ISSN 1330-9862
(FTB-2943)

original scientific paper

Antilisterial Activity of Bacteriocin Isolated from *Leuconostoc mesenteroides* ssp. *mesenteroides* IMAU:10231 in the Production of Sremska Sausages: Lactic Acid Bacteria Isolation, Bacteriocin Identification and Meat Application Experiments

Slavica Vesković Moračanin[1]*, *Lazar Turubatović*[1], *Marija Škrinjar*[2] and *Dragojlo Obradović*[3]

[1]Institute of Meat Hygiene and Technology, Kaćanskog 13, RS-11000 Belgrade, Serbia

[2]Faculty of Technology, Bulevar cara Lazara 1, RS-21000 Novi Sad, Serbia

[3]Faculty of Agriculture, Nemanjina 6, RS-11080 Belgrade, Serbia

Received: October 6, 2011
Accepted: March 14, 2012

Summary

Lactic acid bacteria (LAB) have an essential role in the production of fermented meat products. The metabolic activity of LAB affects the ripening process, leading to the formation of the desired sensory characteristics of the products, while inhibiting the growth of undesirable microorganisms. Bacteriocins are extracellular peptides or protein molecules, produced by some LAB, which possess bactericidal properties against specific species or genera of microorganisms, usually related bacteria. Bacteriocin production by LAB can act in a selective and competitive way against the surrounding microbiota, which may contain spoilage bacteria or pathogenic microorganisms including *Listeria monocytogenes*. This pathogen is widely distributed in raw products, it survives in different production areas, and human infections have a high mortality rate, all of which makes the control of this microorganism important in food production.

The aim of this work is to determine the possibilities of utilizing a novel bacteriocin isolated from *Leuconostoc mesenteroides* ssp. *mesenteroides* IMAU:10231 in order to prevent the survival of *Listeria monocytogenes* in the production of traditional Serbian Sremska sausages. The bacteriocin-producing strain of *Leuconostoc* originated from the same sausage, which had been produced in the traditional manner. Bacteriocin was isolated using precipitation procedures with ammonium sulphate, and then its properties (strength and range of activities, relationship to high temperatures and proteolytic enzymes) were determined under laboratory conditions. Also, based on the obtained laboratory results, the antilisterial effect of bacteriocin, included as an additive, was examined in the production of traditional Sremska sausages.

Expressed antilisterial activity of bacteriocin has an interesting food safety potential which can be used in the meat industry in the production of fermented sausages. Further research will contribute to a better understanding of its nature, activities, opportunities for application, but also to discovering new bacteriocin-producing strains of LAB, which could act as natural food preservatives, or bioprotectors, when used in a specific (controlled) manner.

Key words: Leuconostoc mesenteroides ssp. *mesenteroides* IMAU:10231, bacteriocin, Sremska sausage, biopreservation

*Corresponding author; Phone: ++381 11 2650 655; Fax: ++381 11 2651 825; E-mail: slavica@inmesbgd.com

Introduction

The contemporary concept in the production and processing of food is based on the application of different types of protective technologies that are aimed at ensuring and preserving product safety, as well as acceptable, and at the same time, unchanging quality from the end of production until consumption. On the other hand, the direct beneficiaries, today's consumers, exhibit considerable consistency in terms of a negative attitude towards the use of chemical substances as additives in the food production process. There is an expressed need for eating food that has not undergone extensive processes of preservation and that does not contain numerous chemical preservatives. Such a trend (so-called 'green technology'), on the one hand, and the continuous development of protective technologies in the twentieth and the twenty--first century on the other, stimulate the development and application of advances in the field of biological food protection.

The growing need for naturally safe and healthy food has led to increased interest in the use of bacteriocin-producing strains of lactic acid bacteria (LAB), which are used as protective cultures for the production of fermented products (*1–3*). The principle on which biological protection relies is based on reducing risks to consumer's health by acting primarily on the undesirable bacteria or foodborne pathogens without changing the quality of the product.

Bacteriocins are extracellular proteins or peptide molecules that possess some bactericidal properties against particular kinds of microorganisms which are usually related to the bacteriocin producers (*4*), *i.e.* they can be found in similar ecological niches (*5,6*). LAB bacteriocins are natural antimicrobial peptides or proteins with very interesting potential application in the food industry as bioprotectors (*7*), in order to preserve people's health (*8*) while enhancing the sustainability of food (*9*). Hurst (*10*) described bacteriocins as 'biological food preservatives' and that term was soon generally accepted.

These bacteriocins are ribosome-synthesized polypeptides that have bactericidal activity and are rapidly digested by proteinases in the human digestive tract (*11*). They are often compared with antibiotics in literature because of their expressed antibacterial properties (*10,12*). However, in contrast to therapeutic antibiotics, their application as a rule does not provoke undesired allergic reactions in humans (*7*). As the discovery of penicillin by Alexander Fleming in the year 1929 had direct importance for the human race, so the discovery of bacteriocins, in an indirect sense, has an importance in terms of natural health care and food safety.

Investigation of the applicability of LAB bacteriocins as a supplement to the hurdle technology, used to ensure food safety, was the basis of the research conducted in this study. Similar research has been carried out by other authors (*13–15*).

Bacteriocin-producing strain of LAB, *Leuconosotoc mesenteroides* ssp. *mesenteroides* IMAU:10231 was isolated from the natural, indigenous (wild) microbiota of Serbian traditional fermented Sremska sausages. After laboratory examinations of the spectrum of antimicrobial effects, the strength of bacteriocin activities and resistance to higher temperature and proteolytic enzymes, the bacteriocin isolated from *Leuconostoc* was included as an additive under novel industrial conditions in the production of traditional Sremska sausage.

Materials and Methods

In accordance with the defined test method, the experiment was divided into two independent phases. After the production of Sremska sausage in accordance with traditional principles, the first phase of the experiment included the isolation and genetic determination of lactic acid bacteria, as well as testing their ability to produce bacteriocin. The second phase of the study was based on determining the intensity of antilisterial activity of the added bacteriocin obtained from *Leuconostoc mesenteroides* ssp. *mesenteroides* IMAU:10231 in the new series of the production of Sremska sausage.

Sausage production and sampling procedure

Sremska sausage was produced under industrial conditions (YUHOR meat industry, Jagodina, Serbia) using the basic principles of traditional manufacturing and in accordance with the applicable national regulations governing the quality of meat products. The composition of the sausage was: frozen pork shoulder 38.3 %, chilled pork ham 33.5 % and frozen solid fatty tissue – back fat 23.9 %, salt 2.49 % with $NaNO_2$ 0.6 %, dextrose 0.48 %, and the following natural spices: crushed garlic 0.76 % and ground sweet and hot peppers 0.57 %.

In the first phase of the experiment, the isolation and molecular genetic determination of LAB, as well as testing the ability to produce bacteriocin, was carried out. In the second phase of the experiment, in accordance with the defined test method, the total amount of stuffing was divided into three experimental groups, as follows: (*i*) one third of the stuffing was separated from the total mass and was immediately filled into pig small intestines; these sausages were used as a control group and were labelled K; (*ii*) the rest of the stuffing was inoculated with *Listeria monocytogenes* ATCC 19111 in a manner ensuring that the final concentration of the pathogen per mass of stuffing was 10^4–10^5 cells/g. After the inoculation with *L. monocytogenes* (conducted with appropriate safeguards), half of the stuffing was separated and filled into pig small intestines (this group was a control sample reflecting the effectiveness of bacteriocins and was marked KO); (*iii*) bacteriocin derived from *L. mesenteroides* ssp. *mesenteroides* IMAU:10231 was added to the remaining amount of the stuffing to achieve a final amount per mass of stuffing of 2560 AU/kg. After homogenization, the stuffing was filled in the same way into pig small intestines. Samples of this group were labeled as test O_1.

In both phases of the experiment, the ripening process of Sremska sausages was the same. After filling, the sausages were hung on rods. After partial draining (a few hours), the sausages were smoked for 3 days at 18 °C and a relative humidity of 65–85 %. The traditional smoking process was carried out, using beech wood in an open fire. The smoking phase was followed by drying and ripening processes at 12–20 °C and a relative humidity of 70–80 %. The complete sausage production

process lasted 21 days. Due to the specificities of the added pathogen, the study was carried out under strictly controlled conditions. Samples for laboratory examinations were taken on days 0, 3, 7, 14 and 21. Three samples were collected at each step of sampling and used for analysis. The sausage manufacture and fermentation process were repeated three times.

Determination of the LAB isolates

At different stages of Sremska sausage ripening, during the three repeated cycles of production, LAB were isolated using conventional microbiological techniques. The LAB isolates from MRS were first checked by Gram staining and catalase reaction. Gram-positive and catalase-negative isolates were then identified by a commercially available biochemical identification system API 50CHL (bioMérieux, Marcy l´Etoile, France) and molecular methods. The second step was screening for their antimicrobial activity. All LAB isolates were tested for their ability to produce bacteriocin.

Genotypic identification of the LAB isolates

Total DNA from the overnight cultures of LAB was extracted from a single colony by using the DNeasy Blood and Tissue Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's protocol for Gram-positive bacteria. PCR was performed in a final volume of 50 μL containing 1×PCR buffer (10×PCR buffer: 500 mM KCl, 100 mM Tris-HCl, 0.8 % Nonidet P 40 (octylphenoxylpolyethoxylethanol; Shell Chemicals, London, UK)), 2.5 mM $MgCl_2$, 200 μM of each dNTP (deoxyribonucleotide triphosphate: dATP, dCTP, dGTP and dTTP), 2.5 μM of each primer, 1 unit (U) of Taq DNA polymerase (Fermentas UAB, Vilnius, Lithuania) and 0.1–1 μg of DNA template (Fermentas UAB). The samples were amplified in a DNA thermal cycler (Techne Flexigene, Bibby Scientific Ltd, Stone, UK) with primers P1V1 (GCGGCGTGCCTAATACATGC) and P4V3 (ATCTACGCATTTCACCGCTAC), complementary to the V1–V3 region of the 16S rRNA (*16*), for 5 min at 95 °C, 35 cycles of 1 min at 95 °C, 1 min at 42 °C, 2 min at 72 °C and the final extension of 5 min at 72 °C. PCR products were purified by QIAquick® PCR purification kit (Qiagen GmbH) and sent to a commercial facility for sequencing (IIT Biotech, Bielefeld, Germany). Search of the GenBank with BLAST program (*17*) was performed to determine the closest known relatives of the partial 16S rDNA sequences obtained.

Bacteriocin production by the LAB strains

The agar well diffusion assay, as described by Schillinger and Lücke (*1*), was used in the bacteriocin purification and biochemical characterization procedure. In order to determine the potential production of bacteriocins, the test organisms *Listeria monocytogenes* ATCC 19111, *Staphylococcus aureus* ATCC 6538 and *Escherichia coli* O157:H7 ATCC 43894 were used.

The test microorganisms were added to brain heart infusion (BHI) agar (HiMedia Laboratories, Mumbai, India) to achieve final concentrations of 10^7–10^8 CFU/mL in the medium. A volume of 1 mL of 18-hour-old culture of LAB was centrifuged (10 000×*g*, 4 °C for 15 min). Neutralization of acid products of metabolism was conducted using 1 M NaOH to pH=6.5–7.0. Neutralization of H_2O_2 was done by the addition of catalase enzyme (C-3515, Sigma-Aldrich, St. Louis, MO, USA) at 5 mg/mL, and the experiment was confirmed by the proteinase test: 50 μL of proteinase K (Sigma-Aldrich) were added to 1 mL of the neutralized broth culture. After 1 h of incubation at 37 °C, the antimicrobial activity was determined. The existence of the growth inhibition zone around test microorganisms was considered as a positive result.

Bacterial strains and growth conditions

Leuconostoc mesenteroides ssp. *mesenteroides* IMAU:10231 isolated from Serbian dry fermented Sremska sausage was used throughout this study. It was kept frozen at –20 °C in de Man-Rogosa-Sharpe (MRS) broth (Merck, KGaA, Darmstadt, Germany) supplemented with 20 % glycerol. Prior to use, the microorganism was subcultured twice in 10 mL of MRS broth (1 % inoculum, 24 h, 30 °C).

Isolation of semi-purified bacteriocin and determination of its strength

Isolation of semi-purified bacteriocin from *L. mesenteroides* ssp. *mesenteroides* IMAU:10231 was achieved by saturated precipitation with ammonium sulphate (*1*), adjusted to the individual laboratory conditions (*18*,*19*). Broth culture was seeded with *L. mesenteroides* ssp. *mesenteroides* IMAU:10231 for several days and grown at 30 °C to obtain 10^{10}–10^{11} CFU/mL and then centrifuged at 10 000×*g* for 30 min at 4 °C (MSE 'High Speed 18', Measuring&Scientific Equipment Ltd, London, UK) in order to separate the supernatant. The supernatant was then neutralized to pH=6.5–7.0 with 10 M NaOH, and bacteriocin was precipitated with ammonium sulphate. Separated bacteriocin in the shape of whitish pellets was suspended in 0.05 M of sodium phosphate buffer, pH=7, to produce the bacteriocin suspension.

The activity of the isolated, semi-purified bacteriocin of *L. mesenteroides* ssp. *mesenteroides* IMAU:10231 was determined by the critical dilution method against the selected test microorganism, *Listeria monocytogenes* ATCC 19111, using the agar well diffusion assay. Bacteriocin suspension was filtered (by using Acrodisc® syringe filter 200/cs, Pall Corporation, Port Washington, NY, USA), and 50-μL volumes of suitable dilutions in sterile deionized water were then placed in previously made and labelled agar wells. After one-hour incubation at 4 °C, in order to stimulate bacteriocin diffusion, the plates were incubated at 30 °C for 24 h. Bacteriocin activity was expressed as the absolute value, marked as the arbitrary unit (AU) per mL. Arbitrary units were calculated using the following formula:

$$AU=2^n\times(1000\ \mu L/50\ \mu L) \qquad /1/$$

where *n* represents the maximum dilution of the bacteriocin suspension which produced a growth inhibition zone of *L. monocytogenes* greater than 2 mm (*20*).

The influence of increased temperatures on the activity of isolated bacteriocin

The influence of high temperatures on bacteriocin activity was investigated in order to determine the functional

properties of the isolated bacteriocin as well as its suitability for application during the production of meat products exposed to increased temperatures.

Volumes of 1 mL of bacteriocin suspension were exposed to the following temperatures: 65, 80, 90 and 100 °C, as well as to the high temperature (121 °C) and high pressure ($1.2 \cdot 10^5$ Pa) during autoclaving. Cuvettes containing 1 mL of bacteriocin suspension were dipped into a water bath heated at 65, 80 and 90 °C for 10 and 30 min. The influence of the temperature of 100 °C was also measured after 60 min. The effect of high temperature (121 °C) combined with increased pressure ($1.2 \cdot 10^5$ Pa) during autoclaving (sterilization), was determined after 15 min. After the prescribed exposure time, bacteriocin activity was determined by the agar well diffusion assay with the selected test microorganism (*L. monocytogenes* ATCC 19111). The results were read after 24 h of incubation at 30 °C.

The influence of proteolytic enzymes on bacteriocin activity

The proteinaceous nature of the isolated bacteriocin was proven by the reaction with proteolytic enzymes. Pepsin TS (with the activity of 1:10 000, NF Galenika, Zemun-Belgrade, Serbia), papain (water soluble, 30 000 U/mL; Merck, KGaA) and proteinase K (minimum 600 milliAnson units per mL; AppliChem, St. Louis, MO, USA) were used. Enzyme preparations were added separately at concentrations of 1 mg/mL to 1 mL of bacteriocin suspension and incubated for 1 h at 37 °C. After that, a suitable volume (50 μL) of the sample (bacteriocin and enzyme) was tested in the agar well diffusion assay in order to determine the antilisterial activity (as described previously). All experiments were performed in triplicate. The results were read after 24 h of incubation at 30 °C.

Microbiological analyses

Sremska sausage samples (25 g) were each transferred to a sterile stomacher bag, 225 mL of saline peptone water (8 g/L of NaCl and 1 g/L of bacteriological peptone; Oxoid, Cambridge, UK) were added and mixed for 30 s in the stomacher. Further decimal dilutions with the same diluents were made and the following analyses were carried out on duplicate agar plates: (*i*) total viable count on peptone agar (Oxoid); incubated under aerobic conditions for 48–72 h at 30 °C, and (*ii*) LAB count on MRS agar (Oxoid), incubated in a double layer for 48 h at 30 °C.

Numbers of inoculated *L. monocytogenes* were determined during the Sremska sausage ripening and fermentation to ascertain the effect of the added antilisterial bacteriocin. Determination of the presence of *Listeria* spp. was carried out following the procedure of the ISO 11290-1 and 11290-2 metods (*21*,*22*). A mass of 25 g of each sample was homogenized with 225 mL of sterile Fraser broth (Biolife, Bolzano, Italy) in a stomacher for 2 min. The homogenates were incubated at 20 °C for 1 h in order to resuscitate the stressed microorganisms. For the enumeration of *L. monocytogenes*, 0.1 mL of each homogenate was spread directly onto each of the two PALCAM agar (Oxoid) plates and incubated at 37 °C for 24–48 h. The homogenates were then supplemented with Fraser half selective supplement (primary enrichment; Biolife) and incubated at 30 °C for 24 h for the detection of *L. monocytogenes*. Afterwards, 0.1 mL of the primary enrichment was inoculated into 10 mL of Fraser broth supplemented with Fraser selective supplement (secondary enrichment; Biolife) and incubated at 37 °C for 24 h. Cultures were streaked onto Oxford plates (Oxoid) and incubated at 30 °C. From each plate of the primary and secondary enrichment, five colonies presumed to be *Listeria* spp. were streaked onto tryptone soy yeast extract agar (TSYEA) plates (Biolife) and incubated for 24 h at 37 °C. Colonies were selected for typical appearance on TSYEA and submitted to Gram staining, catalase and oxidase tests. Haemolytic activity and CAMP tests on sheep blood agar were performed for *L. monocytogenes* confirmation. Biochemical characterization of all the isolates was performed using the API Listeria identification system (bioMérieux).

Results and Discussion

Basic and molecular identification of the LAB isolates

As a result of basic microbiological tests, which included examinations of the morphology of microorganisms, Gram staining and catalase reaction, and ended with biochemical confirmation using the commercial kit API 50CHL (bioMérieux), 50 different species of LAB were isolated from various stages of fermentation and ripening of Sremska sausage. The results of comparative analysis of API identification and the molecular genetic test (sequencing analysis) are shown in Table 1.

Classical microbiological test results from biochemical API identification as a final confirmation of LAB allowed good identification of the genera; however, the correct identification of LAB species was possible only when applying molecular genetic studies (*23*,*24*).

For the purpose of identification of the isolates by molecular methods, the most often targeted gene is the 16S rRNA (*25*). The application of molecular methods for the identification of LAB strains highlighted the presence of *L. mesenteroides* in traditional sausages, which was common to all fermentations. In previous studies (*16*), it had been shown that among LAB, the species most commonly found in meat and meat products, including dry sausages processed with different technologies, are *Lactobacillus sakei*, *L. curvatus* and *L. plantarum* (*1*,*24*,*26*–*30*), with *L. sakei* being the most frequently isolated species.

Detection of antimicrobial activity

By eliminating potential inhibitory components that result from metabolic activity, two strains of leuconostoc, *L. mesenteroides* ssp. *mesenteroides* IMAU:10231 and *L. mesenteroides* ssp. *mesenteroides* J9, demonstrated the ability to produce bacteriocins. With regard to confirmatory tests of bacteriocin activities, both *Leuconostoc* spp. examined for activity against *L. monocytogenes* ATCC 19111 showed a typical bacteriocin profile. The 18-hour-old broth culture of the examined *Leuconostoc* species and its neutralized broth (pH=7), in the agar well diffusion assay, gave growth inhibition zone against the test microorganisms larger than 2 mm. Also, catalase test was positive (presence of inhibition zone), while proteinase test was negative (absence of inhibition zone).

Table 1. Comparison of API identification and sequencing analyses

Day	API	Sequencing
0	*Lactococcus lactis*	*Leuconostoc mesenteroides* ssp. *mesenteroides* strain J9
	Pediococcus pentosaceus	*Pediococcus pentosaceus* strain YTX32BMX
	L. mesenteroides ssp. *mesenteroides*	*L. mesenteroides* strain SK kimchi 6
	L. mesenteroides ssp. *mesenteroides*	*L. mesenteroides* strain SK kimchi 6
	Lactobacillus fermentum	*Lactobacillus plantarum* strain IMAU40091
	L. mesenteroides ssp. *mesenteroides*	*L. mesenteroides* strain SK kimchi 6
	Lc. lactis ssp. *lactis*	*L. mesenteroides* strain SK kimchi 2
	L. mesenteroides ssp. *mesenteroides*	*Lc. lactis* ssp. *cremoris* strain IMAU60024
	Pediococcus pentosaceus	no significant similarity found
	Pediococcus pentosaceus	*Pediococcus pentosaceus* strain CTSPL1
	Lc. lactis spp. *lactis*	*Pediococcus pentosaceus* strain CTSPL1
2	*Lactobacillus curvatus*	*Weissella viridescens* strain H
	L. curvatus	*L. mesenteroides* strain SK kimchi 2
	L. mesenteroides ssp. *mesenteroides*	*Leuconostoc carnosum* strain SACB703a
	Lactobacillus delbruecki ssp. *delbruecki*	*L. mesenteroides* ssp. *mesenteroides* strain J9
	Lc. lactis	*Lc. lactis* strain zmjh8
	L. mesenteroides ssp. *mesenteroides*	*L. mesenteroides* ssp. *mesenteroides* strain J9
	L. curvatus	*Lactobacillus sakei* strain IMAU80189
4	*L. mesenteroides* ssp. *mesenteroides*	*L. mesenteroides* strain SK kimchi 2
	L. mesenteroides ssp. *mesenteroides*	*L. mesenteroides* strain SK kimchi 6
	L. mesenteroides ssp. *mesenteroides*	*L. mesenteroides* strain F170
	L. mesenteroides ssp. *mesenteroides*	*L. mesenteroides* strain SK kimchi 6
	L. mesenteroides ssp. *mesenteroides*	*L. mesenteroides* strain SK kimchi 6
	L. mesenteroides ssp. *mesenteroides*	*L. mesenteroides* ssp. *mesenteroides* strain J9
	L. mesenteroides ssp. *mesenteroides*	*L. mersenteroides* strain Leu1
	L. mesenteroides ssp. *mesenteroides*	*L. mesenteroides* ssp. *mesenteroides* strain J9
	L. delbruecki ssp. *delbruecki*	uncultured bacterium clone 100p7-2314
	Lactobacillus fructivorans	*Leuconostoc gasicomitatum* strain J13
	L. mesenteroides ssp. *mesenteroides*	*L. mesenteroides* strain SK kimchi 6
7	*L. mesenteroides* ssp. *mesenteroides*	*L. mesenteroides* ssp. *mesenteroides* IMAU:10231
	Lactobacillus celobiosus	*L. mesenteroides* strain AaD8
	L. mesenteroides ssp. *mesenteroides*	*L. mesenteroides* ssp. *mesenteroides* strain J9
	L. celobiosus	*L. mesenteroides* strain SK kimchi
14	*L. celobiosus*	*L. mesenteroides* strain AaD8
	L. delbruecki ssp. *delbruecki*	*L. mesenteroides* strain SK kimchi 6
	L. delbruecki ssp. *delbruecki*	*L. curvatus* IMAU:10284
	L. celobiosus	*L. mesenteroides* strain C12
	L. mesenteroides ssp. *mesenteroides*	*L. mesenteroides* ssp. *mesenteroides* strain J9
	Lactobacillus linderi	*L. mesenteroides* strain SK kimchi 6
	L. celobiosus	*L. mesenteroides* subsp. *mesenteroides* strain J9
	L. curvatus	*Pediococcus pentosaceus* strain CTSPL1
	L. curvatus	*Pediococcus pentosaceus* strain CTSPL1
	L. curvatus	*Weissella viridescens*
21	*L. delbruecki* ssp. *delbruecki*	*L. mesenteroides* strain SK kimchi 6
	Lactobacillus brevis	*L. mesenteroides* strain CTC6578
	L. mesenteroides ssp. *cremoris*	*L. mesenteroides* strain LHICA-53-31
	L. brevis	*Lactobacillus alimentarius* strain IMAU80147
	L. brevis	*L. mesenteroides* ssp. *mesenteroides* strain J9
	L. curvatus	*Weissella viridescens*
	L. delbruecki ssp. *delbruecki*	*L. sakei* strain IMAU80189
	L. fermentum	*L. sakei* strain IMAU80168

Neither of these novel bacteriocins showed any activity against *Escherichia* or *Staphylococcus* (data not shown), although antilisterial activity was clearly expressed. For the remainder of our study, *L. mesenteroides* ssp. *mesenteroides* IMAU:10231 was used exclusively.

Production of bacteriocins by *Leuconostoc* spp. was first observed in 1950. However, only since 1984 numerous studies have been published on their antimicrobial compounds, which were at first described as substances similar to bacteriocin (*31*). The inhibitory activity of bacteria of the genus *Leuconostoc* has been shown towards the growth of other LAB, food spoilage bacteria and certain pathogens, in particular *L. monocytogenes*.

Through isolation and complete characterization, it was found that bacteriocins produced by *Leuconostoc* spp. belong to subclasses IIa (pediocin-like bacteriocins) with a strong antilisterial effect (*32*). *L. mesenteroides* ssp. *mesenteroides* is a significant producer of mesenterocin Y105 (*33*). Furthermore, *L. mesenteroides* UL5 isolated from Cheddar cheese (*34*) produced the thermostable bacteriocin, mesenterocin 5, with an antilisterial effect, as did *L. mesenteroides* ssp. *dextranicum* ST99 isolated from boza, a fermented beverage made from corn flour, yeast and sugar (*35*). Leucocin *A*-UAL 187 isolated from *L. gelidum* (originating from vacuum-packed meat) showed an antagonistic effect against other LAB, *L. monocytogenes* and *Enterococcus facealis* (*36*).

The results of our examinations were similar. The antibacterial activity of the bacteriocin-producing *L. mesenteroides* ssp. *mesenteroides* IMAU:10231 strain showed intense inhibitory activity towards *L. monocytogenes* NCTC 10527, but no inhibitory effect towards *S. aureus* NCBF 1499 or *E. coli* 0157:H7 NCTC 12079.

As a result of its physiological characteristics (the creation of mucus, *i.e.* exopolysaccharides, and other metabolic products such as acetoin, diacetate, ethanol, *etc.*), which are unacceptable in terms of quality in the meat industry, the technological application of *Leuconostoc* sp. is mainly limited to the direct application of synthesized and purified bacteriocins (*18*,*19*).

The strength of the isolated bacteriocin

The maximum dilution of bacteriocin isolated from *L. mesenteroides* ssp. *mesenteroides* IMAU:10231 which produced an antilisterial effect was 1:128, giving a calculated activity of approx. 2560 AU/mL. A typical well diffusion assay of bacteriocin in a lawn of *L. monocytogenes* is shown in Fig. 1.

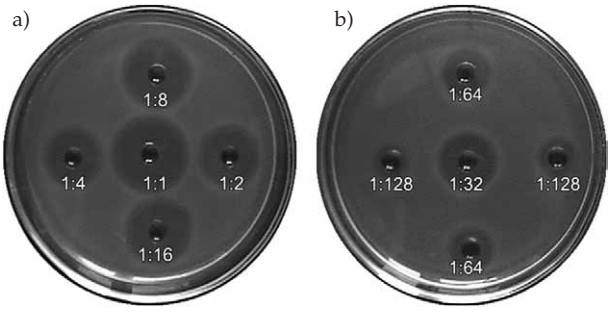

Fig. 1. Agar well diffusion assay for determination of activity of bacteriocin isolated from *Leuconostoc mesenteroides* ssp. *mesenteroides* IMAU:10231: a) 1:1 (bacteriocin/distilled water) – initial dilution (control), 1:2 – first bacteriocin dilution, 1:4 – second bacteriocin dilution, 1:8 – third bacteriocin dilution, 1:16 – fourth bacteriocin dilution; b) 1:32 – fifth bacteriocin dillution, 1:64 – sixth bacteriocin dilution, 1:128 – seventh (the last one) bacteriocin dilution that gave inhibition zone of *L. monocytogenes* growth

Effects of temperature and proteinases on bacteriocin activity

The results of the examination of the effect of high temperatures on the activity of bacteriocin isolated from *L. mesenteroides* ssp. *mesenteroides* IMAU:10231 emphasized its significant thermal resistance (Table 2). Generally, bacteriocin activity was stable at higher temperatures, up to 90 to 100 °C. Antilisterial activity of this bacteriocin, although slightly diminished, was noticeable even after autoclaving (121 °C, $1.2 \cdot 10^5$ Pa for 15 min). Therefore, the use of *L. mesenteroides* ssp. *mesenteroides* IMAU: 10231 bacteriocin in fermented sausages seems to be an obvious choice. Other bacteriocins isolated from *L. mesenteroides* have similar features. Mesentericin Y105 and mesentericin E131 (*33*,*37*) are heat stable (at 60 °C for 120 min and at pH=4.5) and can be partially inactivated only by autoclaving or heating at 100 °C for 120 min. The established thermoresistance indirectly reveals the nature of the isolated bacteriocin.

Actually, literature data show that bacteriocins isolated from *L. mesenteroides* ssp. *mesenteroides* mostly belong to classes I and IIa (*38*,*39*), which are classes of very small globular proteins with high thermostability. Similar results were obtained by other authors (*16*,*37*,*40–42*) examining bacteriocins isolated from different strains of *L. mesenteroides* and *L. sakei*. Also, the bacteriocins produced by *Pediococcus* spp. are classified as class IIa bacteriocins and have high thermostability and antimicrobial activity, especially against *L. monocytogenes* (*43–45*).

Table 2. Activity of bacteriocin isolated from *Leuconostoc mesenteroides* ssp. *mesenteroides* IMAU:10231 after exposure to increased temperatures and pressure

	Temperature/°C									
	65		80		90		100			121
										p=1.2·10^5 Pa
	t/min									
Residual activity/%	10	30	10	30	10	30	10	30	60	15
	100	100	100	100	100	80	80	80	60	60

Residual activity=(total activity of subsequent step/total activity of crude preparation)×100

In the current study, the influence of proteolytic enzymes (pepsin TS, papain and proteinase K) on the activity of the isolated bacteriocin showed the destruction of the antilisterial effect; the bacteriocin lost its antimicrobial activity. This confirmed the proteinaceous nature of the isolated bacteriocin, which is basically the property of bacteriocins produced by LAB (*5,37,40,46*). The proteinaceous nature of bacteriocins determines their fate in the human digestive tract. Humans are the final consumers of food products with added bacteriocins or bacteriocins produced by LAB. Experts in this area of food safety (*11*), while defining the bacteriocins from LAB, emphasize their proteinaceous nature and demonstrate the fact that they can be degraded by human digestive proteinases.

This brings optimism to the application of these bacteriocins as bioprotectors of food, because any other outcome in the human organism (accumulation, partial degradation, resorption, *etc*.) would not be desirable, but on the contrary, would be harmful. However, the role of bacteriocins in the suppression of pathogenic bacteria in the digestive tract of humans is questionable because of the above-mentioned degradation of bacteriocins in contact with proteolytic enzymes. Nevertheless, their use in the production of certain food products could have a potential antimicrobial effect on pathogens (*40,47,48*).

The antimicrobial activity of the added bacteriocin

L. monocytogenes was not detected in any sample of the control group of Sremska sausage (group K, Sremska sausage prepared without added bacteriocin, and without inoculated *L. monocytogenes*) in all stages of testing (days 0, 3, 7, 14 and 21) during the triplicate fermentations. The results of this group are not presented, because the aim of this study was to determine the potential antilisterial effect of the bacteriocin isolated from *Leuconostoc* which was added during the production of traditional Sremska sausage.

The changes in the number of the total viable count of LAB and *L. monocytogenes* in the examined experimental groups of sausages (KO and O_1), are shown in Tables 3 and 4. The antilisterial effect of the newly isolated bacteriocin from *L. mesenteroides* ssp. *mesenteroides* IMAU: 10231 during the manufacturing process of Sremska sausage is presented in Table 4. The results obtained during the analysis of the samples of Sremska sausage inoculated only with *L. monocytogenes* were used for comparison (Table 3).

Total viable counts in both examined groups of sausages show relatively high values ranging from (5.3±0.2) to (5.59±0.09) log CFU/g. The obtained values represent the microbiological status of raw materials on the one hand, and on the other, the hygienic status of the traditional manufacturing process, which is mainly based on manual preparation, mixing and filling the sausage casings. Within the experimental group of sausages, O_1, there was no statistically significant difference in the number of aerobic mesophilic bacteria, which was determined at baseline, at day 0 ($p>0.05$). In the KO group of sausages the highest number of aerobic mesophilic bacteria was identified on the 14th day of testing ((6.5±0.1), (6.5±0.1) and (6.5±0.2) log CFU/g during the 1st, 2nd and 3rd fermentation, respectively), while at the end of the ripening process, the total number in log CFU/g was almost two logs lower (4.7±0.3, 4.7±0.2 and 4.6±0.3). In the O_1 group of sausages, the number of aerobic mesophilic bacteria increased, so that on the 7th day the maximum value in log CFU/g was established (6.0±0.02, 6.0±0.02 and 6.1± 0.2, for the 1st, 2nd and 3rd fermentation, respectively). This was followed by a sudden drop in the number of bacteria; at the end of the ripening process, the established number in log CFU/g was 4.1±0.2, 4.3±0.2 and 4.3±0.3. The reduction of the number of bacteria at the end of the fermentation process in relation to the beginning, in both examined groups of samples, was statistically highly significant ($p<0.01$).

Table 3. The number of bacteria in the samples of Sremska sausage inoculated with *Listeria monocytogenes* (KO samples)

Microorganism	*t*/day				
	0	3	7	14	21
	N(bacteria)/(log CFU/g)*				
Total viable count					
fermentation I	5.3±0.2	5.3±0.2	5.6±0.1	6.5±0.1	4.7±0.3
fermentation II	5.3±0.2	5.3±0.1	5.7±0.1	6.5±0.1	4.7±0.2
fermentation III	5.3±0.1	5.45±0.04	5.7±0.1	6.5±0.2	4.6±0.3
LAB					
fermentation I	5.3±0.2	6.9±0.02	8.39±0.07	9.5±0.1	6.9±0.2
fermentation II	5.2±0.1	7.1±0.1	8.4±0.1	9.9±0.1	7.0±0.1
fermentation III	5.3±0.2	6.9±0.02	8.3±0.2	9.5±0.1	7.3±0.4
L. monocytogenes					
fermentation I	3.2±0.1	3.4±0.1	2.9±0.1	2.5±0.1	110 CFU/g
fermentation II	3.3±0.1	3.5±0.03	3.1±0.2	2.5±0.1	90 CFU/g
fermentation III	3.2±0.1	3.6±0.1	3.1±0.1	2.4±0.3	100 CFU/g

*each result is expressed as the average of measurements from three samples±standard deviation (S.D.)
CFU=colony forming units, LAB=lactic acid bacteria

Table 4. The number of bacteria in the samples of Sremska sausage inoculated with *Listeria monocytogenes* and containing added bacteriocin (O_1 samples)

Microorganism	*t*/day				
	0	3	7	14	21
	N(bacteria)/(log CFU/g)*				
Total viable count					
fermentation I	5.5±0.1	5.5±0.2	6.0±0.02	5.9±0.1	4.1±0.2
fermentation II	5.6±0.1	5.67±0.04	6.0±0.02	6.0±0.04	4.3±0.2
fermentation III	5.3±0.2	5.6±0.1	6.1±0.2	6.0±0.02	4.3±0.3
LAB					
fermentation I	5.5±0.1	6.8±0.1	7.6±0.1	9.1±0.4	6.2±0.1
fermentation II	5.4±0.3	6.6±0.1	7.1±0.2	9.1±0.2	6.2±0.1
fermentation III	4.42±0.08	6.6±0.1	7.98±0.02	9.1±0.1	6.3±0.3
L. monocytogenes					
fermentation I	3.2±0.1	2.4±0.1	2.6±0.1	70 CFU/g	n.d.
fermentation II	3.4±0.1	2.6±0.1	2.63±0.05	80 CFU/g	n.d.
fermentation III	3.4±0.1	2.6±0.1	2.7±0.1	60 CFU/g	n.d.

*each result is expressed as the average of measurements from three samples±S.D.
n.d.=not detected

The total number of LAB (in log CFU/g) in both examined groups of samples progressively increased from day 0. The lowest value was determined in the O_1 group (4.42±0.08, during the third fermentation), while other values were between 5.3±0.2 and 5.5±0.1. The largest number of LAB (in log CFU/g) was determined on day 14 of fermentation in both examined groups of sausage samples (control experiment, KO: 9.5±0.1, 9.9±0.1 and 9.5±0.1 during the 1st, 2nd and 3rd fermentation, respectively; the experimental group of sausages, O_1: 9.1±0.4, 9.1±0.2 and 9.1±0.1 during the 1st, 2nd and 3rd fermentation, respectively). At the end of fermentation, the established number of LAB was three logs lower than on day 14 of testing (KO: 6.9±0.2, 7.0±0.1 and 7.3±0.4 during the 1st, 2nd and 3rd fermentation, respectively; O_1: 6.2±0.1, 6.2±0.1 and 6.3±0.3 during the 1st, 2nd and 3rd fermentation, respectively). A statistically highly significant difference ($p<0.01$) was found between the number of LAB at the beginning and the end of the test in both experimental groups, as well as the number of LAB within the experimental groups.

The particularly important results in this study are those related to the changes in the number of inoculated *L. monocytogenes*. Based on these results, a straight answer about the impact of the added bacteriocins on the growth and the survival of this pathogen was obtained. In the O_1 group of samples of Sremska sausages, inoculated with *L. monocytogenes* and the bacteriocin isolated from *L. mesenteroides* ssp. *mesenteroides* IMAU:10231, the antilisterial capacity of this specific antimicrobial metabolite is particularly visible. The number of *L. monocytogenes*, from the initial value that was common to all experimental samples (approx. 3 logs), decreased to 70, 80 and 60 CFU/g on day 14. At the end of the fermentation and ripening of the tested sausages, we were not able to detect any remaining *L. monocytogenes*. Somewhat different results were determined in the KO group of samples. The number of *L. monocytogenes* (in log CFU/g) on day 0 was 3.2±0.1, 3.3±0.1 and 3.2±0.1, during the three fermentations respectively. The number of *L. monocytogenes* was the largest on the third day, and that value then gradually decreased. By day 21, the number of this pathogen had reduced to 110, 90 and 100 CFU/g.

The results of testing the antilisterial activity of this novel bacteriocin added in the production of traditional sausages confirmed the expected antilisterial properties of bacteriocin derived from our previous laboratory tests. However, as the production of traditional Sremska sausages is based on fermentation that occurs as a result of naturally present microbiota, the antilisterial activity in these conditions is likely the result of cumulative internal and external factors (*18*,*19*,*23*,*48*).

In this chain, the role of LAB is decisive, as, with their metabolic activity, they affect the development of useful sensory changes and simultaneously direct microbial development in the desired direction, thus ensuring safety and quality. In addition to the proven antimicrobial activity of the added bacteriocins, other antimicrobial factors also affect the safety of the product: sodium chloride, nitrite, smoke, smoke components, *etc*. Their cumulative effect was directly visible in the KO group, since the number of inoculated *L. monocytogenes* gradually decreased during fermentation (*49–51*).

Our positive results confirming the antilisterial activity of the added *Leuconostoc* bacteriocin in the production of traditional fermented sausages show clear and interesting potential which should be used to help ensure the complete safety of these fermented meat products for consumers in that respect. Even if bacteriocin-producing *Leuconostoc* strains cannot be used as starter cultures due to the fact that they produce considerable amounts of carbon dioxide during fermentation of carbohydrates, their bacteriocins can be used as biopreservatives in foods to control the growth of *L. monocytogenes* (*52*).

Certainly, for the direct application of bacteriocins and their entry into the full production process, in addition to receiving the status of GRAS (Generally Recognized as Safe) compounds, they must be well studied and harmonized with other technological factors in the production (pH, temperature, salt and nitrite) (*48*,*53*,*54*). Their implementation should be viewed in terms of good alternatives, especially when combined with other natural protectors (*55*) and, of course, good hygienic and manufacturing practices.

Conclusions

The growing need for naturally safe and healthy food has led to increased interest in the use of bacteriocin-producing LAB, which are used as starter cultures for the production of fermented products in the meat industry. The possibility of introducing bacteriocins as a factor in the hurdle technology approach to food protection has attracted scientists in the field of food safety (*50*).

L. mesenteroides ssp. *mesenteroides* IMAU:10231 isolated from Sremska sausage has been shown to produce a proteinaceous substance with antilisterial activity, a quality shared with most of the bacteriocins produced by *Leuconostoc* spp. Our positive results of testing the antilisterial activity of bacteriocin isolated from *L. mesenteroides* ssp. *mesenteroides* IMAU:10231 in the production of traditional fermented sausages confirmed the expected antilisterial properties of bacteriocin derived from our previous laboratory tests. The antilisterial activity of the added bacteriocin under the investigated temperatures prevailing in meat processing makes it a suitable biopreservative with the interesting potential which should be used to help ensure the complete safety of these fermented meat products.

On the other hand, it would be naive to believe that bacteriocins are the solution to all problems in the field of food safety. Their implementation should be viewed in terms of good alternatives, especially when combined with other natural protectors and of course, good hygienic and manufacturing practices. Therefore, our further research in this area will be directed to a better understanding of the nature of bacteriocins, their antimicrobial activities, possibilities for their application, as well as discovering new groups of bacteriocins, which could act as natural food preservatives, or bioprotectors when used in a controlled manner.

Acknowledgement

The results presented in this paper are part of Project III, No 46009: 'Improvement and development of hygienic and technological procedures in the production of foodstuffs of animal origin with the aim of producing high-quality and safe products competitive on the global market' funded by the Ministry of Education and Science of Serbia.

References

1. U. Schillinger, F.K. Lücke, Antibacterial activity of *Lactobacillus sakei* isolated from meat, *Appl. Environ. Microbiol. 55* (1989) 1901–1906.
2. L.M. McMullen, M.E. Stiles, Potential use of bacteriocin-producing lactic acid bacteria in the preservation of meats, *J. Food Protect.* (Suppl.), *59* (1996) 64–71.
3. M. Hugas, Bacteriocinogenic lactic acid bacteria for the biopreservation of meat and meat products, *Meat Sci.* (Suppl.), *49* (1998) 139–150.
4. J.R. Tagg, A.S. Dajani, L.W. Wannamaker, Bacteriocins of Gram-positive bacteria, *Bacteriol. Rev. 40* (1976) 722–756.
5. T.R. Klaenhammer, Genetics of bacteriocins produced by lactic acid bacteria, *FEMS Microbiol. Rev. 12* (1993) 39–85.
6. V.G.H. Eijsink, L. Axelsson, D.B. Diep, L.S. Håvarstein, H. Holo, I.F. Nes, Production of class II bacteriocins by lactic acid bacteria; An example of biological warfare and communication, *Antonie van Leeuwenhoek, 81* (2002) 639–654.
7. J. Cleveland, T.J. Montville, I.F. Nes, M.L. Chikindas, Bacteriocins: Safe, natural antimicrobials for food preservation, *Int. J. Food Microbiol. 71* (2001) 1–20.
8. C. Turcotte, C. Lacroix, E. Kheadr, L. Grignon, I. Fliss, A rapid turbidometric microplate bioassay for accurate quantification of lactic acid bacteria bacteriocins, *Int. J. Food Microbiol. 90* (2004) 283–293.
9. L. De Vuyst, E.J. Vandamme: Antimicrobial Potential of Lactic Acid Bacteria. In: *Bacteriocins of Lactic Acid Bacteria*, L. De Vuyst, E.J. Vandamme (Eds.), Blackie Academic and Professional, London, UK (1994) pp. 91–149.
10. A. Hurst, Nisin, *Adv. Appl. Microbiol. 27* (1981) 85–123.
11. R.D. Joerger, D.G. Hoover, S.F. Barefoot, K.M. Harmon, D.A. Grinstead, C.G. Nettles-Cutter: Bacteriocins. In: *Encyclopedia of Microbiology, Vol. 1*, I.J. Lederberg (Ed.), Academic Press, San Diego, CA, USA (2000) pp. 383–397.
12. J.N. Hansen, Antibiotics synthesized by posttranslational modification, *Annu. Rev. Microbiol. 47* (1993) 535–564.
13. H. Chen, D.G. Hoover, Bacteriocins and their food applications, *Compr. Rev. Food Sci. Food Safety, 2* (2003) 82–100.
14. R.P. Ross, C. Stanton, C. Hill, G.F. Fitzgerald, A. Coffey, Novel cultures for cheese improvement, *Trends Food Sci. Technol. 11* (2000) 96–104.
15. F. Leroy, J. Verluyten, L. De Vuyst, Functional meat starter cultures for improved sausage fermentation, *Int. J. Food Microbiol. 106* (2006) 270–285.
16. L. Cocolin, K. Rantsiou, L. Iacumin, R. Urso, C. Cantoni, G. Comi, Study of the ecology of fresh sausages and characterization of populations of lactic acid bacteria by molecular methods, *Appl. Environ. Microbiol. 70* (2004) 1883–1894.
17. Basic Local Alignment Search Tool (BLAST), National Center for Biotechnology Information (NCBI), Bethesda, MD, USA (*http://www.ncbi.nlm.nih.gov/BLAST*).
18. S. Vesković, The influence of bacteriocins isolated from *Leuconostoc mesenteroides* E 131 and *Lactobacillus sakei* I 154 on *Listeria monocytogenes* during production of Sremska sausage, *MSc Thesis*, Faculty of Agriculture, University of Belgrade, Belgrade, Serbia (2005) (in Serbian).
19. S. Vesković-Moračanin, Influence of *Lactobacillus sakei* I 151, bacteriocin of *Leuconostoc mesenteroides* E 131 and MAP on shelf life of Sremska sausage, *PhD Thesis*, Faculty of Agriculture, University of Belgrade, Belgrade, Serbia (2007) (in Serbian).
20. S.F. Barefoot, T.R. Klaenhammer, Detection and activity of lactacin B, a bacteriocin produced by *Lactobacillus acidophilus*, *Appl. Envion. Microbiol. 45* (1983) 1808–1815.
21. Horizontal Method for the Detection and Enumeration of *Listeria monocytogenes* – Part 1: Detection Method, ISO 11290-1, International Organisation for Standardization (ISO), Geneva, Switzerland (2004).
22. Horizontal Method for the Detection and Enumeration of *Listeria monocytogenes* – Part 2: Enumeration method, ISO 11290-2, International Organisation for Standardization (ISO), Geneva, Switzerland (2004).

23. L. Kozačinski, E. Drosinos, F. Čaklovica, L. Cocolin, J. Gasparik-Reichardt, S. Vesković, Investigation of microbial association of traditionally fermented sausages, *Food Technol. Biotechnol. 46* (2008) 93–106.
24. S. Vesković, B. Borović, B. Velebit, D. Karan, L. Turubatović, D. Obradović, Đ. Okanović, Phenotypisation of LAB strains isolated from 'Užička' sausage, *Proceedings of the 55th International Congress of Meat Science and Technology, (ICoMST)*, Copenhagen, Denmark (2009) pp. 888–892.
25. C.R. Woese, Bacterial evolution, *Microbiol Rev. 51* (1987) 221–271.
26. T. Aymerich, B. Martín, M. Garrida, M. Hugas, Microbial quality and direct PCR identification of lactic acid bacteria and nonpathogenic staphylococci from artisanal low-acid sausages, *Appl. Environ. Microbiol. 69* (2003) 4583–4594.
27. M.S. Champomier, M.C. Montel, F. Grimont, P.A.D. Grimont, Genomic identification of meat lactobacilli as *Lactobacillus sake*, *Ann. Inst. Pasteur Microbiol. 138* (1987) 751–758.
28. Y. Morishita, K. Shiromizu, Characterization of lactobacilli isolated from meats and meat products, *Int. J. Food Microbiol. 3* (1986) 19–29.
29. J.E. Niemand, W.H. Holzapfel, Characterization of lactobacilli isolated from radurised meat, *Int. J. Food Microbiol. 1* (1984) 99–110.
30. B.G. Shaw, C.D. Harding, A numerical taxonomic study of lactic acid bacteria from vacuum-packed beef, pork, lamb and bacon, *J. Appl. Bacteriol. 56* (1984) 25–40.
31. M.E. Stiles, Bacteriocins produced by *Leuconostoc* species, *J. Dairy Sci. 77* (1994) 2718–2724.
32. S. Ennahar, T. Sashihara, K. Sonomoto, A. Ishizaki, Class IIa bacteriocins: Biosynthesis, structure and activity, *FEMS Microbiol. Rev. 24* (2000) 85–106.
33. Y. Héchard, B. Dérijard, F. Letellier, Y. Cenatiempo, Characterization and purification of mesentericin Y105, an anti-*Listeria* bacteriocin from *Leuconostoc mesenteroides*, *J. Gen. Microbiol. 138* (1992) 2725–2731.
34. H. Daba, S. Pandian, J.F. Gosselin, R.E. Simard, J. Huang, C. Lacroix, Detection and activity of a bacteriocin produced by *Leuconostoc mesenteroides*, *Appl. Environ. Microbiol. 57* (1991) 3450–3455.
35. S.D. Todorov, L.M.T. Dicks, Characterization of mesentericin ST99, a bacteriocin produced by *Leuconostoc mesenteroides* subsp. *dextranicum* ST99 isolated from boza, *J. Ind. Microbiol. Biotechnol. 31* (2004) 323–329.
36. J.W. Hastings, M. Sailer, K. Johnson, K.L. Roy, J.C. Vederas, M.E. Stiles, Characterization of leucocin A-UAL 187 and cloning of the bacteriocin gene from *Leuconostoc gelidum*, *J. Bacteriol. 173* (1991) 7491–7500.
37. E.H. Drosinos, M. Mataragas, J. Metaxopoulos, Modelling of growth and bacteriocin production by *Leuconostoc mesenteroides* E131, *Meat Sci. 74* (2006) 690–696.
38. A. Holck, L. Axelsson, S.E. Birkeland, T. Aukrust, H. Blom, Purification and amino acid sequence of sakacin A, a bacteriocin from *Lactobacillus sake* Lb706, *Microbiology, 138* (1992) 2715–2720.
39. D. Guyonnet, C. Fremaux, Y. Cenatiempo, J.M. Berjeaud, Method for rapid purification of class IIa bacteriocins and comparison of their activities, *Appl. Environ. Microbiol. 66* (2000) 1744–1748.
40. N. Xiraphi, M. Georgalaki, K. Rantsiou, L. Cocolin, E. Tsakalidou, E.H. Drosinos, Purification and characterization of a bacteriocin produced by *Leuconostoc mesenteroides* E131, *Meat Sci. 80* (2008) 194–203.
41. D.J. Hartnett, A. Vaughan, D. van Sinderen, Antimicrobial-producing lactic acid bacteria isolated from raw barley and sorghum, *J. Inst. Brew. 108* (2002) 169–177.
42. S. Vesković-Moračanin, D. Obradović, B. Velebit, B. Borović, M. Škrinjar, L. Turubatović, Antimicrobial properties of indigenous *Lactobacillus sakei* strain, *Acta Vet. 60* (2010) 59–66.
43. S. Cosansu, H. Kuleasan, K. Ayhan, L. Materon, Antimicrobial activity and protein profiles of *Pediococcus* spp. isolated from Turkish 'Sucuk', *J. Food Process. Preserv. 31* (2007) 190–200.
44. K. Mattila, P. Saris, S. Työppönen, Survival of *Listeria monocytogenes* on sliced cooked sausage after treatment with pediocin AcH, *Int. J. Food Microbiol. 89* (2003) 281–286.
45. E. Rodríguez, J. Calzada, J.L. Arqués, J.M. Rodríguez, M. Nuñez, M. Medina, Antimicrobial activity of pediocin-producing *Lactococcus lactis* on *Listeria monocytogenes*, *Staphylococcus aureus* and *Esherichia coli* O157:H7 in cheese, *Int. Dairy J. 15* (2005) 51–57.
46. I.F. Nes, H. Holo, Class II antimicrobial peptides from lactic acid bacteria, *Biopolymers, 55* (2000) 50–61.
47. E.H. Drosinos, M. Mataragas, S. Vesković-Moračanin, J. Gasparik-Reichardt, M. Hadžiosmanović, D. Alagić, Quantifying nonthermal inactivation of *Listeria monocytogenes* in European fermented sausages using bacteriocinogenic lactic acid bacteria or their bacteriocins: A case study for risk assessment, *J. Food Protect. 69* (2006) 2648–2663.
48. S. Vesković: *Lactic Acid Bacteria Bacteriocins – Possibilities of Application in Production of Fermented Sausages*, Andrejević Publisher, Belgrade, Serbia (2009) (in Serbian).
49. P.S. Fernández, S.M. George, C.C. Sills, M.W. Peck, Predictive model of the effect of CO_2, pH, temperature and NaCl on the growth of *Listeria monocytogenes*, *Int. J. Food Microbiol. 37* (1997) 37–45.
50. L. Leistner, Basic aspects of food preservation by hurdle technology, *Int. J. Food Microbiol. 55* (2000) 181–186.
51. G. Vignolo, S. Fadda, M.N. de Kairuz, A.P. de R. Holgado, G. Oliver, Effects of curing additives on the control of *Listeria monocytogenes* by lactocin 705 in meat slurry, *Food Microbiol. 15* (1998) 259–264.
52. N. Zdolec, M. Hadžiosmanović, L. Kozačinski, Ž. Cvrtila, I. Filipović, M. Škrivanko, K. Leskovar, Microbial and physicochemical succession in fermented sausages produced with bacteriocinogenic culture of *Lactobacillus sakei* and semi-purified bacteriocin mesenterocin Y, *Meat Sci. 80* (2008) 480–487.
53. J. Cleveland, T.J. Montville, I.F. Nes, M.L. Chikindas, Bacteriocins: Safe, natural antimicrobials for food preservation, *Int. J. Food Microbiol. 71* (2001) 1–20.
54. D. Obradović, S. Vesković-Moračanin, Functional fermented sausages – Present state and perspectives, *Tehnologija mesa, 48* (2007) 93–97 (in Serbian).
55. F. Leroy, L. De Vuyst, Lactic acid bacteria as functional starter cultures for the food fermentation industry, *Trends Food Sci. Technol. 15* (2004) 67–78.