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ORIGINAL ARTICLE

Characterization of a novel bacteriocin produced by *Lactobacillus plantarum* ST8SH and some aspects of its mode of action

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Abstract Bacteriocins produced by lactic acid bacteria (LAB) are ribosomally synthesized antimicrobial peptides, with a diverse mode of bactericidal activity. This study focused on characterization of the bactericidal activity of bacteriocin ST8SH, with special attention to control of *Listeria* and *Enteroccus* species. *Lactobacillus plantarum* ST8SH produces a bacteriocin of the pediocin PA-1 family (sharing 96 % similarity on genetic level) with activity against several LAB, *Enterococcus* spp., *Klebsiella pneumoniae*, *Listeria* spp., *Streptococcus* spp. and some other human and foodborne pathogens. Addition of bacteriocin ST8SH to exponential or stationary phase cultures of *L. monocytogenes* ScottA and *E. faecalis* ATCC 19433 inhibited growth for 12 h. The effects of bacteriocin ST8SH on *L. monocytogenes* ScottA and *E. faecalis* ATCC 19433 were recorded indirectly based on enzyme, protein and nucleotide material leakage. Considering the antimicrobial activity of bacteriocin ST8SH against the tested microorganisms, and the physiological characteristics of *Lb. plantarum* ST8SH, either the bacteriocin or the strain may be used as tools for biopreservation.

Keywords *Lactobacillus plantarum* · *Enterococcus faecalis* · *Listeria monocytogenes* · Anti-*Listeria* and anti-*Enterococcus* activity · Bacteriocins

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Introduction

Bacteriocins are ribosomally synthesized antibacterial peptides that are usually active against genetically related species (Jack et al. 1995). They have been grouped into four classes based on their structure and mode of action (Heng et al. 2007). In the last two decades several reports have focused on the production of bacteriocins from lactic acid bacteria (LAB) isolated from different ecological niches, including fermented products, vegetables, fruits, meat, fish, human and animal gastrointestinal tract (GIT) (Cotter et al. 2005).

The preservation of meat products is increasingly directed towards biocontrol using bacteriocinogenic *Lactobacillus* species as protective microbiota to inhibit the growth of *Listeria monocytogenes* and other undesirable microorganisms (Bredholt et al. 2001; Mataragas et al. 2003; Castellano et al. 2004). Numerous strains of bacteriocinogenic *Lactobacillus plantarum* have been isolated in the last two decades and have been reviewed by Todorov (2009). However, most of these bacteriocins have not been fully characterized.

For proper usage as biopreservatives, the bacteriocins must be characterized by biochemical and molecular methodologies. Bacteriocin production does not always correlate with an increase in cell mass or growth rate of the producer strain (Kim et al. 1997). Higher bacteriocin levels are often recorded in the absence of growth-stimulating nutrients, or at temperatures and pH conditions lower than required for optimal growth (Krier et al. 1998; Todorov et al. 2000). Optimal bacteriocin production is often detected in a medium with limiting concentrations of sugars, nitrogen sources, vitamins and potassium phosphate, or when the medium pH is regulated (Todorov and Dicks 2005).

The objective of this study was to characterize the bacteriocin ST8SH, produced by *Lb. plantarum* strain ST8SH isolated from Bulgarian salami, with the aim of using this strain as a biopreservative culture in fermented food products for control of *L. monocytogenes* and/or *Enterococcus* spp.

Materials and methods

Screening for bacteriocinogenic strains from Shpek

Isolation of bacteriocinogenic strains

"Shpek" is a Bulgarian type of fermented dry or semi-dry sausage produced from mixture of pork and beef meat. Traditionally this product is produced without addition of specific starter cultures. Samples of "shpek" (12 products, manufactured by different producers) were obtained from a local supermarket (Belogratchik, Bulgaria), and 20 g of each sample was cut into small pieces with a sterile scalpel and homogenized with 180 mL 0.85 % (w/v) NaCl in a stomacher (Stomacher® 400 circulator, Sevard, France) for 2 min. For isolation of bacteriocinogenic LAB, samples were surface-plated on agar (1 %) and covered with 10 mL MRS (BD Difco, Franklin Lakes, NJ, USA) supplemented with 1 % agar. After formation of the individual colonies (incubation for 48 h at 37 °C) BHI (Difco) supplemented with 1 % agar and inoculated with *L. monocytogenes* ScottA or *E. faecalis* ATCC 19433 at 10^6 colony forming units (CFU)/mL was added as a third level. These plates were then incubated for 24 h at 37 °C. Colonies with visual inhibition zones were selected for future study. Isolation and cultivation in MRS broth were followed by surface plating on MRS agar. Isolated colonies were identified by morphology, Gram staining and biochemical tests. Gram-positive, non-sporulating, catalase-negative and oxidase-negative isolates were considered as LAB. Pure cultures were maintained at −80 °C in MRS broth supplemented with 30 % (v/v) glycerol. All other strains used as control strains or target microorganisms in antimicrobial tests were cultured in the recommended growth medium and stored at −80 °C in presence of 30 % (v/v) glycerol (Table 1). Before use, all cultures were activated by successive transfer in their respective culture media at 30 °C or 37 °C (Table 1).

Isolated cultures (24 in total) were grown in MRS broth at 37 °C for 24 h. The pH of the cell-free supernatant obtained after centrifugation (8000*g*, 10 min, 4 °C) was adjusted to pH 6.0 with 1 M NaOH, after which it was heat-treated (80 °C for 10 min) and tested for potential bacteriocin production against the indicator strains *L. monocytogenes* ScottA and *E. faecalis* ATCC 19433, according the protocol described by Todorov (2009).

Table 1 Spectrum of activity recorded for bacteriocin ST8SH

Test microorganism	Bacteriocin activity
Enterococcus faecalis	8/8[a]
Enterococcus faecium	6/9
Enterococcus mundtii	1/1
Escherichia coli	0/2
Klebsiella pneumoniae	1/3
Lactobacillus paracasei	2/2
Lactobacillus paraplantarum	0/2
Lactobacillus pentosus	2/2
Lactobacillus plantarum	5/7
Lactobacillus sakei	3/4
Lactobacillus salivarius	2/2
Lactobacillus delbruekii	0/2
Lactobacillus fermentum	0/2
Lactobacillus curvatus	2/5
Lactococcus lactis subsp. *lactis*	3/3
Leuconostoc mesenteroides	0/3
Listeria innocua	4/4
Listeria monocytogenes	103/108
Listeria ivanovii subsp. *ivanovii*	1/1
Pediococcus acidilactici	0/3
Staphylococcus aureus	0/5
Streptococcus infantarius	2/2
Streptococcus agalactiae	0/2
Streptococcus caprinus	2/2

[a] Sensitive strains / total tested strains

Identification of bacteriocin-producing isolates

LAB isolates that showed bacteriocin production against *L. monocytogenes* ScottA or *E. faecalis* ATCC 19433 were differentiated by RAPD-PCR and identified by 16S rRNA sequencing analysis. Total genomic DNA was extracted and purified using the ZR Fungal/Bacterial DNA Kit (Zymo Research, Irvine, CA), according to the manufacturer's protocol. Total DNA was used for differentiation of isolates according to Todorov et al. (2010) with primers OPL-02, OPL-04, OPL-14 and OPL-20. DNA from unique isolates was used as a template for 16S rRNA amplification using universal bacterial primers 8F and R1512 (Felske et al. 1997). PCR products have been purified by QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) and the fragments obtained were sequenced at the Center for Human Genome Studies, Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, Brazil, and compared to sequences available at

GenBank using BLAST (Basic Local Alignment Search Tool, http://www.ncbi.nlm.nih.gov/BLAST).

Characterization of bacteriocin produced by ST8SH

Lactobacillus plantarum ST8SH

Lb. plantarum ST8SH (selection based on the preliminary antimicrobial test and genetic identification) was grown in MRS broth at 37 °C for 24 h. Cell-free supernatant was obtained as described above.

Effects of enzymes, pH, detergents and temperature on bacteriocin stability

To determine and confirm the proteinaceous nature of the active component, the cell-free supernatant was treated with trypsin, proteinase K, pronase, α-amylase and catalase (Sigma, St. Louis, MO), at final concentrations of 0.1 mg/mL and 1 mg/mL and incubated at 37 °C for 2 h. After enzymatic treatment of cell-free supernatants, enzymes were inactivated by thermal treatment (98 °C for 3 min) and the samples tested for presence of antimicrobial activity against *L. monocytogenes* ScottA and *E. faecalis* ATCC 19433 by agar spot on the low assay as described by Todorov (2009).

The effect of the pH on the stability of the antimicrobial substance(s) was investigated by treatment of cell-free supernatant obtained from *Lb. plantarum* ST8SH as described before, and submitted to pH treatment with sterile solutions of 5 *N* NaOH or 5 *N* HCl to reach a range of pH-values from 4.0 to 11.0 with 1.0 intervals. After incubation at each of these pH-values for 1 h, the samples were re-adjusted to pH 6.0 and the antimicrobial activity was determined against *L. monocytogenes* ScottA and *E. faecalis* ATCC 19433 as described by Todorov (2009).

The influence of different temperatures (15, 30, 37, 60, 80 and 100 °C for 10, 30 and 60 min, respectively, and 121 °C for 15 min) on the antimicrobial activity of the cell-free supernatant was also tested. After incubation for 1 h, the samples were re-adjusted to pH 6.0 and the antimicrobial activity was determined against *L. monocytogenes* ScottA or *E. faecalis* ATCC 19433 as described before.

Sterile MRS broth treated with the particular enzyme, pH and temperature and untreated samples were used as controls. All investigations were performed as three independent experiments.

Identification of genes involved in production of bacteriocin by ST8SH

Total DNA from *Lb. plantarum* ST8SH was isolated using the ZR Fungal/Bacterial DNA Kit following the instructions of the manufacturer and subjected to PCR using the primers Pedpro and Pedc1041 (Table 2), designed on the basis of information related to the operon encoding Pediocin PA-1/AcH (accession number M83924) (Todorov et al. 2010). PCR reactions were performed using the GeneAmp® PCR Instrument System 9700 (Applied Biosystems, Foster City, CA) with the following conditions: an initial denaturation step of 94 °C for 1 min, followed by 35 cycles of 1 min at 94 °C, 30 s at 50 °C and 1 min at 72 °C, and final extension at 72 °C for 5 min. The amplified product was visualized in a 1.0 % (w/v) agarose gel stained with ethidium bromide (Sigma). A band of interest, corresponding to the correct size of Pediocin PA-1/AcH, was purified from the gel using the QIAquick PCR purification kit (Qiagen) and DNA fragments were sequenced in an automatic sequencer at the Center for Human Genome Studies, Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, Brazil. Sequences were analyzed using database of GenBank using the BLAST algorithm (http://www.ncbi.nlm.nih.gov/BLAST).

In addition to previous descriptions, total DNA from *Lb. plantarum* ST8SH was tested for presence of genes encoding plantaricin NC8 (primers: planNC8-F and planNC8-R; Maldonado et al. 2003), plantaricin S (primers: planS-F and planS-R; Stephens et al. 1998), plantaricin W (primers: planW-F and planW-R; Holo et al. 2001) and nisin (primers: nis-F and nis-R; Kruger et al. 2013) (Table 2).

Production of bacteriocin by Lactobacillus plantarum ST8SH

Lb. plantarum ST8Sh was cultured overnight in MRS broth at 30 °C and used to inoculate three flasks with 200 mL MRS broth each. These cultures were grown until stationary phase at 25 °C, 30 °C and 37 °C, respectively, for 36 h. Bacterial growth was monitored by turbidity measurements of the cultures at 600 nm (UV-VIS Spectrophotometer, BEL Photonics, Monza, Italy) every hour. In addition, changes in pH were recorded. Every 3 h, samples were drawn for determination of the production of bacteriocin, expressed in AU/mL, against *L. monocytogenes* Scott A and *E. faecalis* ATCC 19433 as described by Todorov (2009).

Adsorption of bacteriocin produced by Lactobacillus plantarum ST8SH to producer cells

Adsorption of bacteriocin produced by *Lb. plantarum* ST8SH to its own producer cells was studied according to the method proposed by Yang et al. (1992). *Lb. plantarum* ST8SH was grown in 200 mL MRS broth for 24 h at 37 °C, followed by adjustment of the pH to 6.5 with 10 M NaOH and harvesting of the cells by centrifugation (5000*g*, 4 °C, 15 min). Cells were washed three times with 100 mM phosphate buffer at pH 6.5 and resuspended in 20 mL 100 mM NaCl, pH 2.0 and stirred for 2 h at 4 °C. Cells were separated by centrifugation (5000*g*, 4 °C, 15 min) and the pH of the supernatant adjusted

Table 2 Primers used in this study

Primer	Primer	Sequence (5′-3′)	Reference
RAPD-PCR	OPL-02	TGGGCGTCAA	Todorov et al. 2010
	OPL-04	GACTGCACAC	
	OPL-14	GTGACAGGCT	
	OPL-20	ACCCGGTCAC	
16s rRNA	8F	CACGGATCCAGACTTTGAT(C/T)(A/C)TGGCTCAG	Felske et al. 1997
	R1512	GTGAAGCTTACGG(C/T)TAGCTTGTTACGACTT	
pediocin PA-1	Pedpro	CAAGATCGTTAACCAGTTT	Todorov et al. 2010
	Pedc1041	CCGTTGTTCCCATAGTCTAA	
plantaricin S	planS-F	GCCTTACCAGCGTAATGCCC	Stephens et al. 1998
	planS-R	CTGGTGATGCAATCGTTAGTTT	
plantaricin NC8	planNC8-F	GGTCTGCGTATAAGCATCGC	Maldonado et al. 2003
	planNC8-R	AAATTGAACATATGGGTGCTTTAAATTCC	
plantaricin W	planW-F	TCACACGAAATATTCCA	Holo et al. 2001
	planW-R	GGCAAGCGTAAGAAATAAATGAG	
nisin	nis-F	ATGAGTACAAAAGATTTCAACTT	Kruger et al. 2013
	nis-R	TTATTTGCTTACGTGAACGC	

to 6.5 with 10 M NaOH. Bacteriocin activity was determined at all stages of the experiment against *L. monocytogenes* ScottA and *E. faecalis* ATCC 19433.

Isolation and partial purification of bacteriocin produced by Lactobacillus plantarum ST8SH

Lb. plantarum ST8SH was cultured in 200 mL MRS broth (Difco) for 24 h at 37 °C. The cells were harvested (8000*g*, 10 min, 4 °C), the cell-free supernatant was adjusted to pH 6.0 with 1 M NaOH, heat-treated (80 °C for 10 min) and the bacteriocin ST8SH precipitated with 60 % ammonium sulfate (4 h at 4 °C with permanent mixing). The obtained precipitate (10,000*g*, 60 min, 4 °C) was re-suspended in 20 mL 25 mM ammonium acetate (pH 6.5) and the level of antimicrobial activity determined using the indicator strains *L. monocytogenes* ScottA and *E. faecalis* ATCC 19433.

Total proteins were pre-separated by reversed-phase chromatography on SepPak C18 columns (Waters, Milford, MA, USA), eluted with ammonium acetate buffer (25 mM, pH 6.5) containing increasing concentrations of iso-propanol (20 %, 40 %, 60 % and 80 %). Bacteriocin activity of each fraction was tested against *L. monocytogenes* ScottA and *E. faecalis* ATCC 19433. Fractions showing activity were pooled and dehydrated under reduced pressure (Speed-Vac, Savant, France) and stored at −20 °C. For future experiments material was redissolved in sterile ultra-pure water.

Molecular weight of bacteriocin produced by Lactobacillus plantarum ST8SH

Separation of the bacteriocin produced by *Lb. plantarum* ST8SH in Tris-Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed in continuous gradient gel designed for low molecular weight proteins as described by Schägger and Van Jagow (1987). A standard low molecular weight marker ranging from 2.5 to 45.0 kDa (Amersham Biosciences, Freiberg, Germany) was used. The position of the bacteriocin was determined by antagonistic test with *L. monocytogenes* ScottA (10^5–10^6 CFU/mL) as described by Todorov et al. (2010).

Mode of action of bacteriocin produced by *Lactobacillus plantarum* ST8SH

Growth of the test-microorganisms in presence of bacteriocin produced by Lactobacillus plantarum ST8SH

A 20 mL aliquot of bacteriocin-containing filter-sterilized (0.20 μm, Minisart®, Sartorius, Bohemia, NY, USA) supernatant (pH 6.0) was added to 100 mL culture of *L. monocytogenes* ScottA and *E. faecalis* ATCC 19433 in the early exponential phase (OD_{600nm}=0.052) and incubated for 12 h. Optical density readings (at 600 nm) were recorded at 1-h intervals.

Cell lysis of test microorganisms in presence of bacteriocin produced by Lactobacillus plantarum ST8SH

Cell lysis was measured using sterile flat-bottom 96-well TPP (Zellkultur Testplatte, Trasadingen, Switzerland). Cells of *L. monocytogenes* ScottA and *E. faecalis* ATCC 19433 were obtained by centrifugation (5000*g*, 4 °C, 15 min) of 20 mL 24-h cultures that were washed and re-suspended in 10 mL potassium phosphate buffer (20 mM, pH 6.5). Each bacterial suspension (100 μL) was placed individually in microtiter plate wells and 50 μL potassium phosphate buffer containing semi-purified bacteriocin ST8SH (as previously described) at different two-fold serial dilutions. Plates were incubated at 37 °C for up to 24 h. Absorbance at 655 nm was measured

using a Microplate reader (VERSA*max* Microplate Reader, Molecular Devices, Sunnyvale, CA, USA). The percentage of cell lysis was calculated as [100 – (At/Ao × 100)], where Ao and At were absorbance measured at 0 and 3, 9 and 18 h of incubation, respectively.

Determination of cell lysis by measuring the levels of intracellular materials

Extracellular levels of β-galactosidase activity were monitored. An 11-h-old culture of 20 mL *L. monocytogenes* ScottA (100 mL) was harvested (5 000*g*, 4 °C, 15 min) and the cells were washed twice with 0.03 M sodium phosphate buffer (pH 6.5) and re-suspended in 20 ml of the same buffer. Equal volumes of partial purified bacteriocin (60 % iso-propanol fraction, 12 800 AU/mL) and cell suspension of *L. monocytogenes* ScottA (2 mL) were mixed for 5 min at 25 °C, followed by the addition of 0.2 mL 0.1 M ONPG (*O*-nitrophenyl-β-D-galactopyranoside, Sigma) in 0.03 M sodium phosphate buffer (pH 6.8). After 10 min at 37 °C, the reaction of β-galactosidase was stopped by the addition of 2.0 mL 0.1 M sodium carbonate. The cells were harvested (5000*g*, 15 min, 25 °C) and absorbance readings of the supernatant recorded at 420 nm. Cells of *L. monocytogenes* Scott A cultured under the same conditions that were mechanically disrupted with 0.1 mm diameter glass beads (vortexed for 5 min) served as controls. All experiments were conducted in duplicate at two independent stages, also with *E. faecalis* ATCC 19433 as test microorganism.

In addition, in order to investigate the effect of bacteriocin produced by *Lb. plantarum* ST8SH on cell integration, cells from 20 mL of 11-h cultures of *L. monocytogens* ScottA and *E. faecalis* ATCC 19433 were harvested by centrifugation (5000*g*, 4 °C, 15 min), followed by washing with sterile 5 mM phosphate buffer (pH 6.0). Bacteriocin ST8SH (60 % iso-propanol fraction, 12 800 AU/mL) was added at a ratio of 0.1:1.0 to a cell suspension of *L. monocytogens* ScottA and *E. faecalis* ATCC 19433 and the mixture incubated at 37 °C for 60 min. Cells were removed after centrifugation (5 000*g*, 4 °C, 15 min) and cell-free supernatant was filtered thought a 0.20 μm membrane (Minisart®, Sartorius). Absorbance readings of the presence of liberated intracellular material were recorded at 260 nm. *L. monocytogens* ScottA and *E. faecalis* ATCC 19433 suspended in 5 mM phosphate buffer without bacteriocin STSH served as controls, while a third control comprised the same buffer containing bacteriocin ST8SH without cells of the two mentioned test microorganisms. All experiments were performed in duplicate on three independent occasions.

Reduction of viable cells of test microorganisms in the presence of bacteriocin produced by Lactobacillus plantarum ST8SH

Early stationary phase (18 h) culture of *L. monocytogenes* ScottA and *E. faecalis* ATCC 19433 was harvested (5000*g*, 5 min, 4 °C), washed twice with sterile saline water and re-suspended in 10 mL saline water. Equal volumes of the cell suspensions and filter-sterilized (0.20 μm, Minisart®, Sartorius) bacteriocin produced by *Lb. plantarum* ST8SH containing cell-free supernatant were mixed. Viable cell numbers were determined before and after incubation for 1 h at 37 °C by plating onto MRS agar. Cell suspension of *L. monocytogenes* ScottA and *E. faecalis* ATCC 19433 with no bacteriocin added served as a control.

Adsorption of bacteriocin produced by Lactobacillus plantarum ST8SH to test microorganisms

Adsorption to sensitive and resistant test-microorganisms Adsorption to target cells (Table 1) was tested according to Todorov (2008). The bacterial strains (*L. innocua* 2030C, *L. ivanovii* subsp. *ivanovii* ATCC 19119, *L. monocytogenes* ScottA and *E. faecalis* ATCC 19433) were grown overnight in BHI broth at 37 °C to OD_{600nm}=0.1–0.2 and the cells harvested (5000*g*, 15 min, 4 °C). Cells were washed twice in 5 mM phosphate buffer (pH 6.5) and re-suspended in the same buffer to the original volume. Each cell suspension (0.7 mL) was mixed with an equal volume of bacteriocin produced by *Lb. plantarum* ST8SH and incubated at 37 °C for 1 h. The cells were harvested (5000 *g* for 15 min) and activity of unbound bacteriocin produced by *Lb. plantarum* ST8SH in the cell-free supernatant determined using the agar-spot method as described above. Adsorption of bacteriocin produced by *Lb. plantarum* ST8SH to the target cells was calculated according to the following formula: % Adsorption = 100 – [(bacteriocin ST8SH activity after treatment/original bacteriocin ST8SH activity) x 100]. The experiment was performed in triplicate.

Effect of pH and temperature on adsorption of bacteriocin produced by Lactobacillus plantarum ST8SH

Cells suspensions of *L. innocua* 2030C, *L. ivanovii* subsp. *ivanovii* ATCC 19119, *L. monocytogenes* ScottA and *E. faecalis* ATCC 19433 were prepared as described in the previous paragraph, but resolved in sterile physiological water and the pH was corrected to values between 2.0 and 10.0 with sterile 1 M NaOH or 1 M HCl (Todorov 2008). Bacteriocin produced by *Lb. plantarum* ST8SH was added to the cell suspension and incubated at 4, 10, 25, 30, 37, 45 and 60 °C for 1 h. The cells were harvested (5000*g*, 15 min, 25 °C) and the pH of the supernatants adjusted to 6.0 with sterile 1 M NaOH or 1 M lactic acid. Bacteriocin activity was determined

as described before. All experiments were performed on three independent occasions in duplicate.

Effect of surfactants, inorganic salts and organic compounds on the adsorption of bacteriocin produced by Lactobacillus plantarum ST8SH

Cell suspensions of *L. innocua* 2030C, *L. ivanovii* subsp. *ivanovii* ATCC 19119, *L. monocytogenes* ScottA and *E. faecalis* ATCC 19433 were prepared as described before and treated with 1 % (*w/v*) Tween 80, Tween 20, ascorbic acid, potassium sorbate, sodium nitrate and 0.5, 1.0, 1.5 and 2.0 % sodium chloride (Todorov 2008). The pH of each suspension was adjusted to 6.5 with 1 M NaOH or 1 M HCl. Bacteriocin produced by *Lb. plantarum* ST8SH was added to the treated cells, as before, and incubated at 37 °C for 1 h. The cells were harvested (5000 *g*, 15 min, 25 °C) and activity of the cell-free supernatant was determined as before. Duplicate experiments were performed on three independent occasions.

Growth and bacteriocin production by *Lactobacillus plantarum* ST8SH in a mixed culture with *Listeria monocytogenes* ScottA

Overnight cultures of *Lb. plantarum* ST8SH and *L. monocytogenes* Scott A were inoculated into MRS broth at concentrations of 2.0 % (v/v) and 0.1 % (v/v), respectively. Low cell numbers of *L. monocytogenes* Scott A were used to simulate conditions normally expected in food contamination. *Lb. plantarum* ST8SH of 10^7 CFU/mL was chosen to represent optimal cell numbers during application in food fermentation processes involving starter cultures. The mixed culture was incubated at 37 °C for 32 h. At different time intervals samples were taken to follow changes in pH while cell numbers of *L. monocytogens* Scott A were determined on listeria enrichment broth (LEB) and of total cell numbers on MRS agar. Bacteriocin production was determined by using the agar-spot method as described previously. The experiment was repeated three times.

Results

The screening for potential bacteriocin producers among bacterial isolates from 12 different branded "shpek" samples, enabled the selection of 24 strains with inhibitory activity against *L. monocytogenes* ScottA or *E. faecalis* ATCC 19433. All selected strains were Gram-positive, catalase-negative and presented rod-shaped morphology, and in fact originated from only two samples. However, after comparing the generated profile obtained from RAPD-PCR analysis with four different primers, it was found that most of the isolates were replicas of the same strain so that only three stains were finally selected (data not shown). Based on the inhibitory analysis and activity exhibited against *L. monocytogenes* ScottA or *E. faecalis* ATCC 19433, strain ST8SH was selected for future study.

No significant differences in growth and production of bacteriocin ST8SH were observed when the *Lb. plantarum* ST8SH was cultured for 24 h in MRS broth at 26, 30 or 37 °C (Fig. 1). At these three different cultivation temperatures, the recorded antimicrobial activity was 25,600 AU/mL against *L. monocytogenes* ScottA and *E. faecalis* ATCC 19433, with a very small variation in reaching the maximal activity.

The bacteriocin produced by ST8SH was active against food spoilage bacteria and foodborne pathogens as listed in Table 1. Similar results were recorded for the cell-free supernatant and for the semi-purified bacteriocin (60 % isopropanol fraction).

In the test for identification of genes encoding bacteriocin(s) from total DNA extracted from *Lb. plantarum* ST8SH, we did not record positive results for any of the tested target genes for plantaricin W, plantaricin S, plantaricin NC8 or nisin. However, when PCR targeting the pediocin PA-1/AcH gene (PedPro and Pedc1041 primers) was performed, an amplicon corresponding in size to that of pediocin PA-1/AcH (not shown) was generated.

Based on the Tricine-SDS-PAGE results from the Coomassie Blue stained gel, and gel overlaid with *L. monocytogenes* Scott A, the molecular weight of bacteriocin ST8SH was estimated to be around 5 kDa.

Addition of bacteriocin ST8SH (obtained from a 24-h culture of *Lb. plantarum* ST8SH) to a 3-h culture of *L. monocytogenes* ScottA ($OD_{600nm}\approx0.075$), *Lb. sakei* ATCC 15521 ($OD_{600nm}\approx0.033$) and *E. faecalis* ATCC 19433 ($OD_{600nm}\approx0.068$) inhibited cell growth over a 12 h period (Fig. 2). When bacteriocin ST8SH was added to a 7-h-old test culture, similar inhibition of *L. monocytogenes* ScottA, *Lb. sakei* ATCC 15521 and *E. faecalis* ATCC 19433 was observed (data not shown). No viable cells were determined at 10 h or 12 h in cultures of *L. monocytogenes* ScottA, *Lb. sakei* ATCC 15521 and *E. faecalis* ATCC 19433 treated with bacteriocin ST8SH, pointing to the bactericidal mode of action of this bacteriocin.

The antagonistic effect of the semi-purified bacteriocin ST8SH (60 % iso-propanol fraction) could be observed as early as within 3 h of incubation, when *L. monocytogenes* ScottA, *Lb. sakei* ATCC15521 or *E. faecalis* ATCC19433 were cultured in MRS broth in the presence of concentrations of bacteriocin ST8SH ranging from 12,800 to 200 AU/mL (Fig. 3). For *L. monocytogenes* Scott A, this inhibitory effect, presented in percentage of the cell lyses, ranged from 10.33 % to 32.84 %, for *Lb. sakei* ATCC 15521 from 20.76 % to 39.64 %, and for *E. faecalis* ATCC 19433 from 31.19 % to 46.44 %, depending on the concentration of bacteriocin (from 200 to 12,800 AU/mL). A better inhibitory effect of

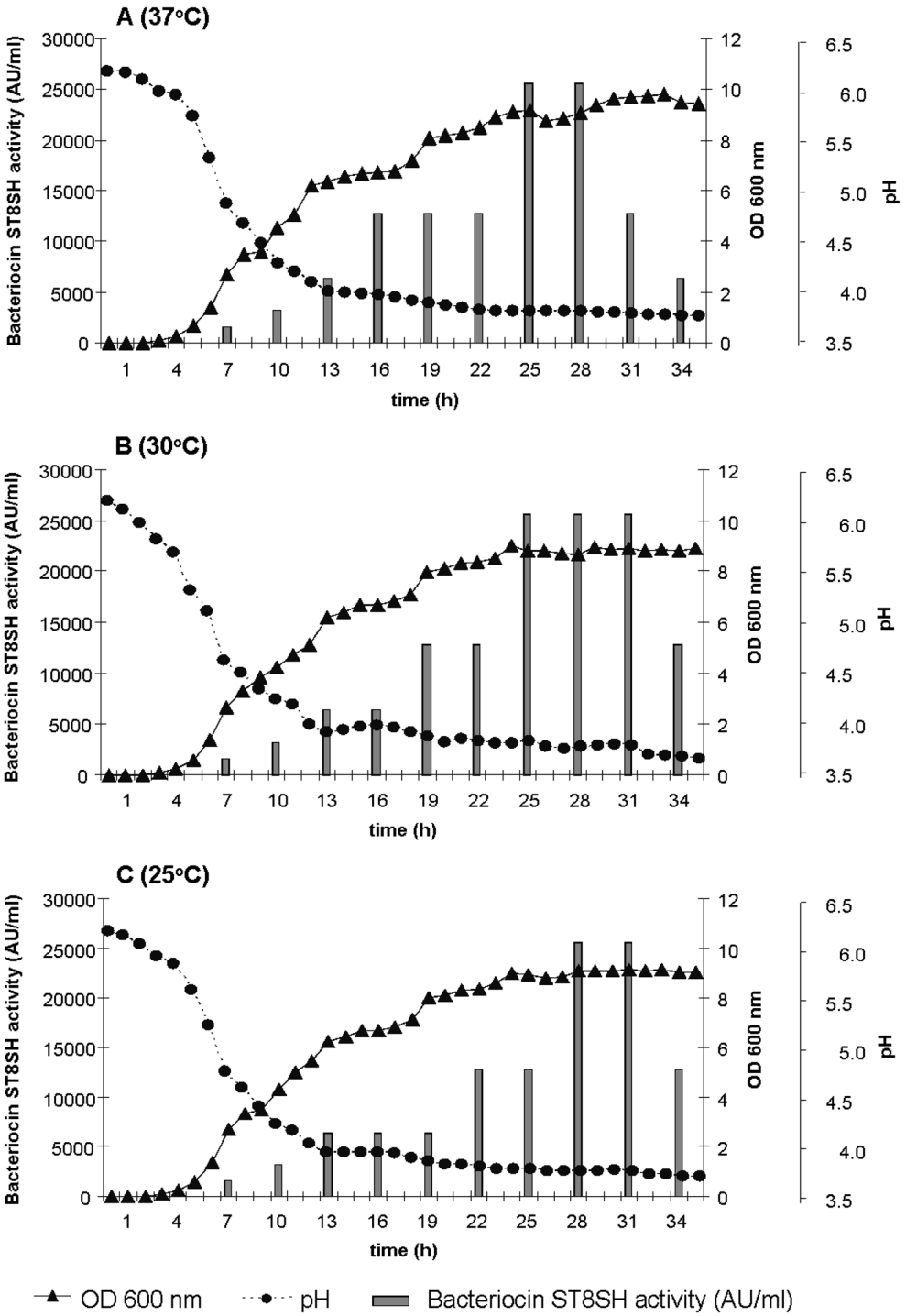

Fig. 1a–c Growth of *Lactobacillus plantarum* ST8SH in MRS broth (▲), changes in pH (●) and production of bacteriocin expressed in AU/mL (*bars*) at different temperatures. **a** 37 °C, **b** 30 °C, **c** 25 °C

bacteriocin ST8SH was observed after 9 and 18 h of incubation (Fig. 3). After 18 h, this inhibitory effect, expressed as percentage of cell lysis, varied from 85.27 % to 97.56 % for *L. monocytogenes* Scott A, from 86.61 % to 98.65 % for *Lb. sakei* ATCC 15521, and from 87.95 % to 99.74 % for *E. faecalis* ATCC 19433.

Treatment of cells of *L. monocytogenes* ScottA and *E. faecalis* ATCC 19433 with bacteriocin ST8SH resulted in leakage of β-galactosidase, DNA, RNA and proteins, detected at 420 nm and 260 nm, respectively (Table 3). Results obtained by DNA, RNA, protein and the β-galactosidase test (Table 3), confirmed that the bactericidal mode of action of bacteriocin ST8SH is via destabilization of cell membrane permeability.

Treatment of stationary phase cells of *L. monocytogenes* ScottA (10^{10} CFU/mL) and *E. faecalis* ATCC 19433

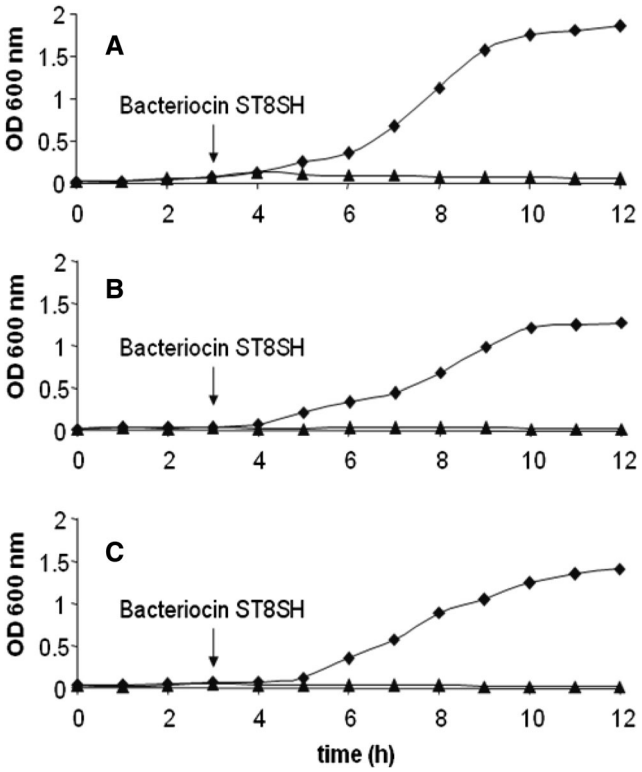

Fig. 2 Effect of bacteriocin ST8SH on exponentially growing **a** *Listeria monocytogenes* ScottA, **b** *Lactobacillus sakei* ATCC 15521 and **c** *Enterococcus faecalis* ATCC 19433. ♦ Without added bacteriocin, ▲ with added 10 % (v/v) bacteriocin ST8SH

(10^9 CFU/mL) with bacteriocin ST8SH resulted in bacterial killing. After 1 h of contact time, only 10^2 CFU/mL of viable cells of *L. monocytogens* ScottA and 10^1 CFU/mL viable cells of *E. faecalis* ATCC 19433 were detected (Table 4). No significant changes in cell numbers of *L. monocytogenes* Scott A and *E. faecalis* ATCC 19433 were recorded in the untreated (control) sample. Similar results were recorded when bacteriocin ST8SH was added to low concentrations of *L. monocytogenes* ScottA and *E. faecalis* ATCC 19433 cultures (data not shown).

Bacteriocin ST8SH adsorbed to both sensitive and non-sensitive cells of indicator bacteria (Table 5). Seventy-five percent of the peptide adsorbed to *L. innocua* 2030C, *L. ivanovii* subsp. *ivanovii* ATCC 19119, *L. monocytogenes* Scott A and *E. faecalis* ATCC 19433 when incubated at 37 °C.

Treatment of cells of *L. innocua* 2030C, *L. ivanovii* subsp. *ivanovii* ATCC 19119, *L. monocytogenes* ScottA and *E. faecalis* ATCC 19433 with bacteriocin ST8SH at 4–45 °C and at pH values 3.5, 5.5 and 7.0 led to significant changes in adsorption (Table 5). The highest levels of adsorption of bacteriocin ST8SH to *L. innocua* 2030C was recorded at pH 5.5 and 7.0 (75 %) and temperatures 15, 30 and 37 °C (75 %). When *L. ivanovii* subsp. *ivanovii* ATCC 19119 was used as the target strain for adsorption of bacteriocin ST8SH, the highest levels of adsorption were recorded at 45 °C (87.5 % adsorption) and pH 5.5 and 7.0 (75 % adsorption). For *L. monocytogenes* ScottA, 100 % adsorption was recorded at 45 °C and 75 % at pH 7.0. For the test organism *E. faecalis* ATCC 19433, 75 % adsorption was recorded at pH 7.0 and incubation temperatures of 15, 30, 37 and 45 °C (Table 5).

The presence of ascorbic acid and potassium sorbate did not interfere with the adsorption of bacteriocin ST8SH to *L. innocua* 2030C. However, NaCl, Tween 20 and Tween 80 reduced adsorption, whereas sodium nitrate increased adsorption to 87.5 % of bacteriocin ST8SH to *L. innocua* 2030C (Table 5). When *L. ivanovii* subsp. *ivanovii* ATCC 19119 and *L. monocytogenes* Scott A were used as target strains for adsorption of bacteriocin ST8SH, similar reduction of the interaction was observed in presence of Tween 20, Tween 80 and NaCl. However, ascorbic acid and potassium sorbate reduced the adsorption of bacteriocin ST8SH to *L. ivanovii* subsp. *ivanovii* ATCC 19119 and *L. monocytogenes* Scott A, pointing to the strain-specific importance of this interaction (Table 5). Adsorption of bacteriocin ST8SH to *E. faecalis* ATCC 19433 was reduced in the presence of Tween 20, Tween 80 and NaCl, as observed with *L. innocua* 2030C, *L. ivanovii* subsp. *ivanovii* ATCC 191198 and *L. monocytogenes* Scott A, but was not affected in the presence of ascorbic acid, potassium sorbate and sodium nitrate (Table 5).

Production of bacteriocin ST8SH increased to 51,200 AU/ml when the strain was cultured in the presence of either viable or dead cells of *L. monocytogenes*. High cell numbers of *Lb. plantarum* ST8SH and *L. monocytogenes* ScottA were recorded on MRS plates when co-cultured (Fig. 4). However, results recorded on selective LEB medium showed that the cell numbers of *L. monocytogenes* ScottA decreased from 1.42×10^4 to 1.02×10^2 CFU/mL within 9 h and to undetectable levels after 36 h (Fig. 4).

Discussion

Appropriate identification of LAB stains with potential for application in biopreservation is essential. Presently, 16S rRNA is considered as the gold standard in taxonomy. Strain ST8SH showed high similarity of 99 % to *Lb. plantarum* from GenBank and was therefore designated to this species. Previously, *Lb. plantarum* strains were isolated from various habitats, including meat, fish, dairy, plant products and also the GIT and reproductive organs of humans and animals (Todorov and Franco 2010). Some researchers have evaluated the application of different plantaricins to control the growth of the foodborne (opportunistic) pathogens *L. monocytogenes* and some *Enterococcus* spp. (Atrih et al. 2001; Powell et al. 2007; Todorov 2008).

Maximal antimicrobial activity of 25,600 AU/mL against *L. monocytogenes* ScottA and *E. faecalis* ATCC 19433 was

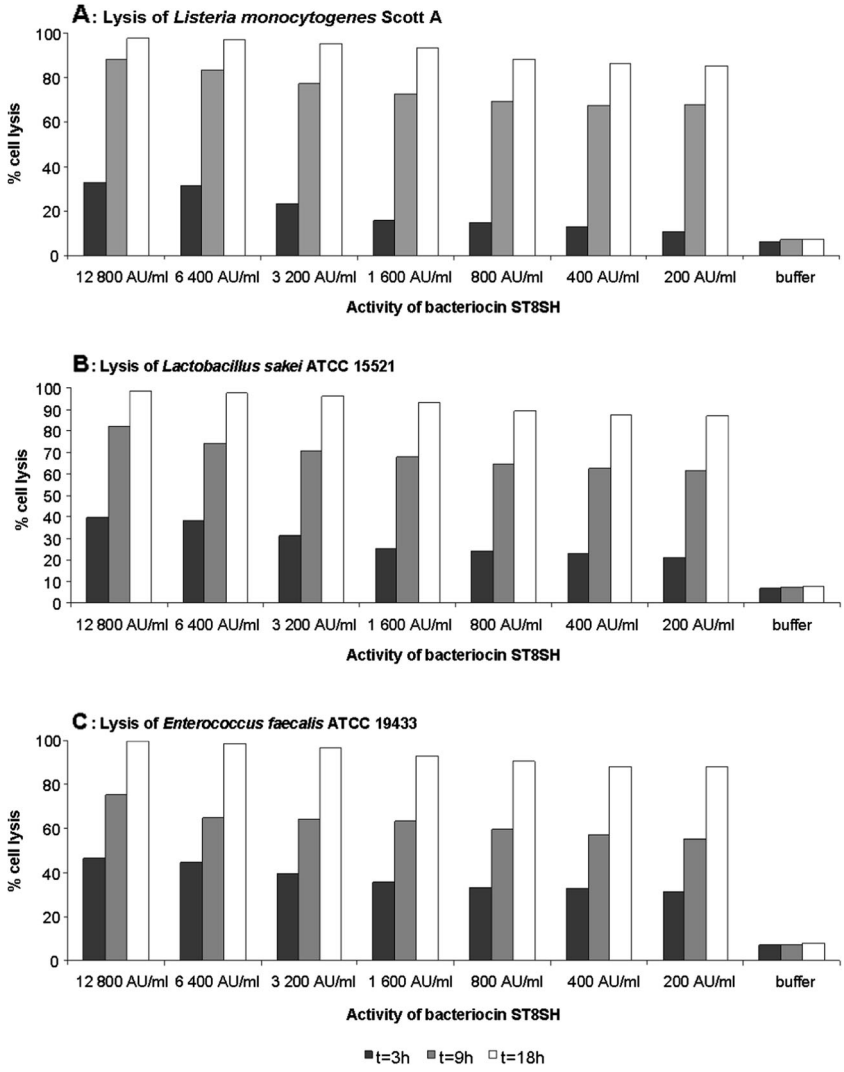

Fig. 3 Effect of different concentrations of bacteriocin ST8SH from *Lactobacillus plantarum* ST8SH on **a** *Listeria monocytogenes* ScottA, **b** *Lactobacillus sakei* ATCC 15521 and **c** *Enterococcus faecalis* ATCC 19433 recorded at 3, 9 and 18 h. Results are presented as % of lysis of test microorganisms

recorded when *Lb. plantarum* ST8SH was cultured for 24 h in MRS broth at 26 °C, 30 or 37 °C. This is in agreement with results recorded for plantaricin ST31 (Todorov et al. 1999) and mundticin ST4SA (Todorov and Dicks 2009). In contrast, several other bacteriocins have been reported to express only at specific temperatures. Furtado et al. (2014) reported on a strain of *Lactococcus lactis* subsp. *lactis*, able to express nisin only at 30 °C and not at 37 °C. Perin and Nero (2014) observed that temperature plays an important role in the expression of bacteriocins produced by several *Lactococcus* spp. and *Enterococcus* spp. with optimum production at 25 °C. Based on the results obtained and the potential future application of *Lb. plantarum* ST8SH not only as a bacteriocin producer, but both as bioprotective culture and a potential probiotic strain, all further experiments were conducted at 37 °C.

Similar results were recorded with cell-free supernatant and semi-purified bacteriocin produced by *Lb. plantarum* ST8SH.

Table 3 Effect of bacteriocin ST8SH on *Listeria monocytogenes* ScottA and *Enterococcus faecalis* ATCC 19433 determined by detection of the extracellular levels of DNA, RNA, proteins (at 260 nm) and β-galactosidase (at 420 nm)

	Listeria monocytogenes ScottA			*Enterococcus faecalis* ATCC19433		
	Treated	Control (cells + buffer)	Control (bacteriocin)	Treated	Control (cells + buffer)	Control (bacteriocin)
DNA, RNA, proteins (260 nm)	3.75	0.12	0.11	4.6	0.09	0.11
β-galactosidase (420 nm)	0.198	0.019	0.044	0.217	0.011	0.044

Table 4 Effect of bacteriocin ST8SH on stationary cells of *Listeria monocytogenes* ScottA and *Enterococcus faecalis* ATCC 19433. Bacteriocin (50 %, v/v) was added to each cell suspension of *Listeria monocytogenes* ScottA and *Enterococcus faeccium* ATCC 19433. Viable cell numbers were determined before and after treatment

	CFU/mL before treatment	CFU/mL after treatment
Bacteriocin ST8SH vs *L. monocytogenes* ScottA	7.45×10^{9}	1.74×10^{2}
Bacteriocin ST8SH vs *E. faecalis* ATCC 19433	3.7×10^{9}	2.45×10^{1}

It is important to underline the strong activity of this bacteriocin against *Enterococcus* spp. and various serological groups of *Listeria monocytogenes*, yet activity against some *Lactobacillus* spp. was also observed (Table 1). Inhibition of LAB can be both a positive and negative feature for the bacteriocins produced by *Lb. plantarum* ST8SH, depending on the situation. On the one hand, some LAB can be considered as undesirable because of their role in the spoilage of processed meats, while some strains may cause deterioration of quality during food fermentation processes. On the other hand, LAB used as starter cultures are applied for their beneficial metabolic activities and thus their inhibition may negatively influence the outcome of the conversion process. Activity against *L. monocytogenes* is frequently addressed as a marker to designate new bacteriocins as class IIa in the classification of bacteriocins (Heng et al. 2007). However, bacteriocins from other groups can also be active against *Listeria* spp. A classical example are the lantibiotics, in particular nisin—a class I bacteriocin from the same classification system (Heng et al. 2007). In this case, it is most important to perform gene or protein sequencing to confirm the presence of the N-terminal region typical of class IIa bacteriocins containing the conserved YGNGV/L or "pediocin box" motif (Fimland et al. 2005). Frequently, type IIa bacteriocins are referred to as pediocin-like bacteriocins (pediocin PA-1, carnobacteriocin B2, listerocin 743A and ubericin A) (Favaro et al. 2015).

When PCR targeting the pediocin PA-1/AcH gene (PedPro and Pedc1041 primers) was performed, an amplicon corresponding in size to that of pediocin PA-1/AcH was generated. The sequences of the generated amplicon showed 96 % similarity to that previously reported for pediocin PA-1/AcH (Marugg et al. 1992). Several pediocins with molecular mass between 2867 and 4685 Da have been reported in the literature and all present strong anti-*Listeria* activity (Gonzales and Kunka 1987; Bhunia et al. 1988; Daba et al. 1991; Henderson et al. 1992; Motlagh et al. 1994; Diep et al. 1996; Fimland et al. 2002; Bauer et al. 2005). Analysis of the generated nucleotide sequence showed a high similarity to plantaricin 423, a pediocin PA-1 family bacteriocin with possible amino

Table 5 Effect of temperature, pH and chemicals on adsorption of bacteriocin ST8SH to *Listeria innocua* 2030C, *Listeria ivanovii* subsp. *ivanovii* ATCC 19119, *Listeria monocytogenes* ScottA and *Enterococcus faecalis* ATCC 19433. Values are shown as percent adsorption

	Listeria innocua 2030C	*Listeria ivanovii* susbp. *ivanovii* ATCC 19119	*Listeria monocytogenes* ScottA	*Enterococcus faecalis* ATCC 19433
Effect of temperature (°C):				
4	50	50	50	50
15	75	75	75	75
30	75	75	75	75
37	75	75	75	75
45	50	87.5	100	75
pH				
3.5	50	50	50	50
5.5	75	75	50	50
7.0	75	75	75	75
Chemicals (1 %)				
Tween 80	25	25	25	50
Tween 20	25	25	50	50
Ascorbic acid	75	50	50	75
Potassium sorbate	75	50	50	75
Sodium nitrate	87.5	75	87.5	75
NaCl (0.5, 1.0, 1.5 and 2.0 %)	50	50	50	50

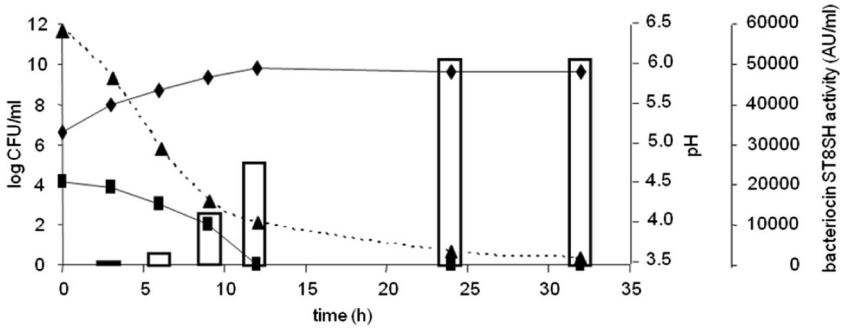

Fig. 4 Growth of a mixed culture of *Lactobacillus plantarum* ST8SH and *Listeria monocytogenes* ScottA (♦) and *Listeria monocytogenes* ScottA (■). ▲ Changes in pH, *histogram bars* production of bacteriocin ST8SH

acid differences in the C-terminal part of the molecule. The N-terminal "pediocin box" is intact and this can explain the high activity of bacteriocin ST8SH against *Listeria* spp. Based on the observed difference between bacteriocin produced by *Lb. plantarum* ST8SH and the previously described pediocin PA-1 and plantaricin 423, we suggest "bacteriocin ST8SH" as a designation for the new bacteriocin. The recorded difference in the genetic sequence can be responsible for changes in a few amino acids. Differences in 1 or 2 amino acids in the structure of bacteriocins have already been used as an argument for a new name of several bacteriocins. Classical examples are the different variants of nisin, indicated by different letters of the alphabet (Perin and Nero 2014). However, presence of gene/s or even an entire operon does not imply that this bacteriocin is expressed. Several examples of the presence of bacteriocin genes have been used as an argument to claim that such bacteriocins were expressed and present in the supernatant, but only a few reports have provided proof of this hypothesis (Todorov et al. 2012).

Van Reenen et al. (1998, 2003) reported that plantaricin 423, belonging to the pediocin PA-1 family, produced by *Lb. plantarum* 423, is a small heat-stable bacteriocin belonging to class IIa (anti-*Listerial* antimicrobial peptides). Plantaricin 423 shares high similarity with pediocin PA-1 and coagulin operons, in fact the *pla*C and *pla*D genes are identical to *ped*C and *ped*D of the pediocin PA-1 operon, as well as *coa*C and *coa*D of the coagulin operon (Van Reenen et al. 2003). When we tested the effect of bacteriocin ST5Ha, previously described as a pediocin PA-1-like bacteriocin (Todorov et al. 2010) against a culture of *Lb. plantarum* ST8SH, no inhibitory effects were observed. This is in agreement with the hypothesis for production of pediocin-like bacteriocins, since immunity genes are located in the same reading frame with the structural gene encoding the mature antimicrobial peptide, and, moreover, *Lb. plantarum* ST8SH shows auto-immunity to pediocin PA-1-like bacteriocins.

The molecular weight of bacteriocin ST8SH was estimated to be around 5 kDa. This detected molecular size for bacteriocin ST8SH was slightly larger than for most bacteriocins previously described for the genus *Lactobacillus* (De Vuyst and Vandamme 1994). However, Martinez et al. (2013) reported similar molecular weight of a bacteriocin produced by *Lb. plantarum* ST71KM. Bacteriocins produced by *Lb. plantarum* ST23LD were estimated to be within approximate sizes of 3.0 and 14.0 kDa (Todorov and Dicks 2005).

Strong antimicrobial activity of bacteriocin produced by *Lb. plantarum* ST8SH against *L. monocytogenes* ScottA, *Lb. sakei* ATCC 15521 or *E. faecalis* ATCC 19433 was observed. In a similar study, applying an equivalent approach to determining the inhibitory effect of bacteriocin ST5Ha produced by *E. faecium* ST5Ha isolated from smoked salmon, a similar inhibitory profile of *Lb. sakei* ATCC 15521 and *E. faecalis* ATCC 19433 was recorded, with even lower concentrations of bacteriocin applied (Todorov et al. 2010). The stronger antimicrobial activity of bacteriocin ST5Ha (Todorov et al. 2010) should be emphasized when compared with that recorded for bacteriocin ST8SH against *Lb. sakei* ATCC 15521 and *E. faecalis* ATCC 19433. Based on the observed inhibitory potential of bacteriocin ST8SH, this antimicrobial protein shows potential for application in the control of listerial or enterococcal food contaminations. It is important to underline that, in the present and other experiments, we tested the efficacy of bacteriocin ST8SH against extremely high numbers (approximately 10^8 CFU/mL) of the indicator microorganisms. However, in real food systems where the level of contamination is much lower, a stronger effect of bacteriocin ST8SH may be expected. An important conclusion is that bacteriocin ST8SH can be effective at low levels in a similar way as if it were to be applied in different concentrations from 12,800 to 200 AU/mL (Fig. 3).

Similar results on detection of leakage of β-galactosidase, DNA, RNA and proteins after treatment of test-organisms with bacteriocin/s were observed previously for plantaricin 423 (Todorov and Dicks 2006), pediocin AcH (Bhunia et al. 1991), mundticin ST4SA (Knoetze et al. 2008) and bacteriocin HV219 (Todorov et al. 2006), using similar methodologies.

In addition, when bacteriocin ST8SH was added to stationary phase cells of *L. monocytogenes* ScottA and *E. faecalis* ATCC 19433 cultures, complete bacterial killing was observed. Previously, a similar effect of bacteriocins HA-6111-2 and HA-5692-2, produced by *P. acidilactici*, on *E. faecium* HKLHS was reported by Albano et al. (2007).

Bacteriocin ST8SH adsorbed to both sensitive and non-sensitive cells of indicator bacteria at different levels. However, 66 % of the macedonocin ST91KM also adsorbed to sensitive *Lb. sakei* LMG13558 and non-sensitive strains of *E. faecalis* (BFE1071 and FAIR-E92) and *Streptococcus caprinus* (ATCC 700066). Limited or very little adsorption (up to 33 %) of macedocin ST91KM was recorded for other resistant strains (Pieterse et al. 2010).

Yildrim et al. (2002) reported a high percentage adsorption of buchericin LB to resistant strains of *Lc. lactis* (94 %), *Ped. cerevisiae* (100 %) and *St. aureus* (80 %). However, Manca de Nadra et al. (1998) reported weak adsorption of pediocin N5p to resistant strains (13–20 %) and higher adsorption to sensitive strains (30 to 100 %). These data indicate that the bactericidal action of pediocin N5p is dependent on specific receptors on sensitive strains.

Adsorption of bacteriocin ST8SH to test-organisms was shown to be temperature- and pH-dependent. Treatment of cells with macedocin ST91KM at 10–60 °C and at pH 8.0 and 10.0 led to a significant increase in adsorption (Pieterse et al. 2010). Adsorption of the peptide to target cells decreased from 66 % to 33 % below 37 °C, and was completely inhibited at 4 °C (Pieterse et al. 2010).

Addition of inorganic and organic salts reduced adsorption of the macedocin ST91KM and $MgCl_2$, while KI and Na_2CO_3 salts completely prevented adsorption of the peptide to target cells (Pieterse et al. 2010). SDS, Triton X-100 and Triton X-114 did not affect adsorption of macedocin ST91KM to target cells, while β-mercaptoethanol, 80 % ethanol and methanol reduced adsorption to 33 %. Chloroform had no effect on adsorption (Pieterse et al. 2010).

Production of bacteriocin ST8SH increased to 51,200 AU/mL when the strain was cultured in the presence of either viable or dead cells of *L. monocytogenes*. The bacteriocin activity recorded represents that of the cell-free peptides and does not represent peptide molecules that may still be bound to the producer cell or test microorganism. Nevertheless, it would seem that bacteriocin ST8SH production is stimulated by the presence of the target organisms, most probably by the protein receptors of their cell wall. High cell numbers of *Lb. plantarum* ST8SH and *L. monocytogenes* ScottA were recorded on MRS plates when co-cultured (Fig. 4). However, results recorded on selective LEB medium showed that the cell numbers of *L. monocytogenes* ScottA decreased from 1.42×10^4 to 1.02×10^2 CFU/mL within 9 h and to undetectable levels after 36 h (Fig. 4). This indicated that the high cell numbers recorded on MRS plates were *Lb. plantarum*. Inhibition of *L. monocytogenes* ScottA cannot be ascribed to lactic acid production or a decrease in pH, since a much more drastic decline in cell numbers was recorded after 9 h fermentation with increased production of bacteriocin ST8SH (Fig. 4). Less than 3 % difference was recorded among three repeated experiments. Most probably bacteriocin activity could be higher, as some active peptides remain bound to the cell surface of the target strain. The latter phenomenon has been reported by Yildrim et al. (2002) and Schirru et al. (2012).

Conclusions

To our knowledge, this is the first report on some aspects of the bacteriocinogenic mode of action of a strain of *Lb. plantarum* isolated from "Shpek". *Lb. plantarum* ST8SH produces a bacteriocin active against several strains of genera *Enterococcus*, *Klebsiella*, *Listeria* and *Streptococcus*, including food-borne pathogens. Based on the physiological characteristics of *Lb. plantarum* and the physico-chemical and biological properties of bacteriocin ST8SH, the strain and bacteriocin may be used in the preservation of foods. Taking into consideration the difference in the amino-acid structure, *Lb. plantarum* ST8SH produces a new bacteriocin (bacteriocin ST8SH) within the pediocin PA-1 family. Further investigation of the technological properties of *Lb. plantarum* ST8SH and additional experiments demonstrating the safety of the strain and its bacteriocin should be conducted.

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