JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

CONTROL OF *LISTERIA MONOCYTOGENES* AND *STAPHYLOCOCCUS AUREUS* IN MEAT AND MEAT PRODUCTS BY CELL-FREE SUPERNATANT OF *BREVIBACILLUS LATEROSPORUS* BGSP7 AND BGSP9

Nemanja Mirkovic[1], Zorica Radulovic[1], Branko Jovcic[2], Nemanja Stanisavljevic[3], Milan Kojic[4], Fatih Oz[5], Charalampos Proestos[6], Volker Heinz[7], Igor Tomasevic[1,7]*

Address(es):
[1]Faculty of Agriculture, University of Belgrade, Belgrade, 11080, Serbia.
[2]Faculty of Biology, University of Belgrade, Belgrade, 11000, Serbia.
[3]Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, 11000, Serbia.
[4]Institute of Virology, Vaccines and Sera "Torlak", University of Belgrade, Belgrade, 11000, Serbia.
[5]Faculty of Agriculture, Ataturk University, Erzurum, 25240, Turkey.
[6]National and Kapodistrian University of Athens Zografou, Athens, 15771, Greece.
[7]German Institute of Food Technologies (DIL e.V.), Prof.-von-Klitzing-Str. 7, Quakenbrück, 49610, Germany.

*Corresponding author: nemanja.mirkovic@agrif.bg.ac.rs

https://doi.org/10.55251/jmbfs.11182

ARTICLE INFO

Received 27. 3. 2024
Revised 1. 9. 2024
Accepted 19. 9. 2024
Published 1. 10. 2024

Regular article

OPEN ACCESS

ABSTRACT

The presence of pathogens in food has increased awareness of food safety, but it also causes large economic losses. Fresh meat and meat products contain a sufficient quantity of proteins, lipids, water, and a favorable pH that stimulates the growth of various microorganisms, including pathogens.
The aim of this study was to investigate the efficacy of *Brevibacillus laterosporus* BGSP7 (CFS-BGSP7) and BGSP9 (CFS-BGSP9) cell-free supernatants in the control of *Listeria monocytogenes* and *Staphylococcus aureus* in raw meat and meat products.
Raw meat and meat products were sliced and then aseptically treated by immersion for 2 minutes into solutions containing: i) CFS-BGSP7; ii) CFS-BGSP9; iii) no treatment. The samples were then artificially contaminated with: Group I – *L. monocytogenes* (~4 log cfu g-1); Group II – *S. aureus* LMM322 (~4 log cfu g^{-1}). Each sample was individually aseptically vacuum-packed and stored at 4°C for 8 weeks.
The number of surviving bacteria in the samples were analyzed immediately after contamination with *L. monocytogenes* and *S. aureus* and at regular time-intervals: after 1, 3, 5 and 8 weeks of storage at 4°C.
Meat samples treated with CFS-BGSP7 and CFS-SP9 showed a significant decrease in the cell counts of *L. monocytogenes* and *S. aureus*. When meat samples treated with CFS-BGSP7 and CFS-BGSP9 are compared, the results show a more intense reduction rate of both *L. monocytogenes* and *S. aureus* in all samples treated with CFS-BGSP7.

Keywords: cell-free supernatant (CFS), *Listeria monocytogenes*, *Staphylococcus aureus*, raw meat, meat products

INTRODUCTION

Foodborne pathogens cause a vast number of diseases with a significant impact on human health and the economy. Unsafe foods containing harmful bacteria, viruses, parasites or chemical substances cause more than 200 diseases (**Bintsis, 2017**), ranging from diarrhea to cancer. An estimated 600 million, or almost one in ten people worldwide fall ill after eating contaminated food and 420 000 die each year. In low- and middle-income countries US$ 110 billion is lost each year in productivity and medical costs resulting due to unsafe food (**WHO, 2022**).
Over the last three decades, pathogenic bacteria have become a major problem in the food industry. The presence of pathogens in food has not only raised awareness of food safety, but also poses a major economic problem.
Raw meat and meat products contain a sufficient quantity of proteins, lipids, carbohydrates and water and, thanks to the optimal pH value, form a suitable environment for the growth of a variety of microorganisms, including bacteria, yeasts, molds and viruses. In the last twenty years, the biggest problem of contamination in the meat industry has been caused by pathogenic bacteria (**Hui and Dykes, 2012; Newell *et al.* 2010**). Among foodborne pathogens, *Listeria monocytogenes* and *Staphylococcus aureus* are very important and ubiquitous pathogens in the meat industry, causing illness or even death in consumers (**Hui and Dykes, 2012; Oxaran *et al.*, 2018**). *Staphylococcus aureus* is responsible for one third of the infections caused worldwide and is the third most common pathogen after *Vibrio parahaemolyticus* (27.8 %) and *Salmonella* (23.1 %) (**Bean *et al.*, 1996; Diep *et al.*, 2006**). %). It multiplies very rapidly at room temperature at a rate of more than 10^5 bacteria per gram and can produce heat-resistant enterotoxin (**Delbes *et al.*, 2006; Masoud *et al.*, 2012; Ryser, 2001**). The symptoms of staphylococcal food poisoning occur after ingestion of low doses of toxin (20-100 ng) (**Normanno *et al.*, 2007; Pelisser *et al.*, 2009; Schelin *et al.*, 2011**) and are responsible for 95% of food poisoning (**Carfora *et al.*, 2015**). On the other hand, the ability of *Listeria monocytogenes* to grow in cold (refrigerator) conditions, low pH and high salinity can cause major problems in food production. *Listeria monocygenes* causes listeriosis, a disease that affects immunocompromised individuals, newborns and pregnant women, at very low doses in food (10^2 to 10^4 cfu/g or ml) (**Ooi *et al.*, 2005; Vazquez-Boland *et al.*, 2001; Wing *et al.*, 2002**). In general, listeriosis is a relatively rare disease, but it has a high mortality rate in Europe (12%) and in the United States (25%) (Schlech, 2000).
Staphylococcus and *Listeria* are usually found on the surface of meat and can be spread in meat processing plants during mixing or grinding. In addition, the ability of these bacteria to form biofilms (including mixed-species biofilm) allows them to remain a persistent contaminant in meat processing plants (**Gamble *et al.*, 2007; Kushwaha *et al.*, 2009; Oxaran *et al.*, 2018**). The first hurdle in the fight against foodborne pathogens and food contamination in the food industry is the implementation of good manufacturing practices (GMP) and standard operating procedures (SOPs) according to the Hazard and Analysis and Critical Control Point (HACCP) concept. Nevertheless, outbreaks of food poisoning still occur. For this reason, there is a constant need for diverse and effective approaches that can contribute to increased protection in the production and distribution of sensitive food products. One of these approaches is biopreservation. Natural and safe biopreservation can be achieved by antimicrobial ribosomal (bacteriocins) or non-ribosomally synthesized antimicrobial peptides and lipopeptides (**Hugas, 1998; Työppönen *et al.*, 2003**).
Many research groups have used lactococci as bacteriocin producers in various foods to improve the quality of products or to control foodborne diseases (**Dal Bello *et al.*, 2012; Rodriguez *et al.*, 2005**). However, some members of the spore formers, such as *Brevibacillus laterosporus*, are beneficial to humans, animals and plants and can be used as probiotics or sources of antimicrobial compounds (**Sanders *et al.*, 2003**). In a previous study, we isolated *Brevibacillus laterosporus* BGBG7 and BGSP9 from silage and showed inhibitory effect of overnight culture and cell-free supernatant against the foodborne pathogens *Listeria monocytogens* and *Staphylococcus aureus* (**Miljkovic et al., 2019**). Both strains of *B. latersporus* BGSP7 and BGSP9 produce a whole arsenal of antimicrobial molecules that are

active against *L. monocytogens* and *S. aureus* (**Miljkovic et al., 2019**). The most active antimicrobial peptide from *B. laterosporus* BGSP7 was purified from the supernatant and had a mass of 1583.94 Da, while more than 5 antimicrobial peptides with a mass of 1540-1620 Da were purified from the supernatant of *B. laterosporus* BGSP9. All purified antimicrobial peptides showed activity against *L. monocytogenes* and *S. aureus*, with the most abundant peptides from BGSP7 (1583.94) and BGSP9 (1556.31 Da) showing activity at very low concentrations (**Miljkovic et al., 2019**).

Considering that the process of purification of antimicrobial peptides is very costly and it is known from a previous study that the most effective antimicrobial peptides were purified from the cell-free supernatants of BGSP7 and BGSP9, the aim of this work was to investigate the efficacy of cell-free supernatants of *B. latersporus* BGSP7 and BGSP9 in combating *L. monocytogenes* and *S. aureus* in pork and beef and in pork and beef products.

MATERIAL AND METHODS

Bacterial strains, media and cultivation conditions

The strains used in this study and their growth conditions are listed in Table 1. *Brevibacillus laterosporus* BGSP7 and BGSP9, isolated from silage (Miljkovic et al., 2019) were incubated at 37°C in Luria Bertani (LB) medium with aeration. *Staphylococcus aureus* BGPF322 and *Listeria monocytogenes* BGPF112 previously isolated from raw pork meat (Collection of Faculty of Agriculture, University of Belgrade) were incubated at 37°C in Luria Bertani (LB) medium with aeration and Brain Heart Infusion broth, respectively. Solid medium (soft and hard agar mediums) was prepared by adding agar (Torlak, Belgrade Serbia) 7 $g.L^{-1}$ and 15 $g.L^{-1}$, respectively.

Table 1 Strains used in this study

Strain	Growth conditions	Source or references
Brevibacillus laterosporus BGSP7	Luria Bertani broth, with aeration, 37°C	Miljkovic et al., 2019
Brevibacillus laterosporus BGSP9	Luria Bertani broth, with aeration, 37°C	Miljkovic et al., 2019
Listeria monocytogenes BGPF112	Brain Heart Infusion broth, 37°C	Collection of Faculty of Agriculture, University of Belgrade
Staphylococcus aureus BGPF322	Luria broth, with aeration, 37°C	Collection of Faculty of Agriculture, University of Belgrade

Preparation of cell-free supernatant *Brevibacillus laterosporus* BGSP7 and BGSP9

Brevibacillus laterosporus BGSP7 and BGSP9 were inoculated 1% (v/v) in LB broth and incubated for 12 h at 37°C. After centrifugation (8 000 x g, 10°C, 30 min) supernatants were filtered through 0.22 µm pore-size (Millipore Corporation, Bedford, MA, USA) and neutralized to pH 7 with 1M NaOH to avoid a potential inhibitory effect of low pH.

Antimicrobial activity of cell-free supernatants of *Brevibacillus laterosporus* BGSP7 and BGSP9

Antimicrobial activity of cell-free supernatants of *Brevibacillus laterosporus* BGSP7 (CFS-BGSP7) and BGSP9 (CFS-BGSP9) was determined by agar well diffusion assay, described previously (**Kojic *et al*., 1991**). *Staphylococcus aureus* BGPF322 (5 log cfu g^{-1}) and *Listeria monocytogenes* BGPF112 (5 log cfu g^{-1}) were used as indicator strains. Aliquots of CFS- BGSP7 and CFS-BGSP9 were assayed in wells made in soft agar inoculated with indicator strains. Plates were incubated at 37°C, and clear zone of inhibition around the wells indicated antimicrobial production/activity. All experiments were done in triplicate.

Preparation of treatments of meat and meat products

Raw meat and meat products were purchased from Serbian producer of meat and meat products, Zlatiborac, Čajetina, Serbia. The meat samples were labeled as follows: PRM - pork raw meat; PBS – pork boiled sausage; PFS – pork fermented sausage; BRM – beef raw meat; BBS – beef boiled sausage; BP – beef prosciutto. All meat samples were sliced (3 cm diameter and weight 5 g), and then aseptically treated by immersion for 2 min, in: i) Cell free supernatant of *Brevibacillus laterosporus* BGSP7 (CFS-BGSP7); ii) Cell free supernatant of *Brevibacillus laterosporus* BGSP9 (CFS-BGSP9); iii) without treatment. Afterwards, samples were artificially contaminated with: Group I - *Listeria monocytogenes* BGPF112 (~4 log cfu g^{-1}); Group II – *Staphylococcus aureus* BGPF322 (~4 log cfu g^{-1}). Every sample (5g) were individually aseptically vacuum packed using machine Minipack®-torre MV35LA13 (Dalmine, Italy) and stored at 4°C for 8 weeks.

Sampling and microbiological analysis

All samples of raw meat and meat products purchased from the market were analysed on presence of *Listeria monocytogenes* and *Staphylococcus auresu* using EN ISO 11290-1:2017 and EN ISO 6888-2 before being used in the experiment. Artificially contaminated **s**amples were analyzed instantly after contamination with *L. monocytogenes* BGPF112 and *S. aureus* BGPF322, and periodically after 1, 3, 5 and 8 weeks of storage at 4°C. Each sample was aseptically diluted in sterile saline solution (0.85% of NaCl (45 mL)), homogenized for 3 minutes in a stomacher (Interlab, BagMixer 400P) and then tenfold dilutions were prepared for microbiological analysis. For enumeration of *S. aureus* BGPF322, Baird-Parker agar (base) with egg yolk tellurite emulsion (Merck Darmstadt, Germany) was used and incubated at 37°C for 48h. For enumeration *Listeria monocytogenes* BGPF112 Palcam Listeria selective agar base with Palcam Listeria selective supplement (Merck Darmstadt, Germany) was incubated at 37°C for 48h. All analyses were done in triplicate.

Statistical analysis

Obtained results were statistically analyzed using the SPSS 20.0 for Windows. The results are shown as mean values ± standard errors/deviations. The differences between the control and treated groups were compared using Tukey`s t-test. P values less than 0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

Antimicrobial activity of cell-free supernatants of *Brevibacillus laterosporus* BGSP7 and BGSP9

Many different strains of *Brevibacillus laterosporus* can produce antimicrobial compounds, such as bacteriocins, lipopeptides and cyclic peptides (**Zhao *et al*., 2016; Baindara *et al*., 2016; Yang *et al*., 2016; Khaled *et al*., 2018**). The antimicrobial activity of the neutralized cell-free supernatant of *Brevibacillus laterosporus* BGSP7 (CFS-BGSP7) and BGSP9 (CFS-BGSP9) against *Listeria monocytogenes* BGPF112 and *Staphylococcus aureus* BGPF322 was investigated in this study. *B. laterosporus* BGSP7 and BGSP9 were previously isolated from silage and showed very strong antimicrobial activity against a large number of Gram-positive and Gram-negative pathogenic bacteria (**Miljkovic *et al*., 2019**). The CFS-BGSP7 showed stronger antimicrobial activity against *L. monocytogenes* BGPF112 and *S. aureus* BGPF322 compared to CFS-BGSP-9, as evidenced by a larger zone of inhibition (**Figure 1**). The antimicrobial activity results previously published by **Miljkovic *et al*. (2019)** indicated the similar activity of CFS-BGSP7 and CFS-BGSP9 against *L. monocytogenes* ATCC19111 and *S. aureus* ATCC25923, yielding zones of inhibition of 15 mm. Minor differences in the activity of CFS-BGSP7 and CFS-BGSP9 against *L.monocytogenes* and *S.aureus* obtained in this study compared to the results of the previous study can be explained by the use of other *Listeria* and *Staphylococcus* strains.

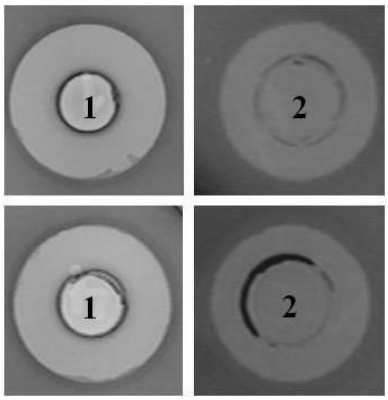

Figure 1 Antimicrobial activity of cell-free supernatant of *Brevibacillus laterosporus* BGSP7 (1) and BGSP9 (2) on different indicator strains: *Listeria monocytogenes* BGPF112 (A); *Staphylococcus aureus* BGPF322 (B). Antimicrobial activity analysed after 16 h of the indicator strains.

Sampling and microbiological analysis of raw meat and meat products

In recent years, some studies have shown very good results on the probiotic properties of *Brevibacillus laterosporus* (**Cao *et al*., 2023, Weng *et al*., 2022**). In

addition, many research results have shown that *Brevibacillus laterosporus* can produce various antibacterial and antifungal agents (**Jiang *et al*., 2015; Hassi *et al*., 2012; Panda *et al*., 2014; Miljkovic *et al*., 2019**). This study aimed to investigate the ability of *Brevibacillus laterosporus* BGSP7 (CFS-BGSP7) and BGSP9 (CFS-BGSP9), which have antilisterial and antistaphylococcal activity, to inhibit the growth of *Listeria monocytogenes* BGPF112 and *Staphylococcus aureus* BGPF322 in raw meat and meat products. *L. monocytogenes* and *S. aureus* were not detected in samples of raw meat and meat products prior to artificial contamination. The results of the antimicrobial activity of CFS-BGSP7 and CFS-BGSP9 against *L. monocytogenes* BGPF112 and *S. aureus* BGPF322 in samples of raw meat and meat products are shown in **Table 2**. and **Table 3**. The statistical analysis showed that both factors (type of treatment and duration of storage) and their interaction had significant effects on the number of *L. monocytogenes* BGPF112 and *S. aureus* BGPF322.

Table 2 Number of *Listeria monocytogenes* in artificially contaminated raw meat and meat products

Sample		PRM	PBS	PFS	BRM	BBS	BP
	Weeks						
Control	0	4.63 ± 0.56^{aA}	4.47 ± 0.03^{aA}	4.47 ± 0.05^{aA}	4.82 ± 0.04^{aA}	4.68 ± 0.04^{aA}	4.62 ± 0.07^{aA}
	1	5.39 ± 0.09^{bcA}	5.11 ± 0.09^{bA}	4.36 ± 0.07^{abA}	5.29 ± 0.10^{bA}	4.84 ± 0.06^{aA}	4.38 ± 0.07^{bA}
	3	5.61 ± 0.05^{bA}	5.77 ± 0.07^{cA}	4.08 ± 0.04^{cA}	5.25 ± 0.20^{bA}	5.52 ± 0.04^{bA}	4.31 ± 0.05^{bA}
	5	5.23 ± 0.10^{cA}	6.25 ± 0.05^{dA}	4.14 ± 0.12^{bcA}	5.20 ± 0.03^{bA}	5.74 ± 0.09^{cA}	4.18 ± 0.04^{bcA}
	8	5.11 ± 0.08^{cA}	6.88 ± 0.03^{eA}	3.91 ± 0.03^{cA}	5.27 ± 0.14^{bA}	5.88 ± 0.09^{cA}	4.00 ± 0.05^{cA}
CFS-BGSP7	0	4.60 ± 0.02^{aA}	4.49 ± 0.05^{aA}	4.45 ± 0.05^{aA}	4.84 ± 0.11^{aA}	4.78 ± 0.01^{aA}	4.58 ± 0.03^{aA}
	1	4.06 ± 0.21^{bB}	4.19 ± 0.06^{bB}	4.01 ± 0.02^{bB}	4.13 ± 0.16^{bB}	4.34 ± 0.05^{bB}	4.37 ± 0.09^{aA}
	3	3.97 ± 0.06^{bcB}	4.04 ± 0.04^{bB}	4.07 ± 0.09^{bA}	4.13 ± 0.08^{bB}	4.14 ± 0.11^{cB}	4.22 ± 0.04^{aA}
	5	3.77 ± 0.07^{cB}	3.43 ± 0.08^{cB}	3.69 ± 0.09^{cB}	4.12 ± 0.10^{bB}	4.05 ± 0.02^{cB}	4.06 ± 0.06^{aA}
	8	3.55 ± 0.04^{cB}	2.69 ± 0.08^{dB}	3.10 ± 0.09^{dB}	3.74 ± 0.12^{cB}	3.96 ± 0.04^{cB}	3.30 ± 0.06^{bB}
CFS-BGSP9	0	4.60 ± 0.04^{aA}	4.47 ± 0.05^{aA}	4.45 ± 0.03^{aA}	**4.82 ± 0.07^{aA}**	4.73 ± 0.03^{a}	4.57 ± 0.11^{aA}
	1	4.07 ± 0.11^{bB}	4.48 ± 0.01^{aC}	4.34 ± 0.11^{aA}	4.30 ± 0.04^{bB}	4.70 ± 0.01^{aA}	4.38 ± 0.08^{aA}
	3	4.05 ± 0.05^{bB}	4.10 ± 0.07^{bB}	3.69 ± 0.09^{bB}	4.34 ± 0.12^{bB}	4.60 ± 0.04^{abC}	4.20 ± 0.01^{bA}
	5	4.09 ± 0.04^{bC}	4.08 ± 0.09^{bC}	3.50 ± 0.11^{bB}	4.37 ± 0.08^{bB}	4.48 ± 0.01^{bcC}	4.11 ± 0.10^{bA}
	8	4.04 ± 0.04^{bC}	3.01 ± 0.06^{cC}	3.23 ± 0.20^{cB}	4.37 ± 0.08^{bC}	4.41 ± 0.09^{cC}	3.87 ± 0.09^{cA}

Legend: Small letter indicated statistical significant difference in cell number of *L. monocytogenes* BGPF112i n same sample and treatment during storage. Capital letter indicated statistically significant differences in cell number of *L. monocytogenes* BGPF112 in same sample, between treatments, at the same week of storage.

Table 3 Number of *Staphylococcus aureus* in artificially contaminated raw meat and meat products

Sample		PRM	PBS	PFS	BRM	BBS	BP
	Weeks						
Control	**0**	4.56 ± 0.12^{aA}	4.37 ± 0.11^{aA}	4.06 ± 0.07^{aA}	4.21 ± 0.08^{aA}	4.35 ± 0.05^{aA}	4.60 ± 0.10^{aA}
	1	4.38 ± 0.09^{abA}	4.27 ± 0.20^{aA}	4.17 ± 0.11^{aA}	4.04 ± 0.07^{aA}	3.98 ± 0.03^{bA}	4.36 ± 0.07^{bA}
	3	4.29 ± 0.03^{bA}	5.07 ± 0.09^{bA}	4.07 ± 0.12^{aA}	4.17 ± 0.03^{aA}	5.01 ± 0.06^{cA}	4.29 ± 0.04^{bA}
	5	4.29 ± 0.10^{bA}	5.75 ± 0.04^{cA}	3.52 ± 0.08^{bA}	4.13 ± 0.13^{aA}	5.53 ± 0.03^{dA}	4.16 ± 0.04^{dbA}
	8	4.37 ± 0.09^{abA}	5.75 ± 0.06^{cA}	2.54 ± 0.06^{cA}	4.16 ± 0.09^{aA}	5.66 ± 0.16^{eA}	4.05 ± 0.04^{edA}
CFS-BGSP7	**0**	4.51 ± 0.02^{aA}	4.39 ± 0.08^{aA}	3.97 ± 0.07^{aB}	4.17 ± 0.11^{aA}	4.36 ± 0.06^{aA}	4.55 ± 0.03^{aA}
	1	4.07 ± 0.07^{bcB}	3.95 ± 0.05^{bB}	3.79 ± 0.08^{aB}	3.35 ± 0.16^{bB}	4.11 ± 0.16^{abA}	4.32 ± 0.08^{bA}
	3	4.08 ± 0.09^{cA}	3.37 ± 0.10^{cB}	3.42 ± 0.10^{bB}	3.26 ± 0.24^{bB}	3.98 ± 0.03^{bB}	4.20 ± 0.03^{bcA}
	5	3.69 ± 0.09^{dB}	3.30 ± 0.10^{cB}	2.13 ± 0.16^{cB}	2.98 ± 0.03^{bcB}	2.37 ± 0.12^{cB}	4.05 ± 0.04^{cA}
	8	2.45 ± 0.15^{eB}	3.11 ± 0.11^{cB}	1.23 ± 0.21^{dB}	2.89 ± 0.16^{cB}	2.01 ± 0.06^{cB}	3.28 ± 0.05^{dB}
CFS-BGSP9	**0**	4.50 ± 0.03^{aA}	4.40 ± 0.07^{aA}	3.97 ± 0.02^{aA}	4.23 ± 0.07^{aA}	4.39 ± 0.12^{aA}	4.55 ± 0.10^{aA}
	1	3.97 ± 0.03^{bcB}	4.11 ± 0.10^{bAB}	3.97 ± 0.03^{aAB}	3.62 ± 0.13^{bA}	4.10 ± 0.17^{aA}	4.37 ± 0.10^{abA}
	3	3.88 ± 0.03^{cB}	4.03 ± 0.05^{bC}	3.64 ± 0.03^{bB}	3.83 ± 0.12^{bA}	4.16 ± 0.27^{aB}	4.18 ± 0.01^{bcA}
	5	3.58 ± 0.05^{dB}	3.86 ± 0.04^{bC}	2.57 ± 0.05^{cC}	3.95 ± 0.05^{abA}	2.52 ± 0.07^{bB}	4.10 ± 0.09^{cA}
	8	3.11 ± 0.16^{eC}	3.86 ± 0.03^{bC}	1.63 ± 0.07^{dC}	3.93 ± 0.10^{ab}	2.12 ± 0.12^{cB}	3.85 ± 0.08^{dC}

Legend: Small letter indicated statistical significant difference in cell number of *S. aureus* BGPF322 in same sample and treatment during storage. Capital letter indicated statistically significant differences in cell number of *S. aureus* BGPF322 in same sample, between treatments, at the same week of storage.

In the control and treated samples, the number of *L. monocytogenes* BGPF112 and *S. aureus* BGPF322 immediately after artificial contamination (point 0) was ~4 log cfu g^{-1}.

In the control samples PRM, PBS, BRM and BBS, artificially contaminated with *L. monocytogenes* BGPF112, the number of pathogens increased significantly at the end of storage. These results are consistent with previously published results on the growth of *L. monocytogenes* on raw meat and cooked meat products during storage at 4°C (**Beumer *et al*., 1996**). In the other two control samples, PFS and BP, the number of *L. monocytogenes* BGPF112 decreased significantly during storage. This decrease in *Listeria* during storage could be due to the fact that these products have a lower aw value, a lower pH value, a higher salt concentration and the presence of starter cultures, which influences the growth and multiplication of *L. monocytogenes* (**Foegeding *et al*., 1996**). The results were similar in the control samples artificially contaminated with *S. aureus* BGPF322: in PRM, PBS, BRM and BBS the number of *S. aureus* BGPF322 increased, while in the control samples PFS and BP the number of sataphylococci decreased. **Mansur *et al*. (2016)** showed that *S. aureus* has a slight tendency to grow in samples of raw pork and pork ham during storage at 10 degrees, while a slight tendency to decrease the number of *S. aureus* is observed in samples of dry sausages.

A significant reduction of *L. monocytogenes* BGPF112 and *S. aureus* BGPF322 was observed in all treated samples compared to the control samples. However, the intensity of the reduction rate varied depending on the treatment and sample. At the end of storage (8 weeks), the number of *L. monocytogenes* BGPF112 was at the same level in the sample PFS (fermented pork sausage) treated with CFS-BGSP7 and CFS-BGSP9. In all other treated samples, CFS-BGSP7 showed better antilisterial activity than the CFS-BGSP9 treated and control samples. Similar results were obtained in the treated samples artificially contaminated with *S. aureus* BGPF322. In the BBS sample (boiled beefsausage), the number of *S. aureus* BGPF322 was the same in both treated samples at the end of storage. In all other samples, CFS-BGSP7 showed a higher reduction rate in the inhibition of *S. aureus* BGPF322 compared to the control and CFS-BGSP9 treated samples. According to a previous study, more small antimicrobial compounds were found in the supernatant of *B. laterosporus* BGSP9 strain than in the supernatant of *B. laterosporus* BGSP7 strain (**Miljkovic *et al*., 2019**). However, an extremely strong antimicrobial compound (1583 Da) was found in the supernatant of strain *B. laterosporus* BGSP7, which could be a possible reason for the stronger activity of CFS-BGSP7 in raw meat and meat products. CFS-BGSP7 also showed stronger activity compared to CFS-BGSP9 (**Figure 1** in agar well diffusion assay, which correlates with the results of the reduction of *L. monocytogenes* BGPF112 and *S. aureus* BGPF322 on raw meat and meat products.

CFS-BGSP7 and CFSBGSP9 showed different activity over time in different samples of raw meat and meat products. In the samples of raw pork and beef meat (PRM and BRM) treated with CFS-BGSP7 and CFS-BGSP9, similar results were obtained in the reduction of *Listeria* after 8 weeks of storage. The number of *L. monocytogenes* BGPF112 was reduced by ~1.5 log cfu g^{-1} and ~1.00 log cfu g^{-1} by CFS-BGSP7 and CFS-BGSP9 treatment, respectively. The efficacy of cell-free supernatants against *S. aureus* BGPF322 in raw pork samples was better, compared to the reduction of *L. monocytogenes* in raw pork and beef samples. The number of staphylococci was reduced by ~1.9 log cfu g^{-1} and ~1.2 log cfu g^{-1} by treatment with CFS-BGSP7 and CFS-BGSP9, respectively. In a previous study on the

control of *L. monocytogenes* in Spanish raw meat using bacteriocin PA1, a bactericidal effect of antilisterial compounds was obtained, which is consistent with our results (**Nieto-Lozano *et al*., 2006**). In some studies, complete inhibition of *Listeria* was achieved in the control of *L. monocytogenes* by using *Carnobacterium piscicola* in vacuum-packed meat (**Schöbitz *et al*., 1999**). The reason for this result could be the lower initial number of *L. monocytogenes*, as it has been shown that the efficacy of antimicrobial compounds depends on the number of pathogen cells and the amount of antimicrobial compounds (**Hugas *et al*., 1995; Mendoza *et al*., 1999**).

The efficacy of CFS-BGSP7 and CFS-BGSP9 was very high in boiled pork sausages (PBS) and boiled beef sausages (BBS) compared to other samples. In the samples of PBS artificially contaminated with *L. monocytogenes* BGPF112 and treated with CFS-BGSP7 and CFS-BGSP9, the best results in the reduction of *Listeria* were obtained compared to all other products. The number of *Listeria* was reduced by ~4.2 log cfu g^{-1} when treated with CFS-BGSP7 and by ~3.9 log cfu g^{-1} when treated with CFS-BGSP9, compared to the control. Staphylococci were also reduced by ~3.65 log cfu g^{-1} and ~3.54 log cfu g^{-1} in samples of BBS artificially contaminated with *S. aureus* BGPF322 after treatment with CFS-BGSP7 and CFS-BGSP9, respectively. Compared to previous studies, CFS-BGSP7 and CFS-BGSP9 achieved better results in reducing *Listeria* and *Staphylococcus* in cooked meat products (**Vijayakumar *et al*., 2017; Jofre *et al*., 2008**). This could be due to the fact that the cell-free supernatants used in this study contain a large number of different types of antimicrobial compounds (bacteriocins, lipopeptides, etc.), whereas cell-free supernatants in previous studies contained only one bacteriocin or purified bacteriocin.

In other treated samples, fermented pork sausages (PFS) and beef prosciutto (BP), the effects of CFS-BGSP7 and CFS-BGSP9 on the reduction of *L. monocytogenes* BGPF112 and *S. aureus* BGPF322 were statistically significant, but the intensity of the reduction is weaker compared to the other samples. This effect could be due to a higher retention of antimicrobial compounds by meat and fat components as well as a more difficult distribution of antimicrobial compounds in the meat matrix at higher dry matter content. In the dry sausage environment, a higher concentration of antimicrobial compounds may be required to balance the adsorption of antimicrobial molecules to the meat matrix. Previous studies (**Ananou *et al*., 2005 a, b**) have shown that the efficacy of antimicrobial compounds in raw sausages against *L. monocytogenes* and *S. aureus* depends on the concentration of target bacteria and bacteriocin molecules.

COCNLUSION

The results presented in this study are the first application of the cell-free supernatant of *B. laterosporus* to control *L. monocytogenes* and *S. aureus* in raw meat and meat products. The results showed that the antimicrobial compounds found in the cell-free supernatant of these two strains are very active against *L. monocytogenes* and *S. aureus* in raw meat and meat products. However, since a large number of antimicrobial compounds were isolated from the supernatant of these two strains, additional tests need to be performed to investigate effect of each of these compounds and their safety for use in food.

Acknowledgments: This research was funded by the Ministry for Science and Technological Development and Innovation of the Republic of Serbia through agreement 451-03-65/2024-03/200116 and 451-03-66/2024-03/200042

REFERENCES

Ananou, S., Garriga, M., Hugas, M., Maqueda, M., Martínez-Bueno, M., Gálvez, A., & Valdivia, E. (2005). Control of Listeria monocytogenes in model sausages by enterocin AS-48. *International Journal of Food Microbiology*, *103*(2), 179–190. https://doi.org/10.1016/j.ijfoodmicro.2004.12.024

Ananou, S., Maqueda, M., Martínez-Bueno, M., Gálvez, A., & Valdivia, E. (2005). Control of Staphylococcus aureus in sausages by enterocin AS-48. *Meat Science*, *71*(3), 549–556. https://doi.org/10.1016/j.meatsci.2005.04.039

Baindara, P., Singh, N., Ranjan, M., Nallabelli, N., Chaudhry, V., Pathania, G. L., Sharma, N., Kumar, A., Patil, P. B., & Korpole, S. (2016). Laterosporulin10: a novel defensin like Class IId bacteriocin from Brevibacillus sp. strain SKDU10 with inhibitory activity against microbial pathogens. *Microbiology*, *162*(8), 1286–1299. https://doi.org/10.1099/mic.0.000316

Bean, N. H., Goulding, J. S., Lao, C., & Angulo, F. J. (1996). Surveillance for foodborne-disease outbreaks--United States, 1988-1992. *MMWR. CDC Surveillance Summaries: Morbidity and Mortality Weekly Report. CDC Surveillance Summaries*, *45*(5), 1–66. https://pubmed.ncbi.nlm.nih.gov/8890258/

Beumer, R.R., Giffel, M.C., Boer, E.M., & Rombouts, F.M. (1996). Growth of Listeria monocytogenes on sliced cooked meat products. *Food Microbiology*, *13*, 333-340. https://doi.org/10.1006/fmic.1996.0039

Bintsis, T. (2017). Foodborne pathogens. *AIMS Microbiology*, *3*(3), 529–563. https://doi.org/10.3934/microbiol.2017.3.529

Cao, Y., Wang, Z., Dai, X., Zhang, D., Zeng, Y., Ni, X., & Pan, K. (2024). Evaluation of probiotic properties of a *Brevibacillus laterosporus* strain. *˜The œFASEB Journal*, *38*(5). https://doi.org/10.1096/fj.202302408r

Carfora, V., Caprioli, A., Marri, N., Sagrafoli, D., Boselli, C., Giacinti, G., Giangolini, G., Sorbara, L., Dottarelli, S., Battisti, A., & Amatiste, S. (2015). Enterotoxin genes, enterotoxin production, and methicillin resistance in *Staphylococcus aureus* isolated from milk and dairy products in Central Italy. *International Dairy Journal*, *42*, 12-15. https://doi.org/10.1016/j.idairyj.2014.10.009

Dal Bello, B., Cocolin, L., Zeppa, G., Field, D., Cotter, P. D., & Hill, C. (2012). Technological characterization of bacteriocin producing *Lactococcus lactis* strains employed to control *Listeria monocytogenes* in cottage cheese. *International Journal of Food Microbiology*, *153*(1-2), 58–65. https://doi.org/10.1016/j.ijfoodmicro.2011.10.016

Delbes, C., Alomar, J., Chougui, N., Martin, J. F., & Montel, M. C. (2006). *Staphylococcus aureus* growth and enterotoxin production during the manufacture of uncooked, semihard cheese from cows' raw milk. *Journal of Food Protection*, *69*(9), 2161–2167. https://doi.org/10.4315/0362-028x-69.9.2161

Diep, D. B., Godager, L., Brede, D., & Nes, I. F. (2006). Data mining and characterization of a novel pediocin-like bacteriocin system from the genome of *Pediococcus pentosaceus* ATCC 25745. *Microbiology*, *152*(6), 1649–1659. https://doi.org/10.1099/mic.0.28794-0

Foegeding, P. M., Thomas, A. B., Pilkington, D. H., & Klaenhammer, T. R. (1992). Enhanced control of *Listeria monocytogenes* by in situ-produced pediocin during dry fermented sausage production. *Applied and Environmental Microbiology*, *58*(3), 884–890. https://doi.org/10.1128/aem.58.3.884-890

Gamble, R., & Muriana, P. M. (2007). Microplate fluorescence assay for measurement of the ability of strains of *Listeria monocytogenes* from meat and meat-processing plants to adhere to abiotic surfaces. *Applied and Environmental Microbiology*, *73*(16), 5235–5244. https://doi.org/10.1128/AEM.00114-07

Hassi, M., Guendouzi, S. E., Haggoud, A., David, S., Ibnsouda, S., Houari, A., & Iraqui, M. (2012). Antimycobacterial activity of a *Brevibacillus laterosporus* strain isolated from a moroccan soil. *Brazilian Journal of Microbiology*, *43*(4), 1516–1522. https://doi.org/10.1590/S1517-83822012000400036

Hazard analysis and critical control point principles and application guidelines. Adopted August 14, 1997. National Advisory Committee on Microbiological Criteria for Foods. (1998). *Journal of Food Protection*, *61*(9), 1246–1259.

Hugas, M. (1998). Bacteriocinogenic lactic acid bacteria for the biopreservation of meat and meat products. *Meat Science*, *49*, 139–150. https://doi.org/10.1016/S0309-1740(98)90044-4

Hugas, M., Garriga, M., Aymerich, M., & Monfort, J.M. (1995). Inhibition of Listeria in dry fermented sausages by the bacteriocinogenic *Lactobacillus sake* CTC494. *Journal of Applied Microbiology*, *79*, 322-330. https://doi.org/10.1111/J.1365-2672.1995.TB03144.X

Hui, Y. W., & Dykes, G. A. (2012). Modulation of cell surface hydrophobicity and attachment of bacteria to abiotic surfaces and shrimp by Malaysian herb extracts. *Journal of Food Protection*, *75*(8), 1507–1511. https://doi.org/10.4315/0362-028X.JFP-12-062

Jofré, A., Garriga, M., & Aymerich, T. (2008). Inhibition of *Salmonella* sp. *Listeria monocytogenes* and *Staphylococcus aureus* in cooked ham by combining antimicrobials, high hydrostatic pressure and refrigeration. *Meat Science*, *78*(1-2), 53–59. https://doi.org/10.1016/j.meatsci.2007.06.015

Jiang, H., Wang, X., Xiao, C., Wang, W., Zhao, X., Sui, J., Sa, R., Guo, T. L., & Liu, X. (2015). Antifungal activity of *Brevibacillus laterosporus* JX-5 and characterization of its antifungal components. *World Journal of Microbiology & Biotechnology*, *31*(10), 1605–1618. https://doi.org/10.1007/s11274-015-1912-4

Khaled, J. M., Al-Mekhlafi, F. A., Mothana, R. A., Alharbi, N. S., Alzaharni, K. E., Sharafaddin, A. H., Kadaikunnan, S., Alobaidi, A. S., Bayaqoob, N. I., Govindarajan, M., & Benelli, G. (2018). *Brevibacillus laterosporus* isolated from the digestive tract of honeybees has high antimicrobial activity and promotes growth and productivity of honeybee's colonies. *Environmental Science and Pollution Research International*, *25*(11), 10447–10455. https://doi.org/10.1007/s11356-017-0071-6

Kojic, M., Svircevic, J., Banina, A., & Topisirovic, L. (1991). Bacteriocin-Producing Strain of *Lactococcus lactis* subsp. *diacetylactis* S50. *Applied and Environmental Microbiology*, *57*(6), 1835–1837. https://doi.org/10.1128/aem.57.6.1835-1837

Kushwaha, K., & Muriana, P. M. (2009). Adherence characteristics of Listeria strains isolated from three ready-to-eat meat processing plants. *Journal of Food Protection*, *72*(10), 2125–2131. https://doi.org/10.4315/0362-028x-72.10.2125

Mansur, A. R., Park, J. H., & Oh, D. H. (2016). Predictive Model for Growth of *Staphylococcus aureus* on Raw Pork, Ham, and Sausage. *Journal of Food Protection*, *79*(1), 132–137. https://doi.org/10.4315/0362-028X.JFP-15-227

Masoud, W., Vogensen, F. K., Lillevang, S., Abu Al-Soud, W., Sørensen, S. J., & Jakobsen, M. (2012). The fate of indigenous microbiota, starter cultures, *Escherichia coli*, *Listeria innocua* and *Staphylococcus aureus* in Danish raw milk and cheeses determined by pyrosequencing and quantitative real time (qRT)-PCR. *International Journal of Food Microbiology*, *153*(1-2), 192–202. https://doi.org/10.1016/j.ijfoodmicro.2011.11.014

Mendoza-Yepes, M.J., Abellan-Lopez, O., Carrion-Ortega, J., & Marin-Iniesta, F. (1999). Inhibition of *Listeria monocytogenes* and other bacteria in Spanish soft cheese made with *Lactococcus lactis* subsp. *diacetylactis*. *Journal of Food Safety*, *19*, 161-170. https://doi.org/10.1111/j.1745-4565.1999.tb00242.x

Moberg, L. (1989). Good Manufacturing Practices for Refrigerated Foods. *Journal of Food Protection*, *52*(5), 363–367. https://doi.org/10.4315/0362-028X-52.5.363
Miljkovic, M., Jovanovic, S., O'Connor, P. M., Mirkovic, N., Jovcic, B., Filipic, B., Dinic, M., Studholme, D. J., Fira, D., Cotter, P. D., & Kojic, M. (2019). *Brevibacillus laterosporus* strains BGSP7, BGSP9 and BGSP11 isolated from silage produce broad spectrum multi-antimicrobials. *PloS One*, *14*(5), e0216773. https://doi.org/10.1371/journal.pone.0216773
Newell, D. G., Koopmans, M., Verhoef, L., Duizer, E., Aidara-Kane, A., Sprong, H., Opsteegh, M., Langelaar, M., Threfall, J., Scheutz, F., van der Giessen, J., & Kruse, H. (2010). Food-borne diseases - the challenges of 20 years ago still persist while new ones continue to emerge. *International Journal of Food Microbiology*, *139*, 3–15. https://doi.org/10.1016/j.ijfoodmicro.2010.01.021.
Nieto-Lozano, J. C., Reguera-Useros, J. I., Peláez-Martínez, M.delC., & Hardisson de la Torre, A. (2006). Effect of a bacteriocin produced by *Pediococcus acidilactici* against *Listeria monocytogenes* and *Clostridium perfringens* on Spanish raw meat. *Meat Science*, *72*(1), 57–61. https://doi.org/10.1016/j.meatsci.2005.06.004
Normanno, G., La Salandra, G., Dambrosio, A., Quaglia, N. C., Corrente, M., Parisi, A., Santagada, G., Firinu, A., Crisetti, E., & Celano, G. V. (2007). Occurrence, characterization and antimicrobial resistance of enterotoxigenic *Staphylococcus aureus* isolated from meat and dairy products. *International Journal of Food Microbiology*, *115*(3), 290–296. https://doi.org/10.1016/j.ijfoodmicro.2006.10.049
Oxaran, V., Dittmann, K. K., Lee, S. H. I., Chaul, L. T., Fernandes de Oliveira, C. A., Corassin, C. H., Alves, V. F., De Martinis, E. C. P., & Gram, L. (2018). Behavior of Foodborne Pathogens *Listeria monocytogenes* and *Staphylococcus aureus* in Mixed-Species Biofilms Exposed to Biocides. *Applied and Environmental Microbiology*, *84*(24), e02038-18. https://doi.org/10.1128/AEM.02038-18
Panda, A. K., Bisht, S. S., DeMondal, S., Senthil Kumar, N., Gurusubramanian, G., & Panigrahi, A. K. (2014). Brevibacillus as a biological tool: a short review. *Antonie van Leeuwenhoek*, *105*(4), 623–639. https://doi.org/10.1007/s10482-013-0099-7
Pelisser, M. R., Klein, C. S., Ascoli, K. R., Zotti, T. R., & Arisi, A. C. (2009). Ocurrence of *Staphylococcus aureus* and multiplex PCR detection of classic enterotoxin genes in cheese and meat products. *Brazilian Journal of Microbiology*, *40*(1), 145–148. https://doi.org/10.1590/S1517-838220090001000025
Rodriguez, E., Arques, J. L., Nuñez, M., Gaya, P., & Medina, M. (2005). Combined effect of high-pressure treatments and bacteriocin-producing lactic acid bacteria on inactivation of *Escherichia coli* O157:H7 in raw-milk cheese. *Applied and Environmental Microbiology*, *71*(7), 3399–3404. https://doi.org/10.1128/AEM.71.7.3399-3404.2005
Ryser, E.T. (2001). Food Microbiology and Safety. *Journal of Food Science*, *66*, 471-471. https://doi.org/10.1111/j.1365-2621.2001.tb16132.x
Sanders, M. E., Morelli, L., & Tompkins, T. A. (2003). Sporeformers as Human Probiotics: Bacillus, Sporolactobacillus, and Brevibacillus. *Comprehensive Reviews in Food Science and Food Safety*, *2*(3), 101–110. https://doi.org/10.1111/j.1541-4337.2003.tb00017.x
Say, T. O., & Lorber, B. (2005). Gastroenteritis Due to *Listeria monocytogenes*. *Clinical Infectious Diseases*, *40*(9), 1327–1332, https://doi.org/10.1086/429324
Schelin, J., Wallin-Carlquist, N., Cohn, M. T., Lindqvist, R., Barker, G. C., & Rådström, P. (2011). The formation of *Staphylococcus aureus* enterotoxin in food environments and advances in risk assessment. *Virulence*, *2*(6), 580–592. https://doi.org/10.4161/viru.2.6.18122
Schlech, W. F., & Acheson, D. (2000). Foodborne Listeriosis. *Clinical Infectious Diseases*, *31*(3), 770–775. https://doi.org/10.1086/314008
Schöbitz, R., Zaror, T.M., León, O., & Costa, M. (1999). A bacteriocin from *Carnobacterium piscicola* for the control of *Listeria monocytogenes* in vacuum-packaged meat. *Food Microbiology*, *16*, 249-255. https://doi.org/10.1006/fmic.1998.0241
Työppönen, S., Petäjä, E., & Mattila-Sandholm, T. (2003). Bioprotectives and probiotics for dry sausages. *International Journal of Food Microbiology*, *83*(3), 233–244. https://doi.org/10.1016/s0168-1605(02)00379-3
Vázquez-Boland, J. A., Kuhn, M., Berche, P., Chakraborty, T., Domínguez-Bernal, G., Goebel, W., González-Zorn, B., Wehland, J., & Kreft, J. (2001). Listeria pathogenesis and molecular virulence determinants. *Clinical Microbiology Reviews*, *14*(3), 584–640. https://doi.org/10.1128/CMR.14.3.584-640.2001
Vijayakumar, P. P., & Muriana, P. M. (2017). Inhibition of *Listeria monocytogenes* on Ready-to-Eat Meats Using Bacteriocin Mixtures Based on Mode-of-Action. *Foods*, *6*(3), 22. https://doi.org/10.3390/foods6030022PMID
Weng, G., Huang, J., Ma, X., Song, M., Yin, Y., Deng, D., & Deng, J. (2022). *Brevibacillus laterosporus* BL1, a promising probiotic, prevents obesity and modulates gut microbiota in mice fed a high-fat diet. *Frontiers in Nutrition*, *9*, 1050025. https://doi.org/10.3389/fnut.2022.1050025
World Health Organization (WHO). (2022). *Food safety*. Who.int; World Health Organization: WHO. https://www.who.int/news-room/fact-sheets/detail/food-safety
Wing, E.J., & Gregory, S. H. (2002). *Listeria monocytogenes:* Clinical and Experimental Update. *The Journal of Infectious Diseases*, *185*, 18–24. https://doi.org/10.1086/338465
Yang, X., Huang, E., Yuan, C., Zhang, L., & Yousef, A. E. (2016). Isolation and Structural Elucidation of Brevibacillin, an Antimicrobial Lipopeptide from *Brevibacillus laterosporus* That Combats Drug-Resistant Gram-Positive Bacteria. *Applied and Environmental Microbiology*, *82*(9), 2763–2772. https://doi.org/10.1128/AEM.00315-16
Zhao, X., & Kuipers, O.P. (2016). Identification and classification of known and putative antimicrobial compounds produced by a wide variety of Bacillales species. *BMC Genomics*, *882*(17). https://doi.org/10.1186/s12864-016-3224-y