" is produced from a combination of pork meat, pork lard, poultry, wheat bread and olive oil mixed with salt, garlic and spices to the desirable taste. The paste is then stuffed into animal or cellulose-based casings and is smoked for a maximum of 8 days. "Alheira" contains mainly lactic acid bacteria and Micrococcaceae. Some pathogens, such as *Escherichia coli*, *Listeria monocytogenes* and *Staphylococcus aureus* have also been found (Ferreira et al., 2006). *L. monocytogenes* are ubiquitous bacteria often present in meat products; its exclusion from foods is not easy (Albano et al., 2007).

Although not used in traditional production, the application of starter cultures of selected strains of lactic acid bacteria (LAB) may help to inhibit spoilage and pathogenic microorganisms by production of organic acids, hydrogen peroxide, diacetyl and bacteriocins as part of their natural hurdle system. Research on antimicrobial substances, mainly bacteriocins produced by LAB, has led to consideration of their use as natural preservatives in meat products (Albano et al., 2008; Aymerich, Hugas, & Monfort, 1998; Castellano, Holzapfel, & Vignolo, 2004; Cleveland, Montville, Nes, & Chikindas, 2001; Dicks, Mellett, & Hoffman, 2004; Hugas, 1998). Although many strains of these species produce bacteriocins, only Nisin, produced by *Lactococcus lactis* subsp. *lactis,* has GRAS (generally recognized as safe) status and remains the only commercially important bacteriocin being approved as a food preservative in over 40 countries (Cleveland et al., 2001). Alta 2341®, a formulation containing a bacteriocin produced by *Pediococcus acidilactici* has just recently been marketed (Papagianni & Anastasiadou, 2009).

P. acidilactici, *Pediococcus pentosaceus* and *Pediococcus parvulus* isolated from meat products produce various bacteriocins. Pediocin AcH (PA-1), produced by *P. acidilactici*, was the first thoroughly characterized Class IIa bacteriocin (Bhunia, Johnson, Ray, & Kalchayanand, 1991; Cintas et al., 1995; Nieto-Lozano, Nissen-Meyer, Sletten, Pelaez, & Nes, 1992; Pucci, Vedamuthu, Kunka, & Vandenbergh, 1988). The same bacteriocin is also produced by strains of *P. parvulus* isolated from vegetables (Bennik, Smid, & Gorris, 1997), a strain of *P. pentosaceus* just recently isolated from a marine

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fermented seafood product (Bagenda, Hayashi, Yamazaki, & Kawai, 2008) and a strain of *Lactobacillus plantarum* isolated from cheese (Loessner, Guenter, Stefan, & Scherer, 2003). Pediocin-like bacteriocins share 40–60% DNA homology (Eijsink, Skeie, Middelhoven, Brurberg, & Nes, 1998; Papagianni & Anastasiadou, 2009) and are all active against *L. monocytogenes* (Bennik, van Overbeeck, Smid, & Gorris, 1999; Cintas, Casaus, Fernández, & Hernández, 1998; Guyonnet, Fremaux, Cenatiempo, & Berjeaud, 2000; Papagianni & Anastasiadou, 2009).

This study aimed to test the antimicrobial activity of LAB isolated from "Alheiras" against *L. monocytogenes* and other selected pathogens and to describe the bacPPK34 produced by *P. pentosaceus* strain K34 isolated from "Alheira".

2. Materials and methods

2.1. Origin of LAB bacterial isolates

Four hundred and eighty-four isolates of LAB stored at −20 °C in de Man, Rogosa Sharpe broth (MRS, Biokar Diagnostics, France) and M17 broth (Biokar Diagnostics) with 30% (v/v) glycerol, isolated between 2005 and 2007 from different producers and in different stages in the production of "Alheira", were cultured in MRS or M17 broth, depending on the medium of origin, for regeneration and the purity of each culture was confirmed by streaking on plates of the respective medium.

2.2. Pathogenic and indicator strains

L. monocytogenes L1, L2, L3 (Escola Superior de Biotecnologia, UCP), *S. aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212 and *Salmonella typhimurium* (Escola Superior de Biotecnologia, UCP) were used as target bacteria for the inhibitory effects of LAB. Bacteria were grown in Tryptone Soy Yeast Extract Broth (TSB-YE; Tryptone Soy Broth (Lab M, UK) + 6 g/l yeast extract (Lab M)), at 30 °C for 24 h. All strains were stored at −20 °C in TSB containing 30% (v/v) glycerol, and sub-cultured twice before use in assays.

2.3. Antibacterial activity

Tryptone Soy Yeast Extract agar plates (TSA-YE; TSB-YE + 12 g/l agar (Lab M)) were evenly spread with each of the target bacteria and drops (10 μl) of LAB cultures, grown in MRS/M17 broth at 30 °C for 24 h, were spotted on the lawns of pathogens and incubated overnight at 30 °C. Inhibition was recorded as positive if a translucent halo zone was observed around the spot. For the inhibitory strains, initial characterization of the antimicrobial activity was performed according to Tomé, Teixeira, and Gibbs (2006). Culture broths were centrifuged (Rotina 35R, Hettich, Germany) at 3382× *g* for 15 min, at 4 °C. The clear supernatants were sterilized by membrane filtration (0.2 μm, Corning Incorporated, Germany). The pH of the cell-free supernatants was adjusted to 6.5 with NaOH (1 N) and then treated with catalase (Sigma, Germany; 500 IU ml/1, sterile) and trypsin (Sigma; 0.1 mg ml/l, sterile), for 1 h at 37 °C. Cell-free supernatant, neutralized cell-free supernatant treated with catalase and neutralized cell-free supernatant treated with catalase and trypsin, were spotted against the target organisms. *P. acidilactici* HA-6111-2, a pediocin PA-1 producer (Albano et al., 2007) was used as an anti-listerial control strain.

The isolates that showed antimicrobial activity were tested for bacteriocin production according to the method described by Van Reenen, Dicks, and Chikindas (1998). Briefly, a doubling dilution series was made of the cell-free culture supernatant. An aliquot of 10 μl of each dilution was spotted onto an agar plate (0.7% w/v agar) seeded with active growing cells of the target organism (approximately 10^6 cells/ml). Plates were incubated at the optimal growth temperature of the target organism. Antimicrobial activity was expressed as arbitrary units (AU) per ml. One AU is defined as the reciprocal of the highest dilution showing a clear zone of growth inhibition (Van Reenen et al., 1998). Cell-free supernatants with antimicrobial activity were treated with proteinase K (1 mg/ml; Roche).

2.4. Identification of bacteriocin-producing strain

The bacteriocin-producing strain K34 was identified by 16S rRNA sequencing by Macrogen Inc. (Seoul, Korea). Total DNA isolation was performed according to the method described by Dellaglio, Bottazzi, and Troatelli (1973). Amplification of the 16S rDNA was carried out with the primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) using the following profile: primary DNA denaturation step at 95 °C for 5 min, followed by 30 cycles of 1 min at 94 °C, 1 min at 55 °C, and 1.5 min at 72 °C, with an extension of the amplified product at 72 °C for 10 min. Amplification reactions were performed in a MyCycler Thermal Cycler System (Bio-Rad, USA). Following amplification, 5 μl of product was separated at 90 V for 50 min in a 1% w/v agarose gel in 1× TAE buffer (4.84 g Tris-base, 1.09 g glacial acetic acid, 0.29 g ethylenediaminetetraacetic acid, 1 l distilled water), and then stained with 0.5 μg/ml of ethidium bromide. A 1-kb ladder molecular weight marker (Bio-Rad) was used. PCR products, used as templates, were previously purified with the GFX PCR DNA and Band Purification kit (GE HealthCare, Amersham Biosciences, Amersham, UK) and sent for sequencing. On-line similarity searches were performed with the BLAST program in GenBank (http://www.ncbi.nlm.nih.gov).

2.5. Kinetics of growth and bacteriocin production

An overnight culture was inoculated (1% v/v into 150 ml of MRS broth) and incubated at 37 °C, without shaking. Samples were taken at regular intervals during 24 h of incubation. Changes in pH and optical density (600 nm) were recorded every hour, bacteriocin activity (AU/ml) and viable counts were calculated every 3 h, as described by Van Reenen et al., (1998). *L. monocytogenes* strain L2 and *E. faecalis* ATCC 29212 were used as target organisms.

2.6. Partial purification and molecular size of bacteriocins

LAB strain K34 was grown in 300 ml MRS broth for 18 h at 37 °C. Cells were harvested by centrifugation (5500× *g* for 30 min, 4 °C) and the bacteriocin precipitated from the cell-free supernatant with 70% saturated ammonium sulphate (Sambrook, Fritsch, & Maniatis, 1989). After 4 h of slow stirring at 4 °C, proteins were harvested (12,000× *g*, 1 h, 4 °C). Precipitated proteins in the pellet and floating on the surface were collected and dissolved in 25 mM ammonium acetate buffer (pH 6.5). All samples were stored at −20 °C.

Separation of the proteinaceous compounds was carried out by tricine-SDS–PAGE on a Bio-Rad Mini Protean 3 Cell apparatus (Bio-Rad), as described by Schägger and Von Jagow (1987). A molecular weight marker with sizes ranging from 2.5 to 16.9 kDa (Amersham Pharmacia Biotech Europe GmbH, Germany) was used. The gels were fixed and one half stained with Coomassie Brilliant Blue G250 (Bio-Rad). The positions of the active bacteriocins were determined by overlaying the other half of the gel (not stained and pre-washed with sterile distilled water) with cells of the test organism (*L. monocytogenes* L2 or *E. faecalis* ATCC 29212), embedded in Brain Heart Infusion (BHI) agar (Conda, Spain; 0.7% w/v agar).

2.7. Effect of enzymes, temperature, pH and surfactants on bacteriocin activity

LAB strain K34 was grown in MRS broth overnight at 37 °C. Cells were harvested (8000× *g*, 10 min, 4 °C) and the cell-free supernatant adjusted to pH 6.5 with 1 M NaOH. One millilitre sterile cell-free supernatant was incubated for 2 h in the presence of 1 mg/ml and 0.1 mg/ml (final concentrations) of each, proteinase K (Bioron, Germany), pepsin, trypsin (Sigma–Aldrich, Germany), lipase (Sigma–Aldrich) and catalase (Sigma–Aldrich), respectively. The remaining antimicrobial activity was monitored by the agar-spot test method (Van Reenen et al., 1998). In a separate experiment, 1% (w/v) sodium dodecyl sulphate (SDS), Tween 20, urea, Triton X-100 and NaCl were added to bacteriocin-containing cell-free supernatants. EDTA was added to cell-free supernatants in final concentrations of 1.0, 2.0 and 5.0 mM. Untreated cell-free supernatants and detergents at these respective concentrations in water served as controls. All samples were incubated at 37 °C for 5 h and then tested for antimicrobial activity.

The effect of pH on the activity of bacteriocins was tested by adjusting the pH of sterile cell-free supernatants from 2.0 to 12.0 (at increments of two pH units) with sterile 1 M NaOH or 1 M HCl. After 1 h of incubation at room temperature (25 °C), samples were re-adjusted to pH 6.5 with sterile 1 M NaOH or 1 M HCl, heated to 80 °C for 10 min to inactivate possible extracellular proteases and tested for antimicrobial activity. The effect of temperature on bacteriocin activity was tested by incubating cell-free supernatants at 60, 80, and 100 °C for 60 and 120 min. Bacteriocin activity was also tested after 15 min at 121 °C and one month of storage at 4 and −20 °C.

The agar-spot test method was used in all experiments. *L. monocytogenes* L2 and *E. faecalis* ATCC 29212 served as target strains.

2.8. Cell lysis

Twenty milliliters of the bacteriocin-containing cell-free supernatant (12,800 AU/ml, pH 6.0 assayed on *L. monocytogenes,* and 3200 AU/ml, pH 6.0 assayed on *E. faecalis*) was filter-sterilized and added to 100 ml early exponential phase (5 h-old; OD ≥ 0.6) cultures of *L. monocytogenes* L2 and *E. faecalis* ATCC 29212, respectively. Optical density readings at 600 nm were taken every hour for 25 h.

L. monocytogenes L2 and *E. faecalis* ATCC 29212 cultures without added bacteriocins were used as controls.

2.9. Adsorption studies

Adsorption of bacteriocins to producer cells was studied according to the method described by Yang, Johnson, and Ray (1992). Strain K34 was cultured for 18 h at 37 °C. The pH of the culture was adjusted to 6.0 with 1 M NaOH to allow maximal absorption of the bacteriocin to the producer cells. The cells were then harvested (12,000× *g*, 15 min, 4 °C) and washed with sterile 0.1 M phosphate buffer (pH 6.5). The pellet was re-suspended in 10 ml 100 mM NaCl (pH 2.0) and agitated for 1 h at 4 °C to allow delaminating bacteriocin from the cells. The cells were then harvested (12,000× *g*, 15 min, 4 °C), the cell-free supernatant neutralized to pH 7.0 with sterile 1 M NaOH and tested for bacteriocin activity as described before.

3. Results and discussion

From the tested strains, 12 exhibited activity against *L. monocytogenes* during the first screenings by developing a translucent halo of at least 2 mm in diameter around the inoculation spot. Of these, six were also active against *E. faecalis* and two others against *S. aureus*. Antimicrobial activity against Gram-negative bacteria was not demonstrated by any of the investigated isolates. This may be attributed to the lipopolysaccharide layer of the cell wall protecting the cell membrane, the site of action of bacteriocins (Stevens, Sheldon, Klapes, & Klaenhammer, 1991). Activity against Gram-negative bacteria is an unusual phenomenon and has rarely been reported (Cheikhyoussef et al., 2009; De Kwaadsteniet, Todorov, Knoetze, & Dicks, 2005; Gong, Meng, & Wang, 2010; Todorov & Dicks, 2005c).

Using this screening method, the observation of an inhibition zone, may result from competition, lactic acid, bacteriocin or hydrogen peroxide production. Following the screening procedure, as shown in Table 1, only strain K34 exhibited activity of a proteinaceous nature, since it was lost only after the digestion with the proteolytic enzyme trypsin. This suggests the inhibitory mechanism of K34 to be bacteriocinogenic, against *L. monocytogenes* and *E. faecalis*. Inhibitory mechanisms of the other screened strains was revealed to be based on substrate competition and/or acidification of the media, as no halos were recorded, respectively, after removing the cells and neutralizing the cell free supernatants.

Strain K34, isolated from “Alheira” during the blending process, was identified as *P. pentosaceus* by 16S rDNA sequencing (similarity of 99%). The majority of LAB isolated from “Alheira” was identified as lactobacilli and enterococci, present at levels of approximately 10^7 cfu/g (Albano et al., 2009; Ferreira et al., 2006). However, Albano et al., (2008) also isolated from “Alheira” a pediocin PA-1 producer (*P. acidilactici* HA-6111-2 strain), that showed several attributes as a potentially bioprotective organism against listeriae in “Alheira” processing.

BacPPK34, the bacteriocin produced by LAB strain *P. pentosaceus* K34, was produced at maximum levels (12,800 AU/ml against *L. monocytogenes* strains L1, L2, L3 and 3200 AU/ml against *E. faecalis* ATCC 29212) after 15–18 h of growth in MRS broth (Fig. 1). The activity of the supernatant remained stable during this period and declined thereafter, this probably being the effect of extracellular proteases (Mezaini, Chihib, Bouras, Nedjar-Arroume, & Hornez, 2009). During 24 h of growth, the medium pH decreased from 6.33 to 4.01 and the culture optical density increased from 0.01 to 5.86 (dilution factor taken into account). By the time of the highest production rate (9–15 h of incubation), pH levels decreased to below 5 and lower (Fig. 2). The post-translational processing of pediocin PA-1 is enzyme dependent and occurs at low pH at which activation of processing enzymes takes place (Papagianni & Anastasiadou,

Table 1
Inhibitory activity of lactic acid bacteria against *L. monocytogenes* L1, L2, L3 and *E. faecalis* ATCC 29212 determined by a spot assay on MRS agar.

LAB strain	*L. monocytogenes* L1, L2, L3 and *E. faecalis* ATCC 29212			
	CS	NF (pH 6.5).	NFC	NFCT
K34	+ and +	+ and +	+ and +	− and −
O18	+ and +	± and ±	− and −	− and −
O40	+ and +	± and ±	− and −	− and −
O55 − 1	+ and +	± and ±	− and −	− and −
O55 − 2	+ and +	± and ±	− and −	− and −
R5	− and −	− and −	− and −	− and −
R12	− and −	− and −	− and −	− and −
R16	− and −	− and −	− and −	− and −
R27	− and −	− and −	− and −	− and −
P. acidilactici HA−6111−2	+ and +	+ and +	+ and +	− and −

− no activity; + zone larger than 2 mm; ± zone smaller than 2 mm; CS, cell-free supernatant; NF, cell-free supernatant adjusted to pH 6.5; NFC, cell-free supernatant adjusted to pH 6.5 and treated with catalase; NFCT, cell-free supernatant adjusted to pH 6.5 and treated with catalase and trypsin.

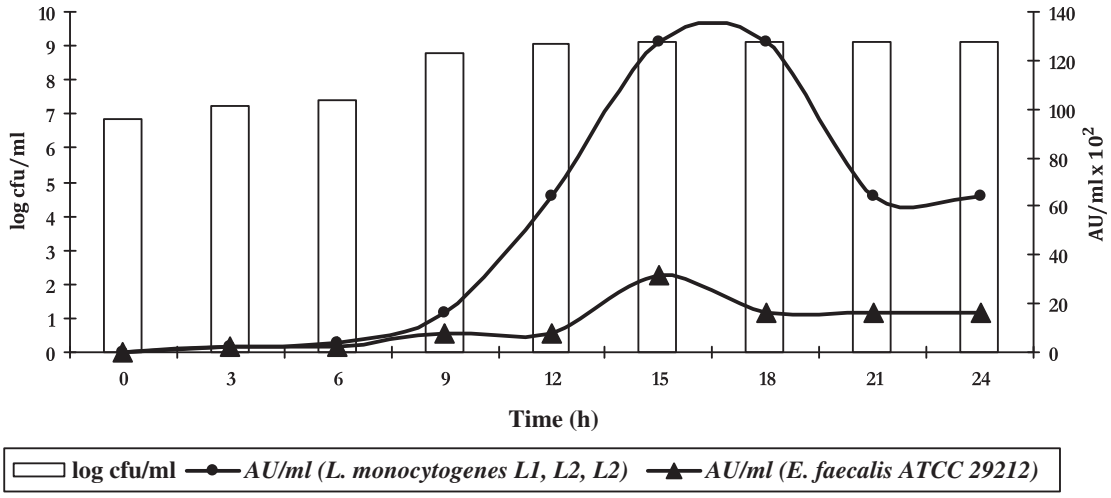

Fig. 1. Growth kinetics and bacteriocin production of strain K34 during 24 h of incubation (37 °C) in MRS broth. Antimicrobial activity of cell-free supernatant is presented as AU/ml. Organism names in parenthesis indicate the test strains used for determination of the AU/ml values.

2009). Low levels of bacPPK34 activity (200 AU/ml against both target organisms) were already recorded after 3 h of growth in MRS broth (Fig. 1). Incubation at 30 °C instead of 37 °C showed no difference in AU-levels (data not shown). All further experiments were conducted at 37 °C. The constant bacteriocin activity levels recorded at pH values below 4.3 and their decrease later may indicate that the production is blocked in some way (Todorov & Dicks, 2005a). Papagianni and Anastasiadou (2009) summarized that the pH decline rate and the final pH reached in cultures appear to be critical factors in pediocin production and re-alkalization cycles might help raising the productivity (Guerra, Agrasar, Macias, & Pastrana, 2005; Guerra, Bernardez, Agrasar, Macias, & Pastrana, 2005). Genetic studies on the expression of the genes encoding bacteriocin production will be necessary to confirm the latter hypothesis.

BacPPK34 is between 2.5 and 6.2 kDa in size, as determined by SDS-PAGE (Fig. 3). This is within the range of most bacteriocins reported for the genus *Pediococcus* (Papagianni & Anastasiadou, 2009). The molecular weight of pediocin PA-1, was calculated from the amino acid sequence as 4629 Da (Fimland et al., 1996).

Antimicrobial activity was completely lost after treatment of the K34 cell-free supernatant with proteinase K and trypsin at concentrations of 0.1 and 1 mg/ml (Table 2). Treatment with pepsin at 0.1 mg/ml reduced the activity levels of the bacteriocin by 44% (only against *L. monocytogenes* target strains), though 1 mg/ml caused complete elimination of activity (Table 2). Some bacteriocins produced by different *Pediococcus* spp. are known to show a slight resistance to proteolytic enzymes (Todorov & Dicks, 2009). No change in activity was recorded when treated with lipase and just a slight difference when treated with catalase (12% reduction of activity against *E. faecalis* ATCC 29212), indicating that H_2O_2 has just a small role in inhibition (Table 2).

BacPPK34 remained relatively stable after incubation for 1 h at pH 4–6 (Table 2). Antimicrobial activity was strongly reduced at pH values of 2.0 (22% decrease against *L. monocytogenes*) and above 8.0 (≥22% against *L. monocytogenes*; no remaining activity against *E. faecalis* ATCC 29212), suggesting that the peptides are sensitive to acidic and alkaline conditions. Similar results have been reported for pediocin PA-1 (Albano, Todorov et al., 2007; Gonzalez & Kunka, 1987). Treatments in the range of 60–80 °C had almost no effect

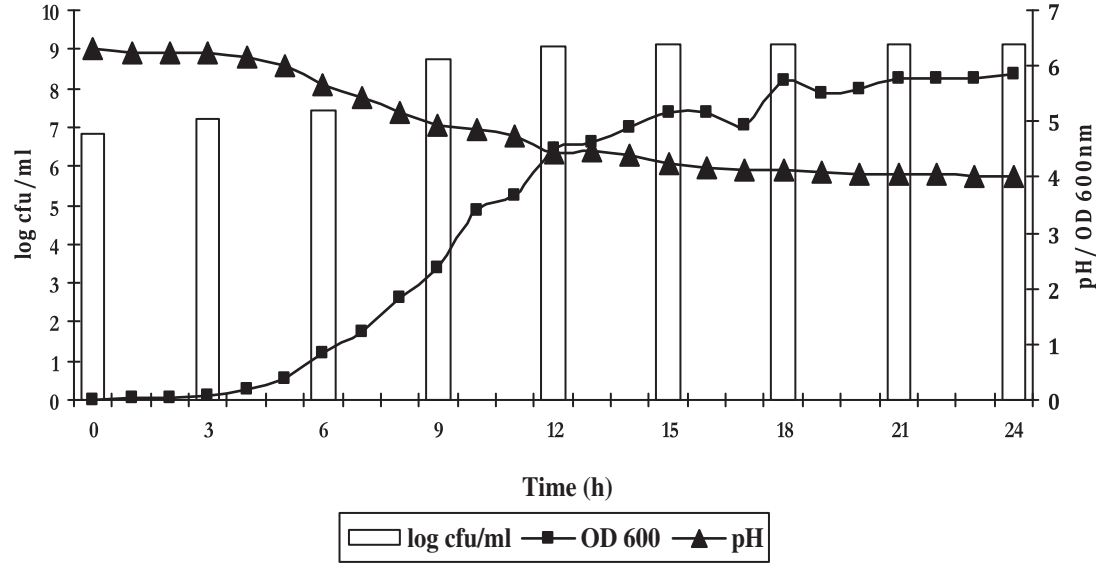

Fig. 2. Growth kinetics and changes in optical density and pH during 24 h of incubation (37 °C).

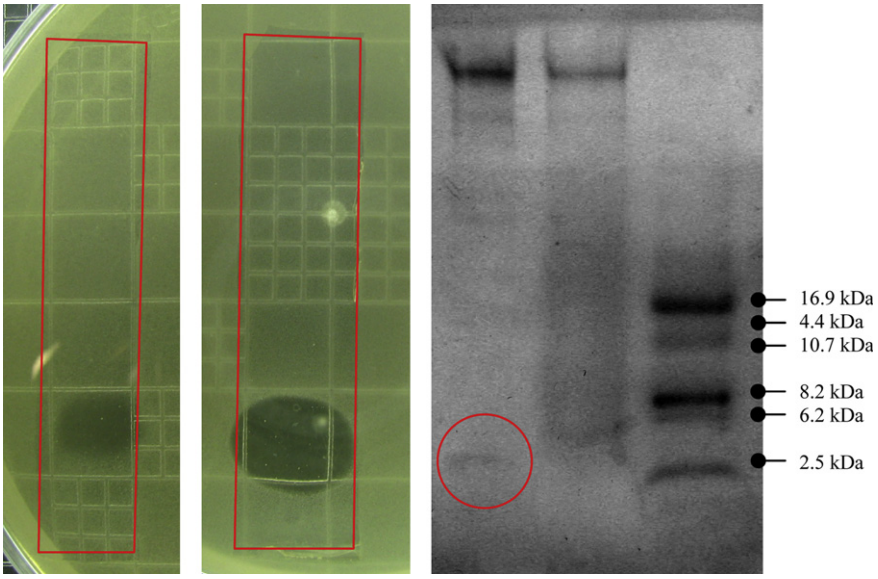

Fig. 3. Tricine-SDS-Gel and overlay (lines = edges of gel). Left: *E. faecalis* ATCC 29212 overlay; Middle: *L. monocytogenes* (L2) overlay; Right: stained gel.

on the antimicrobial activity. Starting from 100 °C to 121 °C (15 min) the antimicrobial activity decreased gradually or disappeared completely (against *E. faecalis* ATCC 29212). Remarkably it could still be recorded against the listerial target strains (L1, L2, and L3) even after 15 min at 121 °C. Similar results have been reported for other bacteriocins of *Pediococcus* spp. (Todorov & Dicks, 2009). Storage of active K34 supernatant for 1 month (12,800 AU/ml against L1, L2, L3; 3200 AU/ml against *E. faecalis* ATCC 29212) at 4 and −20 °C, respectively, did not inactivate the bacteriocin. Storage at −20 °C, proved to provide better conservation conditions, since the activity was lowered only 6% at most. Treatment with different surfactants did not affect the antagonistic activity bacPPK34. SDS, Tween 20, Triton X-100, Urea and NaCl in final concentrations of 1% (w/v) and EDTA in concentrations of 1 mM, 2 mM and 5 mM did not lower the inhibition caused by the K34 supernatant (Table 2). Similar results were reported for pediocins AcH (Todorov & Dicks, 2009) and ALP57 (Pinto et al., 2008).

Table 2
Antimicrobial activity of bacPPK34, after incubation in different conditions (activity expressed in percentage values %).

		bacPPK34	
		L. monocytogenes L1, L2, L3 (%)	*E. faecalis* ATCC 2912 (%)
pH	2	78	100
	4	89	100
	6	94	100
	8	78	0
	10	67	0
	12	44	0
Temp.	4 °C, 1 month	89	87.5
	25 °C, 1 h	100	100
	30 °C and 37 °C, 1 h	100	100
	60 °C, 1 and 2 h	89	100
	80 °C, 1 and 2 h	94	100
	99 °C, 1 and 2 h	83, 78	0
	121 °C, 15 min.	72	0
	− 20 °C, 1 month	94	100
Enzymes	$ProteinaseK_{1.0 \text{ and } 0.1 \text{ mg/ml}}$	0	0
	$Pepsin_{1.0 \text{ mg/ml}}$	0	0
	$Pepsin_{0.1 \text{ mg/ml}}$	56	0
	$Trypsin_{1.0 \text{ and } 0.1 \text{ mg/ml}}$	0	0
	$Catalase_{1.0 \text{ and } 0.1 \text{ mg/ml}}$	100	88
	$Lipase_{1.0 \text{ and } 0.1 \text{ mg/ml}}$	100	100
Detergents	Tween 20	100	100
	Triton X−100	100	100
	SDS	100	100
	$EDTA_{1, 2 \text{ and } 5 \text{ mM}}$	100	100
	Urea and NaCl	100	100

Addition of bacPPK34 (12,800 AU/ml, 3200 AU/ml respectively) to mid-log cultures of *L. monocytogenes* L2 and *E. faecalis* ATCC 29212 ($OD_{600} \approx 0.6$) inhibited the growth for at least 7 h (Fig. 4). Similar results were recorded in treatment with lower concentrations of log-phase cultures of *L. monocytogenes* L2 and *E. faecalis* ATCC 29212 with bacPPK34 (data not shown). Similar results were obtained by Barrena-Gonzales, Huot, and Petitdemange (1996) and Albano, Todorov et al., (2007).

No bacteriocin activity was detected in the cell suspension after treatment of strain K34 in 100 mM NaCl pH 2.0 (data not shown), suggesting that the bacteriocins did not adhere to the surface of the producer cells. Similar results were reported for plantaricin ST31 (Todorov et al., 1999), bozacin B14 (Ivanova, Kabadjova, Pantev, Danova, & Dousset, 2000), pediocin ST18 (Todorov & Dicks, 2005b), and pediocin 05-10 (Huang et al., 2009).

Most bacteriocins (pediocin and pediocin-like produced by *Pediococcus* spp.) are tagged listeria-active, are thermostable and range within 2867–4685 Da in size (Bauer, Chikindas, & Dicks, 2005; Diep, Godager, Brede, & Nes, 2006; Fimland, Sletten, & Nissen-Meyer, 2002; Papagianni & Anastasiadou, 2009). *P. pentosaceus* is able to produce several bacteriocins (Pinto et al., 2008), also including pediocin AcH/PA-1 (Bagenda et al., 2008), being one of the best studied bacteriocins (Chen & Hoover, 2003; Nieto-Lozano et al., 1992). Amplification of DNA of strain K34 with specific-primers for pediocin PA-1 did not yield the specific gene fragment of the bacteriocin (data not shown). Further studies are needed to characterize and identify the present bacteriocin.

Research on the technological and food safety properties of strain *P. pentosaceus* K34 would determine if it has potential as a commercial bioprotective culture in the production of fermented meat sausages. Bacteriocinogenic LAB strains, originally isolated from traditional sausages, are probably the best candidates for improving the microbiological safety of these foods, as they should be better adapted to the specific microenvironment. Their antimicrobial

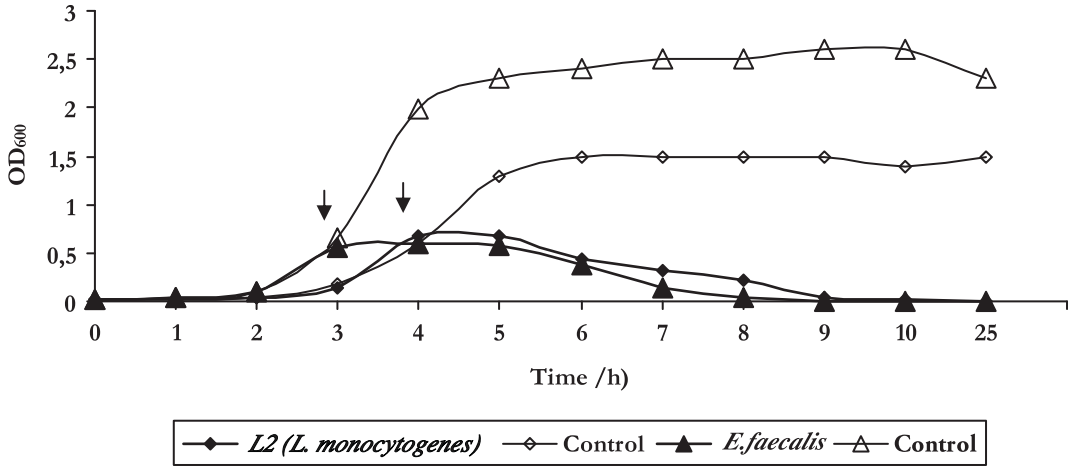

Fig. 4. Activity effect of bacPKK34 on *L. monocytogenes* (L2) (◆) and *E. faecalis* ATCC 29212 (▲). (◇ and △) represent the growth of the test organisms without added bacteriocin (controls). The arrow indicates the point at which the bacteriocin was added (12800 AU/ml for *L. monocytogenes* and 3200 AU/ml for *E. faecalis*).

compounds may be weapons to improve the safety of these products, while answering the need for effective bio-preservation techniques.

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