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ANTILISTERIAL EFFECT OF BACTERIOCIN ISOLATED FROM *ENTEROCOCCUS FAECALIS* DURING THE FERMENTATION OF SOFT WHITE CHEESE

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Abstract

Until recently, genus *Enterococcus* is considered as indicator of fecal contamination. Nowadays, strains of this genus are considered as common microbiota, particularly inautochthonous kinds of cheese, in which they contribute to the development of specific sensory properties. Also, numerous *Enterococcus* strains, isolated from different fermented and non-fermented foodstuffs, produce many types of bacteriocins that induced many investigations due to perceiving its use in foodstuff protection. In this paper, it is investigated the antilisterial effect of bacteriocins produced by *Enterococcus faecalis*, isolated from autochthonous Serbian white cheese (Zlatar cheese), in the aim of *Listeria monocytogenes* growth control during soft white cheese manufacturing.

Isolation of semi purified bacteriocin from *E. faecalis* (enterocin) was done by the method of saturated precipitation with ammonium-sulphate, adjusted to the individual laboratory conditions. The activity of the isolated, semi purified enterocin was determined by the critical dilution method against the selected test microorganism - *L. monocytogenes* ATCC 19111. Its strength was approximately 1280 AU/mL. After milk coagulation on traditional way by commercial rennet with strength of 1 : 5,000 (1.5 - 2 mL/L), the total amount was divided into three equal parts: the first part was control group (K), the second part was inoculated with *L. monocytogenes* (ca. 104 cells/mL) (O-1), in the third part was added *L. monocytogenes* and bacteriocin isolated from *E. faecalis* (1280 AU/mL (O-2)). Manufacturing of these cheese was repeated three times. Samples for laboratory examinations were taken on days: 0, 2, 4, 7, 14 and 21. Determination of the presence of *Listeria* spp. was carried out following the procedure of the ISO 11290 - 1, 2 ([26] and [27]). The software package "Statistica for Windows" (StatSoft, Inc., USA) was used for statistical analysis. Differences between average values are presented on the level of 95% ($P \leq 0.05$).

L. monocytogenes was not detected in any sample of the control group. In the experimental group of cheeses with added bacteriocins (group O-2), at the end of maturation, there was the statistically significant level of reduction of *L. monocytogenes* number ($P \geq 0.05$). From the initial number of 104 CFU/g, at the end of the maturation, the number of this pathogen was reduced under 100 CFU/g.

These results show that enterocin was successful in preventing *L. monocytogenes* growth, despite the possible inactivation by various interactions within the food system. A very important fact is that the strain tested in the present work is isolated from a traditional Zlatar cheese, represents the natural bioprotector isolated from a similar matrix.

***Key words**: Enterocoocus faecalis, Bacteriocin, Antilisterial effect, Cheese.*

1. Introduction

Lactic acid bacteria (LAB) a heterogeneous group of acid-tolerant, Gram-positive, catalase- and oxidase-negative microorganisms which produce lactic acid during homofermentative or heterofermentative metabolism (Klaenhammer *et al.*, [1], Holzapfel and Wood, [2]). Due to their metabolic properties (in addition to lactic acid, they produce: other volatile organic acids, H_2O_2,CO_2, diacetyl, reuterin and bacteriocins - specific antibacterial compounds), LAB are responsible for lactic acid fermentation, the ripening process, flavor development and nutritional characteristics of the fermented product, and have direct effect on the finished product shelf-life (Holzapfel *et al.*, [3]; Beresford *et al.*, [4]; Leroy and De Vuyst, [5]). Due to their positive metabolic and antimicrobial effects, LAB are recognized as "a safe microflora" (Tagg *et al.*, [6], Stiles, [7], De Vuyst

& Vandamme [8], and Caplice and Fitzgerald [9]). The antibacterial spectra of LAB and their metabolites, bacteriocins, are generally associated with Gram-positive spoilage microorganisms and food-borne pathogenic bacteria, with antilisterial effect as the major effect they exert (Drosinos *et al.*, [10]).

Until recently the genus *Enterococcus* was considered an indicator of fecal contamination. Today, however, species within this genus are considered as normal microflora, primarily of autochthonous cheeses, having a positive effect on the development of their specific organoleptic characteristics (Giraffa *et al.*, [11], Fuller, [12], Bulajić and Mijačević, [13]). Numerous *Enterococcus* strains, isolated from different fermented and non-fermented food products, produce more bacteriocins per strain, and this finding has prompted interest in their use as protective cultures in foods (Nes *et al.*, [14]).

Listeria monocytogenes is considered as one of the most frequent food-borne diseases causative. Numerous outbreaks and sporadic cases of listeriosis associated with foods have been reported since 1980 in various parts of the world (Norton and Braden [15]). In European countries the annual incidence of reported listeriosis cases can vary between 0.3 and 7.5 cases/million inhabitants (Swaminathan & Gerner-Smidt [16], EFSA [17]). *L. monocytogenes* is a ubiquitous bacteria usually found in the environment. The pathogen has been found in various food matrices, such as ready-to-eat foods, milk and cheeses, cold-cut meats, smoked fish, seafood, and vegetables (Chao *et al.*, [18], EFSA [17], and Chen *et al.*, [19]). Its presence in foods generally results from post-processing contamination due to the manipulation of foods and contact with contaminated surfaces or other foods from the storage area (Lunden *et al.*, [20]) and due to biofilm formation on the food processing equipment surfaces (Borucki *et al.*, [21]). It is generally known that once introduced into the processing plants, it is able to survive and persist for a long time under adverse conditions (Gram *et al.*, [22]).

The aim of this study was to test bacteriocin isolated from *Enterococcus faecalis* with regard to control *L. monocytogenes* growth during the manufacture of soft white cheese.

2. Material and Methods

2.1 Bacterial strains and growth conditions

Enterococcus faecalis isolated from autochthonous Serbian white cheese (Zlatar cheese), was used throughout this study. The strain *E. faecalis* was kept frozen at -20 ^{0}C in GM17 broth (M17 broth, Merck, KGaA, Darmstadt, Germany, and 0.5% (w/v) glucose) supplemented with 20% glycerol. Prior to use, the microorganism was subcultured twice in 10 mL of GM17 broth (1% inoculum, 24 h, 30 ^{0}C).

2.2 Isolation of semi purified bacteriocin from *Enterococcus faecalis* and determination of its spectrum of antimicrobial activity and strength

Isolation of semi purified bacteriocin from *E. faecalis* was done by the method of saturated precipitation with ammonium-sulphate (Schillinger and Lücke, [23]), adjusted to the individual laboratory conditions. Several days after planting broth culture with the objective of achieving the required *E. faecalis* concentration (10^{10} - 10^{11} CFU/g), it was centrifuged at 10,000 g for 30 minutes at 4 ^{0}C (MSE, "High Speed 18", England). After the separation and neutralization up to pH 6.5 - 7.0 of the supernatant with 10 N NaOH, the precipitation of bacteriocin was achieved with ammonium-sulphate. Separated bacteriocin in the shape of whitish pellets was suspended in 0.05 M of sodium-phosphate buffer pH 7.

The spectrum of antimicrobial activity of isolated bacteriocin was determined using the indicator stains (*Listeria monocytogenes* ATCC 19111, *Staphylococcus aureus* ATCC 6538, and *Esherichia coli* ATCC 11303).

Isolated bacteriocin, as well as its series of suitable dilutions in the amount of 50 µL, after the microbiological filter (Acrodisc, Gelman) of a diameter of 0.22 µm, were spotted into the previously made and marked basins in agar. Dilutions were made with sterile deionized water. After one hour incubation at 4 ^{0}C, in order to stimulate the examined bacteriocin diffusion, the plates were incubated at 30 ^{0}C for 24 h.

The activity of the isolated bacteriocin was expressed as the absolute value, marked as the arbitrary unit (AU/mL). Arbitrary values were determined by the following formula: AU/mL = 2^n x (1,000 µL/50 µL), where n represents the maximum dilution of the bacteriocin portion of 50 µL which gives the growth inhibition zone for *L. monocytogenes* (test-microorganism in this research) as being higher than 2 mm (Barefoot and Klaenhammer, [24]; Ennahar *et al.*, [25]).

2.3 Cheese production and sampling procedure

Raw cow's milk was heat-treated (5 minutes at temperatures above 90 ^{0}C) and then it was cooled to 30 ^{0}C (the optimum temperature for coagulation). Milk coagulation was performed using traditional method i.e. the commercial "Maja" rennet (Čačak, Serbia), strength 1 : 5,000, in the amount of ca. 1.5 - 2 mL/L. The rennet is a mixture of enzymes isolated from the stomach of young ruminants, typically calves (chymosin for the most part, and pepsin in part). The total amount was divided into three equal parts:

i) the first part was control group (K),
ii) the second part was inoculated with *L. monocytogenes* (ca. 10^4 cells/mL) (group E-1), and
iii) in the third part was added *L. monocytogenes* and bacteriocin isolated from *E. faecalis* (1280 AU/mL (group E-2)).

Upon milk coagulation, it took 80 min. for curd formation. After curdling, the curd was placed on a piece of cheese cloth to drain. The draining of the curd was spontaneous at first, without pressing, for about 30 min., and then the curd was kept under pressure (the curd was pressed with a 3 kg stone) for 3 to 4 h. The cheese was sliced, salted and stored in containers during analysis (21 days) at a cool room temperature of 15 - 18 ^{0}C. 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, 2, 4, 7, 14 and 21. Three samples were collected at each step of sampling and used for analysis. The cheese manufacture and fermentation process were repeated three times.

2.4 Microbiological analyses

Determination of the *Listeria* spp. presence was carried out following the ISO 11290 - 1,2 (11290 - 1, 2, [26], [27]) procedure. 25 g of each sample were homogenized with 225 mL sterile Fraser broth base (Biolife, Italy) in a stomacher for 2 min. The homogenates were incubated at 20 ^{0}C for 1 h, in order to resuscitate stressed microorganisms. For *L. monocytogenes* enumeration, a volume of 0.1 mL from each homogenate was directly streaked onto each of 2 Palcam Agar (Oxoid, UK) plates and incubated at 37 ^{0}C for 24 - 48 h. The homogenates were then supplemented by Fraser half selective supplement (primary enrichment) (Biolife, Italy) and incubated at 30 ^{0}C for 24 h for detection of *L. monocytogenes*. Afterwards, 0.1 mL of the primary enrichment was inoculated in 10 mL of Fraser broth supplemented (Biolife, Italy) by Fraser selective supplement (secondary enrichment) (Biolife, Italy) and incubated at 37 ^{0}C for 24 h. Cultures were streaked onto Oxford (Oxoid, UK) plates and incubated at 30 ^{0}C. From each plate of the primary and secondary enrichment, 5 colonies presumed to be *Listeria* spp. were streaked onto TSYEA (Tryptone soya yeast extract agar) plates (Biolife, Italy) and incubated for 24 h at 37 ^{0}C. Colonies were selected for typical appearance on TSYEA and submitted to Gram staining, catalase and oxidase test.

Haemolytic activity and CAMP tests on sheep blood agar were performed for the *L. monocytogenes* confirmation.

The results (a number of colony forming units, CFU) were expressed as average number of CFU g^{-1} cheese. The arithmetic means and standard deviations were calculated.

2.5 Statistical analysis

The results were analyzed using statistical methods to determine measures of central tendency (the arithmetic mean) and measures of variability or dispersion (standard deviation, SD). The results were subjected to a two-way analysis of variance (experimental variants, fermentation period), and the significance of differences was determined by the LSD test (Statistica SPSS 5). The statistical package "Statistica for Windows" (StatSoft.Inc., USA [28]) was used for the analysis.

3. Results and Discussion

3.1 Detection, spectrum of antimicrobial activity and strength of the isolated bacteriocin

Interestingly, three strains of *Enterococcus faecalis* that produce bacteriocin-like antimicrobial compounds were isolated from the traditional Serbian cheese (Zlatar cheese). Inhibitory effects of most of the isolates were attributed to the production of extracellular metabolites, mainly organic acids. Hydrogen peroxide could also act as an anti-*Listeria* agent, but supernatants treatment with catalase allowed the activity of H_2O_2 to be excluded. Test for bacteriocin production revealed a proteinaceous nature of antimicrobial compounds, indicating the possibility that they could be a bacteriocin-like substances. The proteinase test (using the proteolytic enzyme proteinase K) led to bacteriocin inactivation, thus indirectly confirming its proteinaceous nature (Joerger *et al.*, [29]).

Compared to test microorganisms, LAB bacteriocins showed marked antibacterial activity against *Listeria monocytogenes* ATCC 19111, whereas their action against *Staphylococcus aureus* ATCC 6538 and *Escherichia coli* ATCC 11303 was not detected. These results are supported by findings of other authors (Schilinger, [30], Abbe, [31], Vesković Moračanin *et al.*, [32], and [33]) who pointed to the fact that the inhibitory activity of LAB bacteriocins is dominant mostly against Gram-positive bacteria.

Since two bacteriocins very rapidly lost their antilisterial activity in the environment, a bacteriocin whose producer based on 16S rRNA sequencing, was designated as *E. faecalis* ATCC 19433 was used in the research that followed.

The maximum dilution of bacteriocin isolated from *E. faecalis*, which produced an antilisterial effect was 1 : 64, giving a calculated activity of approx. 1,280 AU/mL.

3.2 The antilisterial activity of the added bacteriocin

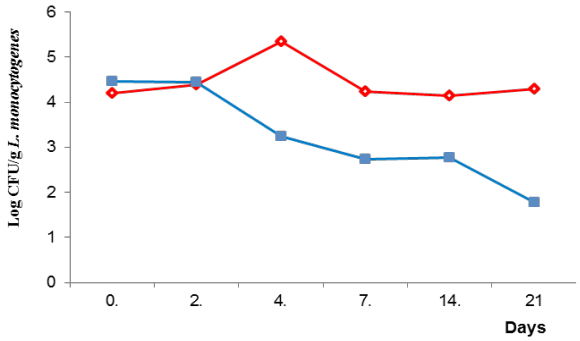
L. monocytogenes was not detected in any sample of the control group of soft white cheese (K-1) in all stages of testing (days: 0, 2, 4, 7, 14 and 21) during the triplicate fermentations. The results of this group are not presented.

The changes in the number of the total viable count of *L. monocytogenes* in the examined experimental groups of cheese (from groups E-1, and E-2) are presented in Table 1 and Figure 1.

Table 1. The total viable count (log CFU/g ± SD)* of *Listeria monocytogenes* in samples of experimental groups E-1 and E-2 soft white cheeses during fermentation

Days	Group E-1	Group E-2
0	$4.19^{bc} \pm 0.19$	$4.47^{ab} \pm 0.01$
2	$4.38^{b} \pm 0.08$	$4.45^{b} \pm 0.95$
4	$5.35^{a} \pm 0.35$	$3.25^{cd} \pm 0.55$
7	$4.24^{bc} \pm 0.23$	$2.74^{de} \pm 0.04$
14	$4.14^{bc} \pm 0.24$	$2.77^{de} \pm 0.17$
21	$4.29^{bc} \pm 0.33$	$1.78^{ef} \pm 0.01$

*each result is expressed as the average of measurements from three samples ± standard deviation (SD).
CFU - colony forming units.
Values followed by different small letters within columns are significantly different (P ≥ 0.05) according to the LSD test.



E-1 (◊) - Experimental group 1 with added *L.monocytogenes*.
E-2 (■) - Experimental group 2 with added *L. monocytogenes* and bacteriocin isolated from *E. Faecalis*.

Figure 1. Growth of *L. monocytogenes* ATCC 19111 in the samples of soft cheese during the fermentation period

Results on changes in the total viable count of *L. monocytogenes* during cheese ripening indicate a high concentration of this pathogen in group E-1 cheese which was on day 21 of ripening close to initial contamination (ca. 10^4 CFU/g). The maximum cell count of *Listeria* was determined on day 4 of testing.

In both experimental group of cheese supplemented with bacteriocin isolated from *E. faecalis* (group E-2), at the end of the ripening process, a statistically significant level of reduction in the count of *L. monocytogenes* (P ≥ 0.05) was determined. The initial count of this pathogen (ca. 10^4 CFU/g) was reduced to below 100 CFU/g (group E-2) at the end of the cheese ripening process.

The obtained results show that the enterocin is successful in preventing the growth of *L. monocytogenes*, despite the possible inactivation by various interactions with the food system (Ennahar *et al.*, [34], Izquierdo *et al.*, [35]).

A very important fact is that the strains tested in the present work were isolated from a traditional Zlatar cheese, and that represents natural bioprotectors isolated from a similar matrix.

The antimicrobial effect of enterococcal bacteriocins (the so-called enterocins) was detected more than fifty years ago in different strains of the genus *Enterococcus* widely distributed in nature (Brock *et al.*, [36]). Most of enterococcal bacteriocins are class II bacteriocins, which are: heat stable, cationic, hydrophobic and low molecular weight peptides with antilisterial activity and with an interesting technological potential (Giraffa, [37], Khan *et al.*, [38], Gálvez *et al.*, [39], and Franz *et al.*, [40]), especially those with a broad-spectrum of inhibition like the cyclic peptide AS-48 (Maqueda *et al.*, [41], Edalatian *et al.*, [42]). To date, a large number of enterococcal bacteriocins that show an antimicrobial effect against foodborne pathogens such as *Listeria* spp. and *Clostridium* spp. have been described (McAuliffe *et al.*, [43], Mendoza *et al.*, [44], Moreno *et al.*, [45], Cocolin *et al.*, [46], Izquierdo *et al.*, [47]). Bacteriocins isolated from enterococci have a very strong antilisterial effect which makes them potential control agents against this pathogen in foods. However, their direct use and the use of *Enterococcus* cells generally lead to reduction or loss of their antibacterial activity due to interactions with food components (Vesković Moračanin, [48], Vesković Moračanin *et al.*, [49]).

Certainly, for the direct application of these bioprotectors and entry into the full production process, in addition to receiving GRAS compounds status (Generally recognized as safe) they must be well studied and harmonized with other technological factors in the production (pH, temperature, salt and nitrite) (Cheveland *et al.*, [50], Vesković and Đjukić, [51]). Their implementation should be viewed in terms of good alternatives, especially when combined with other natural protectors (Leroy and de Vuyst, [52]) and, of course, good hygienic and manufacturing practices.

However, reduction of *Listeria* cells in this way could be a promising tool, although it should be considered only as an additional measure to implement *Listeria* contamination control.

4. Conclusions

- Growing need for naturally safe and healthy food has led to increased interest in the introducing bacteriocins as a factor in the hurdle technology approach to food protection, and has attracted scientists in this food safety field.

- The use of bacteriocins can be interesting and quite desirable since consumers' trust in chemical preservatives has been shaken and even questioned. Food composition i.e. properties (pH, temperature, ingredients and supplements, type and counts of microorganisms) and the technology used during the production process can affect the stability and activity of the added bacteriocins. Future research in this field

should elucidate this unknown aspect regarding their use, thus providing information on the optimization of environmental conditions in order to contribute to the maximum antimicrobial effect of bacteriocins added to food systems and facilitate the search for new producers.

- Moreover, our further research in this field will focus on improving the understanding bacteriocins nature, their antimicrobial activities and potential applications, and searching for new bacteriocin-producing strains of LAB originating from autochthonous fermented products whose controlled and planned use would make them act as natural preservatives or food bioprotectors.

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5. References

[1] Klaenhammer T., Altermann E., Arigoni F., Bolotin A., Breidt F, Broadbent J., Cano R., Chaillou S., Deutscher J., Gasson M., van de Guchte M., Guzzo J., Hartke A., Hawkins T., Hols P., Hutkins R., Kleerebezem M., Kok J., Kuipers O., Lubbers M., Maguin E., McKay L., Mills D., Nauta A., Overbeek R., Pel H., Pridmore D., Saier M., van Sinderen D., Sorokin A., Steele J., O'Sullivan D., de Vos W., Weimer B., Zagorec M., Siezen R. (2002). *Discovering lactic acid bacteria by genomics*. Antonie Van Leeuwenhoek, 82, pp. 29-58.

[2] Holzapfel W. H., Wood B. J. B. (Eds). (2014). *Lactic Acid Bacteria: Biodiversity and Taxonomy*. John Wiley & Sons, Chichester, West Sussex UK.

[3] Holzapfel W., Geisen R., Schillinger U. (1995). *Biological preservation of foods with reference to protective cultures, bacteriocins and food-grade enzymes*. International Journal of Food Microbiology, 24, pp. 343-362.

[4] Beresford M. R., Andrew P. W., Shama G. (2001). *Listeria monocytogenes adheres to many materials found in food-processing environments*. Journal of Applied Microbiology, 90, pp. 1000-1005.

[5] Leroy F., de Vuyst L. (2014). *Lactic acid bacteria as functional starter cultures for the food fermentation industry*. Trends in Food Science and Technology, 15, (2), pp. 67-78.

[6] Tagg J., Dajani A., Wannamaker L. (1976). *Bacteriocins of gram positive bacteria*. Bacteriologicals Reviews, 40, pp. 722-756.

[7] Stiles M. E. (1994). *Bacteriocins Produced by Leuconostoc species*. Journal of Dairy Science, 77, pp. 2718-2724.

[8] De Vuyst L., Vandamme E. J. (1994). *Antimicrobial potential of lactic acid bacteria*. In: De Vuyst L., Vandamme E.J. (eds.), Bacteriocins of Lactic Acid Bacteria, Blacke Academic and Professional, London, UK, pp. 91-149.

[9] Caplice E., Fitzgerald G. F. (1999). *Food fermentations: role of microorganisms in food production and preservation*. International Journal of Food Microbiology, 50, pp. 131-149.

[10] Drosinos E., Mataragas M., Vesković Moračanin S., Gasparik-Reichardt J., Hadžiosmanović M., Alagić D. (2006). *Quantifying Nonthermal Inactivation of Listeria monocytogenes in European Fermented Sausages Using Bacteriocinogenic Lactic Acid Bacteria or Their Bacteriocins: A Case Study for Risk Assessment*. Journal of Food Protection, 69, (11), pp. 2648-2663.

[11] Giraffa G., Canniuti D., Neviani E. (1997). *Enterococci isolated from dairy products: a review of risks and potential tehnological use*. Journal Food Protection, 60, pp. 732-738.

[12] Fuller R. (1989). *Probiotics in man and animals - A Review*. Journal of Applied Bacteriology, 66, pp. 365-378.

[13] Bulajić S., Mijačević Z. (2004). *Enterococci in cheeses-phenotypization and antibiotic resistance*. Acta agriculturae Slovenica, 84, (1), pp. 25-30.

[14] Nes I., Diep Dz., Holo H. (2007). *Bacteriocin Diversity in Streptococcus and Enterococcus*, Journal of Bacteriology, 189, (4), pp. 1189-1198.

[15] Norton D. M., Braden C. R. (2007). *Foodborne listeriosis*. In: E.T. Ryser &E.H. Marth (eds.), Listeria, listeriosis and food safety. New York: Marcel Dekker Inc., USA, pp. 305-349.

[16] Swaminathan B., Gerner-Smidt P. (2007). *The epidemiology of human listeriosis*. Microbes and Infection, 9, pp. 1236-1243.

[17] EFSA, European Food Safety Authority. (2013). *Analysis of the baseline survey on the prevalence of Listeria monocytogenes in certain ready-to-eat (RTE) foods in the EU, 2010 – 2011. Part A: Listeria monocytogenes prevalence estimates*. EFSA Journal, 11, pp. 75.

[18] Chao G., Deng Y., Zhou X., Xu Q., Qian X., Zhou L., Zhu B. (2006). *Prevalence of Listeria monocytogenes in delicatessen food products in China*. Food Control, 17, pp. 971-974.

[19] Chen M., Wu Q., Zhang J., Yan Z., Wang, J. (2014). *Prevalence and characterization of Listeria monocytogenes isolated from retail-level ready-to-eat foods in South China*. Food Control, 38, pp. 1-7.

[20] Lunden J. M., Autio T. J., Sjoberg A. M., Korkeala H. J. (2003). *Persistent and nonpersistent Listeria monocytogenes contamination in meat and poultry processing plants*. Journal of Food Protection, 66, pp. 2062-2069.

[21] Borucki M. K., Peppin J. D., White D., Loge F., Call D. R. (2003). *Variation in biofilm formation among strains of Listeria monocytogenes*. Applied and Environmental Microbiology, 69, pp. 7336-7342.

[22] Gram L., Bagge-Ravne D., Yin N. Y., Gymoese P., Fonnesbech Vogel B. (2007). I*nfluence of food soiling matrix on cleaning and disinfection efficiency on surface attached Listeria monocytogenes*. Food Control, 18, pp. 1165-11671.

[23] Schillinger U., and Lucke F. K. (1989). *Antibacterial activity of Lactobacillus sake isolated from meat*. Applied and Environmental Microbiology, 55, (8), pp. 1901-1906.

[24] Barefoot S. F., Klaenhammer T. R. (1983). *Detection and activity of lactacin B, a bacteriocin produced by Lactobacillus acidophilus*. Applied and Environmental Microbiology, 45, pp. 1808-1815.

[25] Ennahar S., Asou Y., Zendo T., Sonomoto K., Ishizaki A.(2001). *Biochemical and genetic evidence for production of enterocins A and B by cheese-isolated Enterococcus faecium WHE 81*. International Journal of Food Microbiology, 70, pp. 291-301.

[26] ISO 11290-1. (2004). *Horizontal Method for the Detection and Enumeration of Listeria monocytogenes - Part 1*: *Detection Method*. International Organisation for Standardization (ISO), Geneva, Switzerland.

[27] ISO 11290-2. (2004). *Horizontal Method for the Detection and Enumeration of Listeria monocytogenes - Part 2: Enumeration method*. International Organisation for Standardization (ISO), Geneva, Switzerland.

[28] StatSoft.Inc.: STATISTICA for Windows (computer program manual). OK: StatSoft.Inc., Tulsa, USA.

[29] Joerger R. D., Hoover D. G., Barefoot S. F., Harmon K. M., Grinstead D. A., Nettles-Cutter C. G. (2000). *Bacteriocins.* In: Lederberg (ed.), Encyclopeida of microbiology, Vol. 1, 2nd edition, Academic Press, Inc., San Diego, USA, pp. 383-397.

[30] Schillinger U. (1990). *Bacteriocins of lactic acid bacteria,* In: Bills D.D. Kung S.D. (eds.), Biotechnology and Food Safety, Butterworth-Heinemann, Boston, USA, pp. 55-74.

[31] Abbe T. (1995). *Pore-forming bacteriocins of gram-positive and self-protection mechanisms of producer organisms.* FEMS Microbiology Letters, 129, pp. 1-10.

[32] Vesković Moračanin S., Borovic B., Velebit B. (2013). *Basic Characteristics of Natural Isolates of Lactic Acid Bacteria*. International 57th Meat Industry Conference Proceedings, Belgrade, Serbia, pp. 305-308.

[33] Vesković Moračanin S., Turubatović L., Škrinjar M., Obradović, D. (2013). *Antilisterial activity of bacteriocin isolated from Leuconostoc mesenteroides subspecies mesenteroides IMAU:10231 in production of Sremska sausages (traditional Serbian sausage): Lactic acid bacteria isolation, bacteriocin identification, and meat application experiments.* Food Technology and Biotechnology, 51, (2), pp. 247-256.

[34] Ennahar S., Aoude-Werner D., Assobhei O., Hasselmann C., (1998). *Antilisterial activity of enterocin 81, a bacteriocin produced by Enterococcus faecium WHE 81 isolated from cheese*. Journal of Applied Microbiology, 85, pp. 521-526.

[35] Izquierdo E., Marchioni E., Aoude-Werner D., Hasselman C., Ennahar S. (2009). *Smearing of soft cheese with Enterococcus faecium WHE 81, a multi-bacteriocin producer, against Listeria monocytogenes*. Food Microbiology, 26, pp. 16-20.

[36] Brock T. D., Peacher B., Pierson D. (1963). *Survey of the bacteriocins of enterococci*. Journal of Bacteriology, 86, pp. 702-707.

[37] Giraffa G. (1995). *Enterococcal bacteriocins: their potential as antiListeria factors in dairy technology*. Food Microbiology, 12, pp. 291-299.

[38] Khan H., Flint S., Yu, P.L. (2010). *Enterocins in food preservation*. International Journal of Food Microbiology, 141, pp. 1-10.

[39] Gálvez A., Abriouel H., Benomar N., Lucas R. (2010). *Microbial antagonists to food-borne pathogens and biocontrol*. Current Opinion in Biotechnology, 21, pp. 142-148.

[40] Franz C. M., Huch M., Abriouel H., Holzapfel W., Gálvez A. (2011). *Enterococci as probiotics and heir implications in food safety*. International Journal of Food Microbiology, 151, pp. 125-140.

[41] Maqueda M., Gálvez M., Martínez Bueno M., Sanchez-Barrena M. J., González C., Albert A., Rico M., Valdivia E. (2004). *Peptide AS-48: prototype of a new class of cyclic bacteriocins*. Current Protein and Peptide Science, 5, pp. 399-416.

[42] Edalatian M. R., Habibi Najafi M. B., Mortazavi S. A., Alegría Á., Delgado S., Bassami M. R., Baltasar M. (2012). *Production of bacteriocins by Enterococcus spp. isolated from traditional Iranian raw milk cheeses and detection of their encoding genes*. European Food Research and Technology, 234, pp. 789-796.

[43] McAuliffe O., Hill C., Ross R. P. (1999). *Inhibition of Listeria monocytogenes in cottage cheese manufactured with a lacticin 3147-producing starter culture*. Journal of Applied Microbiology, 86, pp. 251-256.

[44] Mendoza F., Maqueda M., Gálvez A., Martínez-Bueno M., Valdivia E. (1999). *Antilisterial activity of peptide AS-48 and study of changes induced in the cell envelope properties of an AS-48 adapted strain of Listeria monocytogenes*. Applied and Environmental Microbiology, 65, pp. 618-625.

[45] Moreno M. R., Sarantinopoulos P., Tsakalidou E., De Vuyst L. (2006). *The role and application of enterococci in food and health*. International Journal of Food Microbiology, 106, pp. 1-24.

[46] Cocolin L., Foschino R., Comi G., Fortina M. G. (2007). *Description of the bacteriocins produced by two strains of Enterococcus faecium isolated from Italian goat milk*. Food Microbiology, 24, pp. 752-758.

[47] Izquierdo E., Marchioni E., Aoude-Werner D., Hasselman C., Ennahar, S. (2009). *Smearing of soft cheese with Enterococcus faecium WHE 81, a multi-bacteriocin producer, against Listeria monocytogenes*. Food Microbiology, 26, pp. 16-20.

[48] Vesković Moračanin S. (2012). *The influence of environmental factors on the intensity of the antimicrobial activity of bacteriocins* (in Serbian). Tehnologija mesa, 53, (2), pp. 157-165.

[49] Vesković Moračanin S, Đukić D., Memiši N. (2014). *Bacteriocins produced by lactic acid bacteria – A review*. Acta periodica technologica, 45, pp. 271-283.

[50] Cleveland L., Montville T. J., Nes, I. F., Chikindas, M. L. (2001). *Bacteriocins: Safe, natural antimicrobials for food preservation*. International Journal of Food Microbiology, 71, pp. 1-20.

[51] Vesković S., Đukić D. (2015). *Bioprotectors in food production* (in Serbian). University of Kragujevac, Faculty of Agronomy Čačak, ISBN 978-86-87611-34-4, pp. 377.

[52] Leroy F., and L. de Vuyst (2004). *Lactic acid bacteria as functional starter cultures for the food fermentation industry*, Trends in Food Science and Technology, 15, pp. 67-78.