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Utilization of bacteriocin-producing bacteria in dairy products

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Abstract

Lactic acid bacteria have been used since ancient times for food preparation and for bio-conservation by fermentation. Selected strains are capable of producing antimicrobial peptides - bacteriocins, which can be natural preservatives, especially in products with short shelf lives. The present study is focused on inhibitory effects of the bacteriocin-producing bacteria strains *Enterococcus faecium*, *Pediococccus acidilactici* and *Lactobacillus plantarum* against *Listeria innocua* as an indicator microorganism. Freeze-dried preparations of bacterial strains producing particular bacteriocins were tested by agar well-diffusion assay and by the traditional spread plate method. Plantaricin exhibited the highest anti-listerial effect among the tested bacteriocins. Pediocin also demonstrated a distinct inhibitory effect, but enterocin appeared to be heat labile and its efficiency was also suppressed under cold storage conditions. Plantaricin reduced *Listeria innocua* counts by 1 log in dairy spread made from cheese and quark. The formation of bacteriocins by various *Lactobacillus plantarum* strains were substantially influenced by the cultivation conditions of the mother culture and by the microbial preparation process before freeze-drying. Bacteriocins introduced into foodstuffs via protective cultures *in situ* offer new perspectives on enhancing food quality and safety.

Key words: bio-conservation, protective culture, lactic acid bacteria, bacteriocin, *Listeria* spp.

Introduction

Food-borne pathogens represent a major problem in the field of public health worldwide. Pathogenic microorganisms take the first place on the list of causes of illness and death in developing countries, with the number of fatalities approximating 1.8 million people per year (Fratamico et al., 2005). The group of bacteria responsible for foodborne diseases include species such as *Campylobacter* spp., *Salmonella* spp., *Staphylococcus aureus*, *Yersinia enterocolitica*, *Vibrio* spp., *Listeria monocytogenes*, *Shigella* spp., *Escherichia coli*, *Clostridium botulinum*, *Clostridium perfringens* and *Bacillus cereus* (McLaughlin, 2006).

The species *L. monocytogenes* is commonly found in soil, surface water, on plants and in food (McLaughlin, 2006). *L. monocytogenes* is an important human pathogen and is a frequent cause of infection, being able to grow even under refrigeration temperatures and in packaged products intended for rapid consumption. Mortality in infected individuals ranges from 20 to 30 % (Vázquez-Boland et al., 2001). The categories most affected in the

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EU are elderly people over 65 years old, children up to 4 years of age, and pregnant women (Vázquez-Boland et al., 2001). Milk and dairy products are important sources of possible listeriosis. *Listeria* spp. bacteria are mainly located on the cow's udder, and are transferred from there to the dairy plant environment and can enter the raw cow's milk during milking (Bell and Kyriakides, 2009). Consumption of contaminated food is the main route of transmission to humans (Blažková et al., 2005).

Modern consumer trends and food legislation set high requirements to food preservation. Consumers demand high quality foods with minimum processing, the absence of preservatives and, at the same time, being safe and having extended shelf lives. In addition, current EU legislation (Commission Regulation (EU) No 1129/2011) modified the conditions for use of chemical preservatives. Therefore, biological conservation using bio-protective cultures, as a possible approach to achieve food safety and shelf-life control, has gained an increasing attention.

Lactic acid bacteria are capable of producing bacteriocins - extracellular bioactive peptides with proteolytic and other activities (Todorov et al., 2011). These compounds have bactericidal or bacteriostatic effects on technologically or hygienically undesirable bacteria such as *Escherichia coli*, *Salmonella typhimurium*, *Campylobacter jejuni* etc. (Deegan et al., 2006; Molloy et al., 2011; Güllüce et al., 2013). However, their narrow spectrum of inhibition is a limiting factor in the application of bacteriocins (Giraffa, 1995). Most bacteriocins are active against Gram positive (G^+) bacteria, but some recently described bacteriocins may also act against Gram negative (G^-) organisms in the gastrointestinal tract of humans and animals. *Pediococcus acidilactici* is capable of producing pediocins that have antimicrobial activity against a broad range of G^+ as well as G^- bacteria (Rodríguez et al., 2005; Papagianni and Anastasiadou, 2009). Enterocins were isolated from the bacterium *Enterococcus faecium*, which is used in the production of fermented sausages, vegetables and dairy products (Giraffa, 1995; Alvarez-Cisneros et al., 2011; Medina and Nunez, 2011; Ghrairi et al., 2012). According to Trmcic et al. (2010 a, b) enterococci and their bacteriocins prevailed in cheese microbiota and exhibited anti-staphylococcal activity. Plantaricin is a thermostable antimicrobial peptide (6.5 kDa) produced by *Lactobacillus plantarum* (Molloy et al., 2011; Sankar et al., 2012; Guidone et al., 2014). This bacteriocin was proven to inhibit growth of *Enterobacter*, *Enterococcus*, *Lactobacillus*, *Pseudomonas*, *Streptococcus* and *Staphylococcus* bacterial strains (Todorov et al., 2011). González et al. (1996) described its bactericidal effect against G^+ bacteria through action against the cytoplasmic membrane. According to Field et al. (2007), Solichová et al. (2012), Trivedi et al. (2012) and Složilová et al. (2014), bacteriocins produced by lactic acid bacteria are not attached to the surface of producing strains but are released into the medium. Cell-free neutralised supernatants of bacteriocin-producing lactic acid bacterial strains were successfully used by Solichová et al. (2012) and Složilová et al. (2014) to test their antimicrobial effects against pathogenic bacteria.

Bacteriocins are considered to be one possible solution against the growing problem of pathogen-resistance to conventional antibiotics (Sang and Blecha, 2008) and they could be applied to a wide range of food products (Nithya et al., 2012). From the perspective of the current legislation, only nisin (a bacteriocin produced by *Lactococcus lactis*) has been approved by the FDA in the USA and in the EU (Commission Regulation (EU) No 1129/2011) (Liu and Hansen, 1990; Ghrairi et al., 2012; Vignolo et al., 2012), though some other bacteriocins may also be safe for use in the food supply (Cleveland et al., 2001).

Generally, there are two basic methods of applying bacteriocins to food - inoculation *in situ* by the bacterial strain used as a protective culture (Holzapfel, 2002) or application *ex situ* which implies the addition of a partially or completely purified bacteriocin preparation. These approaches, however, lack the legislative support that has been established for nisin (Campos et al., 2011).

The aim of this study was to investigate the inhibitory effects of bacteriocins produced by strains *Enterococcus faecium*, *Pediococccus acidilactici* and *Lactobacillus plantarum* against an indicator non-pathogenic strain of *Listeria innocua* in model experiments on cultivation medium and in dairy spreads made from cheese and quark.

Materials and methods

Media and cultures

Agar Listeria according to Ottaviani and Agosti (ALOA) (Bio-Rad); Brain Heart Infusion Agar (BHI) (AES Laboratoire); Brain Heart Infusion Broth (AES Laboratoire) were used for bacterial cultivation and bacteriocin determination.

The following lyophilised cultures of bacteria with presumed bacteriocin production were used:

- A commercial preparation of *Pediococcus acidilactici* named Fargo 37 at a concentration 1×10^6 CFU/g (Amerex Praha spol. s r. o);
- The preparation of *Enterococcus faecium* CCDM 945 at a concentration of 3×10^{10} CFU/g (Czech Collection of Dairy Microorganisms, *Laktoflora®*, MILCOM a.s., CZ);
- A preparation of *Lactobacillus plantarum* CCDM 1078 at a concentration of 1.5×10^9 CFU/g (Czech Collection of Dairy microorganisms, *Laktoflora®*, MILCOM a.s., CZ);
- *Listeria innocua* CCM 4030 (LI CCM4030) (Czech Collection of Microorganisms, Masaryk University, Brno);
- *Listeria innocua* Ln08 (LI Ln08) (Czech Collection of Dairy Microorganisms, *Laktoflora®*, MILCOM a.s., CZ).

Suspensions of bacteriocin-producing bacteria were prepared by mixing a lyophilised microbial culture with sterile brain heart infusion broth. Bacteriocin production by the tested lactic acid bacteria (particularly *Enterococcus mundti* 1282, *Lactobacillus plantarum* DMF30128 and *Lactobacillus plantarum* HV11, *Pediococcus acidilactici* HV12) was proven in Project 2B0850 by polymerase chain reaction (PCR) (Demnerová et al., 2011). According to Wieckowicz et al. (2011) the advantage of this method is rapid detection of genes encoding class IIa bacteriocins. PCR detection is based on the use of primers that allow the identification of previously unidentified bacteriocins. The methodology includes all sequences deposited in the NCBI database.

Effect of thermal treatment and incubation conditions on the antimicrobial activity of selected strains

BHI agar was separately inoculated with *Listeria innocua* CCM4030 (LI CCM4030) and *Listeria innocua* Ln08 (LI Ln08) at a concentration of 10^5 CFU/mL. Microbial preparations containing *Pediococcus acidilactici* (PED), *Enterococcus faecium* (ENT) and *Lactobacillus plantarum* (PLA) were dissolved in 100 mL of sterile water at 0.1 %; 0.2 %; 0.3 %; and 1% concentrations and separated into 2 glass bottles each; one part was heat treated at 72 °C for 5 minutes. The other part of the suspension was not heat treated. Small volumes (0.2 mL) of heat- and non heat-treated suspensions were placed into 8 mm diameter holes on Petri dishes. These were then incubated either at 37 °C for 24 hours or at 7 °C for 5 days. The diameters of zones of inhibition were measured. Each assay was carried out in triplicate.

Antimicrobial activity of selected strains in cheese and quark based spreads

Dairy spreads were prepared in Thermomix laboratory equipment without heat treatment. The cheese dairy spread was composed of smear surface-ripened cheese (*Olomoucke tvaruzky*) (40 g), boiled water (14.2 g), edible salt (0.8 g), butter (5 g), sour cream with 16 % fat (5 g), natural yoghurt with 3.5 % fat (10 g) and quark with dry matter of 23 % (25 g).

The quark based spread consisted of quark with 23 % dry matter (80 g), boiled water (5 g), edible salt (1 g), sour cream with 16 % fat (4 g), natural yoghurt with 3.5 % fat (10 g).

Non heat treated suspensions of tested strains (ENT, PED, and PLA) were added at a concentration of 0.3 % v/v and in the case of PLA, also at a concentration of 1 % v/v. Samples of cheese and quark spreads were artificially contaminated with LI in amounts of 10^1 and 10^3 CFU/g spread and stored in a refrigerator at 6±2 °C for 6 days. Enumeration of LI was performed according to EN ISO 11290-2, Part 2, using ALOA agar.

Detection of genes encoding bacteriocins by polymerase chain reaction (PCR)

The aim of this method is the rapid detection of genes encoding class IIa bacteriocins, to which the tested bacteriocins belong. The expected sizes of the primer sequences and products are given in Table 1.

Lysates of 1 mL of fresh culture were prepared for analysis. Fresh cultures were prepared as follows: 1 mL of deep frozen bacterial culture was inoculated into MRSC or M17 broth and cultivated at 37 °C for 24 hours. New broth was reinoculated with this culture under the same conditions. After washing the centrifuged cells twice with sterile PCR water (18 MΩ), the precipitate was lysed with 100 μL of alkaline lysis solution (0.05M NaOH+0.25% sodium dodecyl sulfate), heated at 95 °C for 30 min and rapidly cooled. Samples were then diluted with 50x PCR water and stored at -20 °C until analysis. Just prior to adding the PCR mixture, samples were reheated for 5 min at 95 °C. The PCR mixtures for strains of *Lactobacillus plantarum* and *Pediococcus acidilactici* were prepared as follows: 9.9 μL of PCR water; 2 μL of 10 x PCR green complete buffer with $MgCl_2$ (Top-Bio, CR); 0.4 μL of dNTPs (10 mM, Fermentas, USA); 0.5 μL of primers BCgr1NSf and BCgr1R1K (Wieckowicz et al., 2011) (10 pmol/μL); 0.4 μL of DMSO; 1.8 μl of $MgCl_2$ (25 mM, Top-Bio, CR); 0.5 μL of CombiTaq polymerase (1 U/μL, Top-Bio, CR) and 4 μL of a template (crude lysate 50x diluted). The total volume of PCR mixture was 20 μL. Amplification was carried out in 32 cycles under the following conditions: 94 °C/45 s - DNA denaturation, 47 °C/45 s - annealing of the primers, 72 °C/60 s - DNA chain synthesis. Before the first cycle of amplification, the mixture was heated at 94 °C for 5 min. In the last cycle, chain synthesis was extended by 5 min. The reaction mixture was then cooled to 10 °C.

The PCR mixture for *Enterococcus faecium* was prepared as follows: 13.4 μL of PCR water; 2 μL of 10x PCR green complete buffer with $MgCl_2$ (Top-Bio, CR); 0.4 μl of dNTPs (10 mM, Fermentas, USA); 0.5 μL of primers Clade4-3F and Clade4-3R (Liu et al., 2014) (10 pmol/μL); 0.8 μL of Taq Purple polymerase (1 U/μL, Fermentas); 0.4 μL of $MgCl_2$ (25 mM, Top-Bio, CR); and 4 μL of a template (HL 50x). The total volume of PCR mixture was 20 μL. Amplification was carried out in 35 cycles under the following conditions: 94 °C/30 s - DNA denaturation, 56 °C/30 s - annealing of the primers, 72 °C/120 s - DNA chain synthesis. Before the first cycle of amplification, the mixture was heated at 94 °C for 5 min. In the last cycle, chain synthesis was extended by 10 min. The reaction mixture was then cooled to 10 °C.

Thermocycler TProfessional (Biometra, DE) was used for PCR mixture amplification. Separation of amplicons was performed using gel electrophoresis; 5 μL of the PCR mixture were mixed together with coating buffer and GelRed (Biotium) fluorescent dye and loaded onto a 2 % w/v agarose gel. Electrophoresis was carried out for 90 min in 0.5x TBE buffer at 90 V. Marker 0'RangeRuler 50 bp DNA Ladder (Thermo Scientific, USA) was added to determine the size of individual fragments. After electrophoresis, the gel was photographed using a UV-transilluminator Genius Gene 12 (Syngene, UK). The results were processed using Zoner Photo Studio 16 (Zoner Software, CZ).

Results and discussion

Effect of thermal treatment and incubation conditions on the antimicrobial activities of selected strains

In the first stage of the study the antimicrobial activities of selected bacteria with presumed bacteriocin production against two strains of *Listeria innocua*, before and after thermal treatment, were studied. Inhibitory activity was expressed as the average size (plus standard deviation) of inhibition zones. All control samples (Petri dishes without suspensions of PLA, PED or ENT) cultivated

Table 1. Combination of primers and sizes of the resulting products (Wieckowicz et al., 2001; Liu et al., 2014)

Clade	Primer	Sequence (5´ to 3´)	Product size (bp)
I	BCgr1NSf	GGT GGT AAA TAC TAT GGT AA	62
	BCgr1R1K	CCC CAG TTA ACA GAG CA	
IV-III	Clade4-3F	GAC ACA CAA CTT ATC TAT GGG GGT	150-200
	Clade4-3R	CCTGGAATTGCTCCACCTAA	

with tested strains of *L. innocua* showed no zones of inhibition. Growth of LI Ln08 at 37 °C for 24 h in the presence of tested strains (ENT, PED, PLA) before and after heat treatment (72 °C/5 minutes) were compared in Figures 1 and 2 (LI Ln08) and in Figures 3 and 4 (LI CCM4030) as well. Accordingly, the zones of *L. innocua* inhibition increased along with the concentrations of tested bacteria in both suspensions. PLA (both without and after heat treatment) clearly had the strongest inhibitory effect, which was similar for both *Listeria* strains. Its inhibition zones were readable even at the lowest concentration i.e. 0.1 %.

At that concentration, PED did not create any inhibitory zone, but at higher concentrations it was more effective than ENT. Furthermore, unheated test strains demonstrated generally higher inhibitory effects than those that were heated.

Based on the above mentioned results, experiments were focused on the antimicrobial activity of selected bacteria against *Listeria innocua* before and after thermal treatment but under different incubation conditions. Figures 5 and 6 demonstrate the sizes of inhibition zones when LI CCM4030 was cultivated in the cold (7 °C/5 days) and at 37 °C/24 h in the presence of heat-treated and untreated bacterial strains. The anti-listerial effects of the tested bacterial strains incubated at 7 °C were very different from that of their counterparts incubated at 37 °C. No inhibitory effect of ENT was evident after incubation at 7 °C for 5 days, before or after heat treatment.

The efficacy of bacteriocin preparations or the addition of bacteriocin-producing cultures were also dependant on the viability of the bacterial strains and on growth conditions such as temperature, pH, water activity etc. The lower efficiency of PLA and PED against *Listeria* appeared to be the result of rapid bacterial proliferation at 37 °C (Tienungoon et al., 2000) where bacterial numbers increased but the level of bacteriocins remained constant. The application of bacteriocins as a protective agent against *Listeria* may be particularly effective at low cell counts of contaminants, low pH and an uninterrupted cold chain (Ravishankar Rai, 2015).

Figure 1. Inhibition of LI Ln08 growth by non-heat treated suspensions of ENT, PED and PLA

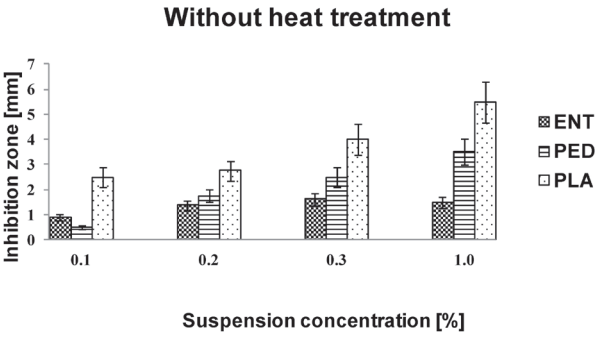


Figure 2. Inhibition of LI Ln08 growth by heat treated suspensions of ENT, PED and PLA

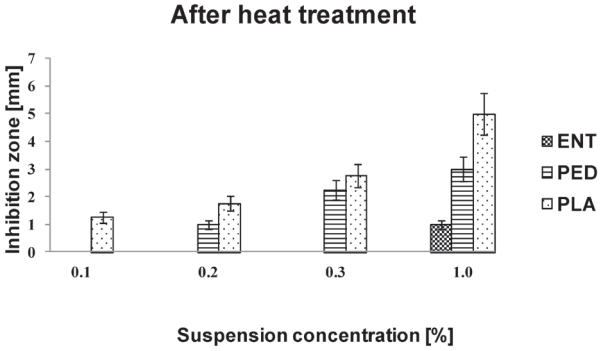


Figure 3. Inhibition of LI CCM4030 growth by non-heat treated suspensions of ENT, PED and PLA

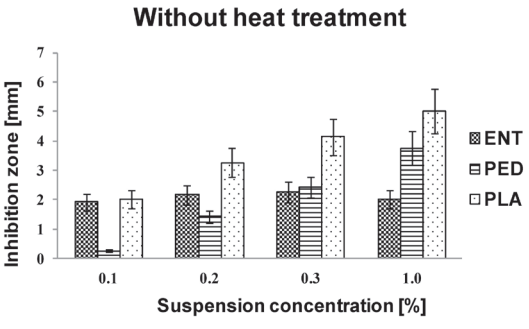


Figure 4. Inhibition of LI CCM4030 growth by heat treated suspensions of ENT, PED and PLA

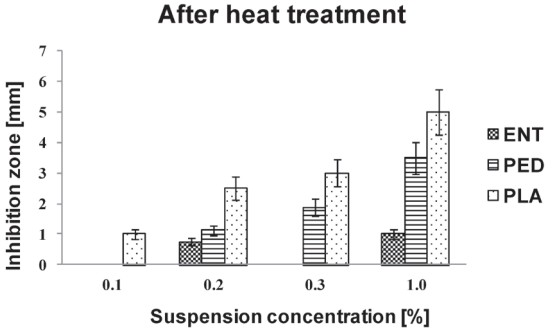


Figure 5. Inhibition of LI CCM4030 growth by non-heat treated suspensions of PED and PLA under different incubation conditions

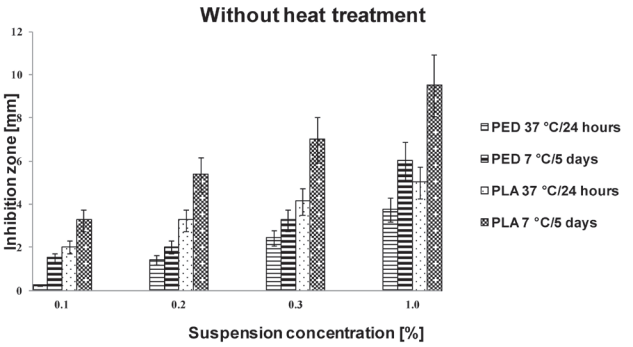
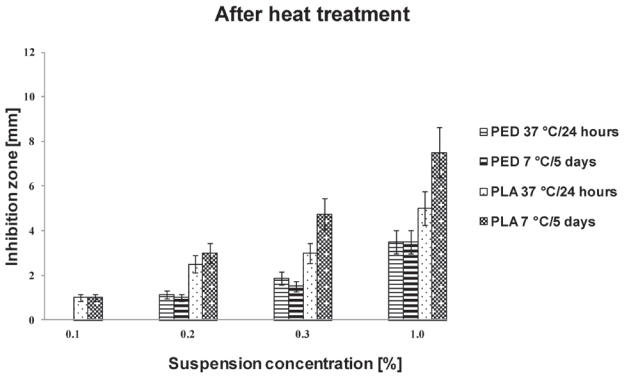


Figure 6. Inhibition of LI CCM4030 growth by heat treated suspensions of PED and PLA under different incubation conditions



PED, after heat treatment, showed nearly the same anti-listerial activity at both temperatures at dosages from 0.2 to 1 %, while with high doses of non-heated PLA preparations the inhibitory effect on *L. innocua* was greater than with heated PLA. The degree of inhibition by PED, similarly to PLA, was higher when incubation took place at 7 °C compared to incubation at 37 °C. PED (0.3 % and 1 %) showed approximately 30-50 % higher values and PLA (0.1 % to 1 %) about 100 % higher values at 7 °C than at 37 °C.

Antimicrobial activity of selected strains in cheese- and quark-based spreads

An evaluation of the antimicrobial activity of selected bacteriocin-producing strains in cheese- and quark-based spreads was also carried out.

The pH of cheese spreads (with and without PLA) varied between 5.55 and 5.98 during 6 days of storage at 7 °C. Samples with PLA did not show any sensory defects compared to spreads without bacterial strains. The addition of PLA (0.3 %) resulted in a reduction in LI CCM4030 from 1.9×10^3 to 6.3 x 10^2 CFU/g (Table 2). The use of 1 % PLA decreased LI CCM4030 counts by one log to 1.0×10^2 CFU/g in comparison with the control sample at 1.9 ×x 10^3 CFU/g.

The pH value of quark spreads ranged from 4.27-4.72 during 9 days of storage at 7 °C. No differences in sensory properties were observed between samples with and without PED or PLA. As shown in Table 3, the addition of PLA (0.3 %) resulted in a reduction in LI CCM4030 to 5.0×10^2 CFU/g. The use of 1 % PLA decreased counts by one log to 3.0×10^2 in comparison with the control. PED (0.3 %) did not exhibit any significant inhibitory effect against LI CCM4030.

Table 2. Counts of LI CCM4030 (CFU/g) in cheese spread three days after manufacture

Sample	LI CCM4030 (CFU/g) after 3 days
Control +LI	$(1.9\pm0.3)\times10^3$
PLA 0.3 %+LI	$(6.3\pm0.7)\times10^2$
PLA 1.0 %+LI	$(1.0\pm0.2)\times10^2$

Detection of genes encoding bacteriocins by polymerase chain reaction (PCR)

In agreement with the demonstrated effect of the tested lactic acid bacterial strains, PCR analysis showed that all three strains contained genes for bacteriocin production (Figures 7 and 8).

Growth inhibition of *Listeria* via application of strains producing bacteriocins was demonstrated in our experiments on model substrates as well as on real samples of dairy products. The similar results in the case of PLA and PED (via well diffusion assay) were found by Puttalingamma et al. (2006). PLA and PED strains may therefore be suitable as protective cultures for dairy products that are perishable and must be stored cold. Zdolec et al. (2007) also observed inhibitory activities of lactic acid bacteria against *L. monocytogenes* NCTC 10527 by agar well diffusion assay. When Bleicher et al. (2010) cultivated *L. monocytogenes* EGDe in cell-free supernatants prepared from red smear cheese containing bacterial consortia, including lactic acid bacteria, almost 20 % of these supernatants exhibited bactericidal activity. Nearly complete eradication on red smear cheese was observed at 10^2 CFU of *L. monocytogenes* WSLC 1364, originating from a cheese-borne outbreak, in the presence of pediocin AcH-producing *Lactobacillus plantarum* (Loessner et al., 2003). Drider et al. (2006) concluded that pediocin-like bacteriocins are the most promising antimicrobial peptides for food as well as for selected medical applications.

The observed absence of ENT effect at 7 °C in the present experiment was not in agreement with conclusions of Kumar and Srivastava (2010) who demonstrated an efficacy of enterocin against *L. monocytogenes* at temperatures from 4 to 37 °C. Ennahar and Deschamps (2000), and Chen and Hoover (2002) also reported anti-listerial effects of enterocin. But those authors also reported that some

Figure 7. PCR amplification of the genes encoding bacteriocins with primers BCgr1NSf and BCgr1R1K. 1: 50 bp ladder, 2: negative control, 3: CCDM 1078 *Lactobacillus plantarum*, 4: CCDM 945 *Enterococcus faecium*, 5: FARGO 37 *Pediococcus acidilactici*. Expected size of fragments, 62 bp

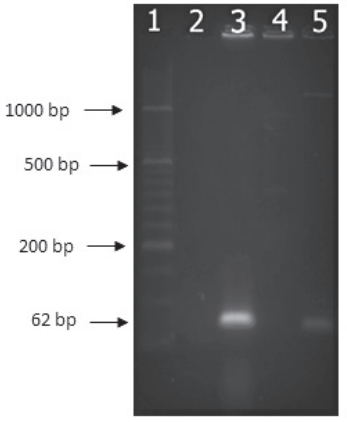


Figure 8. PCR amplification of the genes encoding bacteriocins with primers Clade4-3F and Clade4-3R. 1: 50 bp ladder, 2: negative control, 3: CCDM 945 *Enterococcus faecium*. Expected size of fragments, 150 - 200 bp

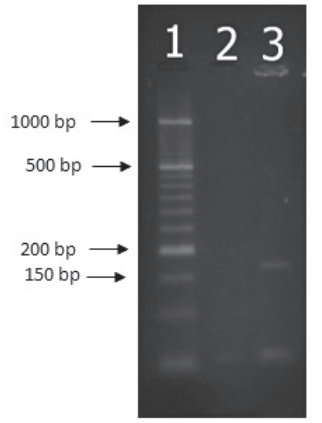


Table 3. Counts of LI CCM4030 (CFU/g) in quark spread three and five days after manufacture

Sample	LI CCM4030 (CFU/g) after 3 days	LI CCM4030 (CFU/g) after 5 days
Control	$<1\times10^1$	$<1\times10^1$
Control + LI	$(1.4\pm0.2)\times10^3$	$(1.2\pm0.5)\times10^3$
PED 0.3 %+LI	$(1.3\pm0.3)\times10^3$	$(8.8\pm0.7)\times10^2$
PLA 0.3 %+LI	$(6.0\pm0.6)\times10^2$	$(5.0\pm0.4)\times10^2$
PLA 1 %+LI	$(4.0\pm0.3)\times10^2$	$(3.0\pm0.2)\times10^2$

bacteriocins may not be effective against *Listeria innocua*, possibly explaining our experimental results.

The application of bacteriocins to real food products often requires higher concentrations of bacteriocins to achieve the same degree of inhibition as seen in model experiments on nutrient medium. Hartman et al. (2011) and Guidone et al. (2014) reported similar findings. On the other hand, lactic acid bacteria may also exhibit antibacterial activity due to other compounds or mechanisms and not only due to bacteriocin production (Ammor et al., 2006). Aguilar and Klotz (2010) reported growth patterns of bacterial systems and their mechanisms of inhibition to be complex and not attributable to only one factor. Bacteriocins have many desirable properties as food preservatives such as non-toxicity, natural origin, thermostability, degradability by digestive enzymes and absence of flavour and odours.

Conclusions

Selected strains of *Pediococcus acidilactici*, *Enterococcus faecium*, *and Lactobacillus plantarum* inhibited growth of *Listeria innocua* CCM4030 and *Listeria innocua* Ln08 under experimental conditions. *Pediococcus acidilactici* and especially *Lactobacillus plantarum* exhibited higher antimicrobial effects against *Listeria innocua* under refrigerated conditions than at 37 °C. Thermal treatment of bacteria (72 °C/5 min) inversely affected their inhibitory activities. Anti-bacterial effects depend on the selected strain, composition of culture medium, dose of inoculum and target microorganisms. *Lactobacillus plantarum* exhibited antimicrobial affects by a reduction in the number of *Listeria innocua* in real samples of cheese- and quark-based spreads.

Upotreba bakterijskih sojeva koji sintetiziraju bakteriocine u proizvodnji mliječnih prerađevina

Sažetak

Bakterije mliječne kiseline od davnina se koriste u proizvodnji hrane te za biološko konzerviranje putem vrenja. Odabrani sojevi bakterija mliječne kiseline mogu sintetizirati bakteriocine - peptide s antimikrobnim djelovanjem, a koji se mogu koristiti kao prirodni konzervansi i to prije svega za proizvode kratkog roka trajanja. Svrha ovog istraživanja bila je ispitati inhibitiorne učinke sojeva *Enterococcus faecium*, *Pediococccus acidilactici* i *Lactobacillus plantarum* koji sintetiziraju bakteriocine naspram soja *Listeria innocua* kao testnog mikroorganizma. Dubokosmrznuti pripravci bakterijskih sojeva koji sintetiziraju specifične bakteriocine ispitivani su dvama metodama - metodom ubrizgavanja kulture u rupice na testnom agaru i klasičnim nacjepljivanjem na hranjivu podlogu. Među svim ispitivanim bakteriocinima plantaricin je pokazao najjači inhbitorni učinak naspram testnog soja *Listeria innouca*. Pediocin je također pokazao određeni inhibitorni učinak, dok se enterocin pokazao termolabilnim te se njegov učinak znatno smanjio u uvjetima hladnog čuvanja. U uzorcima namaza na bazi sira i kvarka plantaricin je uzrokovao redukciju broja živih stanica soja *Listeria innocua* za 1 log. Na sposobnost proizvodnje bakteriocina u različitih sojeva vrste *Lactobacillus plantarum* snažno su utjecali uvjeti uzgoja izvorne kulture kao i primijenjeni procesi pripreme prije postupka dubokog smrzavanja. Bakteriocini koji se namirnicama dodaju *in situ* putem zaštitnih kultura predstavljaju nove mogućnosti za poboljšanje kvalitete i sigurnosti prehrambenih proizvoda.

Ključne riječi: biološko konzerviranje, zaštitne kulture, bakterije mliječne kiseline, bakteriocin, *Listeria* spp.

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