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Inhibitory Effect of Lactococcin BZ Against *Listeria innocua* and Indigenous Microbiota of Fresh Beef

Zeliha Yıldırım[1], Sabire Yerlikaya[2], Nilgün Öncül[3] and Tuba Sakin[1]*

[1]Niğde University, Food Engineering Department, TR-51240 Niğde, Turkey

[2]Karamanoğlu Mehmetbey University, Food Engineering Department, TR-70100 Karaman, Turkey

[3]Gaziosmanpaşa University, Food Engineering Department, TR-60250 Tokat, Turkey

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Summary

In this study, the effect of lactococcin BZ on microbiological quality of fresh beef is investigated. For this purpose, the meat samples were treated with various amounts of lactococcin BZ (200–2500 AU/mL), a bacteriocin produced by *Lactococcus lactis* spp. *lactis* BZ, and kept at 4–5 °C for 12 days. During storage, the microbiological properties of the meat samples with or without lactococcin BZ were determined. Inhibitory effect of lactococcin BZ depended on its amount. The higher the amount of lactococcin BZ, the higher the inhibitory activity. Treatment with lactococcin BZ at the level of 2500 AU/mL resulted in 4.87, 3.50 and 3.94 log cycle decrease in the counts of mesophilic, psychrotrophic and lactic acid bacteria, respectively, and $1.90 \cdot 10^4$ and $1.04 \cdot 10^2$ CFU/g reduction in coliform and faecal coliform bacteria, respectively, at the end of storage as compared to their initial numbers in the control sample. However, the counts of these bacteria in control samples increased during storage. Also, lactococcin BZ at 1600 AU/mL showed very strong antilisterial effect against *Listeria innocua* in fresh meat and reduced the cell numbers from 6.04 log CFU/g to undetectable level on the 6th day of storage. In conclusion, lactococcin BZ has a potential use as a biopreservation agent to improve safety and shelf life of raw beef.

Key words: bacteriocin, lactococcin BZ, beef, microbiological quality, *Listeria innocua*

Introduction

Meat is an excellent environment for microbiological growth due to its biological properties, chemical and nutrient composition. Therefore, fresh meat and meat products can be easily contaminated with food spoilage and foodborne pathogenic bacteria if not properly handled and preserved. In the meat industry, refrigerated storage is generally the most common preservation method used for fresh meat (*1,2*). In order to extend the shelf life of meat under refrigerated conditions, synthetic antimicrobial agents are used. However, usage of chemical additives in meat and other products is perceived by consumers as a health risk. Consumer preferences have been on the rise towards natural and minimally processed foods that are free from foodborne pathogens and chemical additives. Therefore, current trends in the meat industry include alternative non-thermal preservation technologies such as high hydrostatic pressure, biopreservation and active packaging (*3*). Among them, biopreservation is one of the most promising technologies for retaining the shelf life and safety of meat and meat products during the refrigerated storage.

Biopreservation is defined as the preservation of foods by using their natural and controlled microbiota and/or antimicrobial metabolites produced by these microorganisms (*4*). Lactic acid bacteria and their metabolites such as bacteriocins have a major potential for use in biopreservation because most lactic acid bacteria are considered as

*Corresponding author: Phone: +90 388 225 2354; Fax: +90 388 225 0112; E-mail: zeliha.yildirim@nigde.edu.tr

Generally Recognized As Safe (GRAS) and are the predominant microbiota in many foods (*5,6*).

Bacteriocins are generally defined as ribosomally synthesised, cationic and amphipathic antibacterial peptides that inhibit or kill other closely related and unrelated microorganisms. Bacteriocins produced by lactic acid bacteria exert their antimicrobial activity by various mechanisms. These mechanisms include the formation of pores in the cytoplasmic membrane of target cells followed by leakage of low molecular mass cellular compounds (potassium ions, amino acids, *etc.*) and dissipation of the proton-motive force, cell lysis, perturbation of the membrane lipid bilayers, and inhibition of biological processes such as protein, DNA and peptidoglycan synthesis (*7–9*).

It is generally accepted that bacteriocins are less effective in meat and meat products than they are in broth. Inhibitory activity may be reduced by the binding of the bacteriocin molecules to food components such as fat, the destabilising action of proteases, their uneven distribution in the food matrix, and their inhibition by salt and curing agents (*10–12*).

Lactococcin BZ is a bacteriocin produced by *Lactococcus lactis* spp. *lactis* BZ that was isolated from boza in our laboratory. Lactococcin BZ had inhibitory activity against either Gram-positive or Gram-negative bacteria, including some species of *Lactobacillus, Enterococcus, Leuconostoc, Listeria, Bacillus, Enterobacter, Escherichia, Salmonella, Yersinia* and *Citrobacter* (*13*).

Most studies on the application of bacteriocins in food systems are related to the control of foodborne pathogens. There are a few studies about controlling the indigenous spoilage microbiota with bacteriocins. *Listeria innocua* is widely distributed in meat and meat products and is the most closely related to *Listeria monocytogenes*, however, it is generally considered non-pathogenic. The objective of this study is to determine the antimicrobial effect of lactococcin BZ on the microbiological quality and shelf life of fresh beef, in particular the antilisterial activity, with the purpose of evaluating its potential use as a biopreservative.

Materials and Methods

Meat samples, microorganisms and media

Fresh beef samples (aseptically processed) used in this study were purchased at a local market (Tokat, Turkey) and maintained at 4–5 °C.

Lactococcus lactis spp. *lactis* BZ was used as a bacteriocin producer and *Lactobacillus plantarum* DSM 2601 was used as an indicator microorganism to determine bacteriocin activity. Both microorganisms were obtained from our culture collection (Niğde, Turkey), and were cultured in de Mann, Rogosa and Sharpe (MRS) broth (Fluka, Steinheim, Germany) at 32 °C. Both bacteria were maintained in MRS containing 20 % (by volume) glycerol at –80 °C.

Preparation of lactococcin BZ

The cultures of *Lactococcus lactis* spp. *lactis* BZ grown in MRS broth (2 L) at 32 °C for 18 h were centrifuged at 7000×*g* for 20 min and the pellet was discarded. After the pH of cell-free culture supernatant was adjusted to 6.5 by using 10 M NaOH, it was sterilised with membrane filter (d(pore)=0.45 μm) and partially purified by using the method of Moreno *et al.* (*14*). Filter-sterilised supernatant was precipitated with ammonium sulphate (50 % of saturation) and organic solvent (a methanol/chloroform mixture 1:2, by volume). The pellet obtained by centrifugation was stored at –80 °C until use. Bacteriocin activity of lactococcin BZ was determined by a spot-on-lawn method. For this purpose, serial twofold dilutions of lactococcin BZ were made with sterile water and 20 μL of each dilution were put on soft MRS agar (0.8 % agar) seeded with *Lb. plantarum* DSM 2601, the most sensitive indicator organism. After incubation at 30 °C for 24 h, the plates were checked for a clear inhibition zone and bacteriocin activity was defined as the reciprocal of the highest dilution giving a visible zone of inhibition of the indicator lawn and was expressed in AU/mL.

Preparation of meat samples coated with lactococcin BZ

Meat samples were cut into about 5-gram pieces (total surface area of about 6 cm^2) with a sterile knife and put into sterile stomacher bags (VWR, West Chester, PA, USA). After that, they were coated with 1 mL of the partially purified lactococcin BZ at a concentration of 200, 400, 800, 1600 and 2500 AU/mL. Meat samples were stored in sealed bags in refrigerated conditions (4–5 °C) for 12 days. Samples were randomly withdrawn during the experiment. Meat sample without lactococcin BZ was used as a control.

Microbiological analysis

To perform the microbiological analysis, the meat samples were taken at specific time intervals (0, 1, 4, 8 and 12 days) and homogenised in a stomacher for 3 min after the addition of 20 mL of sterile peptone water. Decimal dilutions were prepared using sterile peptone water (0.1 %, by mass per volume) and the following viable cells were counted by the spread plate method: total aerobic psychrotrophic and mesophilic bacteria and lactic acid bacteria. For the count of aerobic psychrotrophic and mesophilic bacteria, plate count agar (PCA; Merck, Darmstadt, Germany) was used as a medium, and aliquots of 0.1 mL of the appropriate dilutions were plated in triplicate on the PCA and incubated aerobically at 30 °C for 24–48 h and at 7 °C for 10 days for the enumeration of mesophilic and psychrotrophic bacteria, respectively (*15*). The viable cell number of lactic acid bacteria in the meat samples was determined by using MRS agar at 30 °C for 24–48 h (*16*).

In addition to these counts, total coliform and faecal coliform analyses were performed by using the most probable number (MPN) technique. MNP method is the most valuable method when expecting low counts of coliforms in the sample. From each dilution, 1-mL aliquots were inoculated into three tubes containing lauryl sulphate tryptose (LST; Merck) broth and incubated at 35 °C for 24–48 h. From each gassing LST tube, a loopful of suspension was transferred to a tube of brilliant green lactose bile broth (BGLB; Merck) and then they were incubated at

(35.0±0.5) °C for 48 h and examined for gas production. MPN of total coliforms was calculated based on the proportion of confirmed gassing LST tubes for three consecutive dilutions. From each gassing LST broth tube, a loopful of each suspension was transferred to a tube of EC (*Escherichia coli*) broth (Merck). EC tubes were incubated at 45.5 °C for 24 h and examined for gas production. Faecal coliform MPN was calculated by using the results of this test (*17*).

Inhibitory effect of lactococcin BZ on Listeria innocua

In this analysis, nalidixic acid-resistant *L. innocua* cultures were used. Therefore, first *L. innocua* ATCC 25401 was adapted to nalidixic acid (50 mg/L; Sigma-Aldrich, Darmstadt, Germany). For this purpose, the culture of *L. innocua* grown overnight at 37 °C in brain heart infusion (BHI) broth was transferred to BHI broth supplemented with an increasing concentration of nalidixic acid (5, 10, 20, 30, 40 and 50 mg/L) for 24 h. At the end of each 24 h, 10 mL of bacterial culture were transferred to the flask containing higher concentration of nalidixic acid and BHI solution (*18*). *L. innocua* cultures were successfully adapted to 50 mg/L of nalidixic acid.

Under sterile conditions, 2-cm deep layer of meat tissue was removed and the remaining meat samples were cut into about 5-gram pieces with a sterile knife and placed in sterile plastic bags. Meat samples were inoculated with 1 mL of nalidixic acid-resistant *L. innocua* suspensions containing about 10^6 CFU/mL of cells and incubated at room temperature for 30 min. After 1 mL of two different bacteriocin preparations (800 or 1600 AU/mL) was coated onto the meat samples, they were stored at refrigeration temperature (4–5 °C) for 6 days. The samples were analysed periodically and the *L. innocua* cell counts were determined on BHI agar with 50 mg/L of nalidixic acid at 35–37 °C for 24–48 h on the pre-poured plates. Meat sample without lactococcin BZ and *L. innocua* was used as a negative control, while the meat sample treated only with *L. innocua* was used as a positive control.

Statistical analysis

The results of the assay were expressed as the average of four independent experiments. Data were subjected to two-way analysis of variance (ANOVA) to estimate the effects of various concentrations of lactococcin BZ and storage time on microbiological quality of beef, and least significant difference at 5 % confidence level was used to evaluate the effects within each treatment.

Results and Discussion

Effect of lactococcin BZ on total aerobic psychrotrophic bacterial count

Total count of aerobic psychrotrophic bacteria in the meat sample without lactococcin BZ (control) was initially 6.12 log CFU/g and increased continuously during the storage period ($p<0.01$). Total count of aerobic psychrotrophic bacteria was high, but still in agreement with Turkish Food Codex Microbiological Criteria, *i.e.* $<5{\cdot}10^6$ CFU/g (*19*). At the end of storage, the level of aerobic psychrotrophic bacteria in the control samples reached 12.21 log cycles (Fig. 1). The treatment of fresh meat samples with lactococcin BZ reduced the counts of psychrotrophic bacteria during storage compared to the control samples (Fig. 1). With 200 AU/mL of lactococcin BZ, reduction was less pronounced ($p>0.05$) and it reached 0.92 log cycles until the 4th day of storage as compared to their initial number in the control sample. After the 4th day of storage, the cell counts of psychrotrophic bacteria increased slightly. Lactococcin BZ at 400 or 800 AU/mL caused a reduction in the counts of psychrotrophic bacteria by 1.12 and 1.56 log cycles, respectively, until the 8th day of storage when compared to the control sample on day 0, and after that, psychrotrophic bacterial counts increased slightly towards the end of storage. Psychrotrophic bacterial counts in meat samples containing lactococcin BZ at the level of 200, 400 or 800 AU/mL reached 6.77, 6.20 and 5.20 log CFU/g, respectively at the end of storage. Based on the results, the control sample and the meat sample containing 200 AU/mL of lactococcin BZ became unacceptable for consumption after the first and 12th day of storage, respectively. In meat samples subjected to lactococcin BZ at 400 or 800 AU/mL, the counts were low and were acceptable at the end of storage. Lactococcin BZ at 1600 or 2500 AU/mL produced very effective inhibitory activity over 12 days storage, reducing the count of psychrotrophic bacteria by 3.20 or 3.50 log units as compared to their initial number in the control sample, respectively (Fig. 1). Lactococcin BZ showed bactericidal effect against psychrotrophic bacteria in fresh beef. The inhibitory effect of lactococcin BZ was proportional to its amount in the samples, with higher levels (1600 and 2500 AU/mL) having a more pronounced effect.

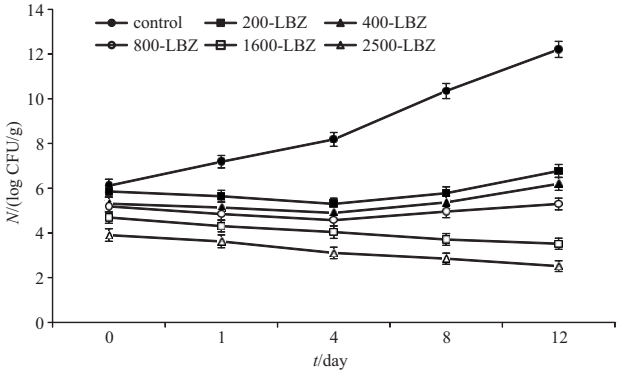

Fig. 1. Inhibitory effect of lactococcin BZ (LBZ) at 200, 400, 800, 1600 and 2500 AU/mL on the total count of aerobic psychrotrophic bacteria in raw meat stored at refrigeration temperature

It was reported that treatment of bovine meat with bacteriocins produced by *Lb. plantarum* BN controlled the growth of aerobic psychrotrophic bacteria and extended the shelf life of the refrigerated raw bovine meat from three to nine days (*20*). Initial psychrotrophic bacterial count in the raw material (day 0) was 3.56 log CFU/g. During the ninth day of storage at 5 °C, the psychrotrophic count in the samples exposed to bacteriocin was 6.32 log units, whereas in control samples without bacteriocin

it was 7.6 log units. It was stated that the microbiological quality of all meat samples was unacceptable at the end of storage (at 5 °C for 12 days). Application of nisin at the level of 200 ppm to raw minced beef resulted in a reduction in total aerobic counts from 9.73 to 7.00 log CFU/g on the first day of the storage, and then total aerobic counts in these samples increased during the cold storage at 4 °C for 6 days (*21*).

Effect of lactococcin BZ on total aerobic mesophilic bacterial count

The initial total mesophilic bacterial count in fresh meat samples was 7.23 log CFU/g and the count of aerobic mesophilic bacteria was very high and not acceptable for human consumption according to Turkish Food Codex Microbiological Criteria, *i.e.* $<5\cdot10^6$ CFU/g (*19*). It was observed that the aerobic mesophilic bacterial count in the control sample without lactococcin BZ increased to 12.58 log CFU/g during storage (Fig. 2). This increment was statistically important ($p<0.01$) from the beginning to the end of the storage period (Fig. 2). It was observed that the inhibitory effect of lactococcin BZ depended on its concentration. The higher concentration of lactococcin BZ, the more inhibitory activity against mesophilic aerobic bacteria was observed. Like psychrotrophic bacteria, the counts of aerobic mesophilic bacteria in the meat samples exposed to lactococcin BZ at the level of 400 and 800 AU/mL decreased until the 8th day of storage, but at the level of 200 AU/mL, the count of aerobic mesophilic bacteria decreased until the 4th day of storage (Fig. 2). After that, the counts of mesophilic bacteria in these samples increased slightly ($p>0.05$). The counts of mesophilic bacteria in the meat samples treated with 200, 400 and 800 AU/mL of lactococcin BZ reached 6.95, 6.11 and 5.37 log CFU/g, respectively, on the 12th day of storage, as compared to their initial numbers in the control sample. Use of 1600 or 2500 AU/mL of lactococcin BZ decreased the cell number of mesophilic bacteria very effectively. The cell number of mesophilic bacteria in the meat samples subjected to 1600 or 2500 AU/mL of lactococcin BZ decreased by 3.65 and 4.87 log cycles at the end of storage, respectively ($p<0.01$).

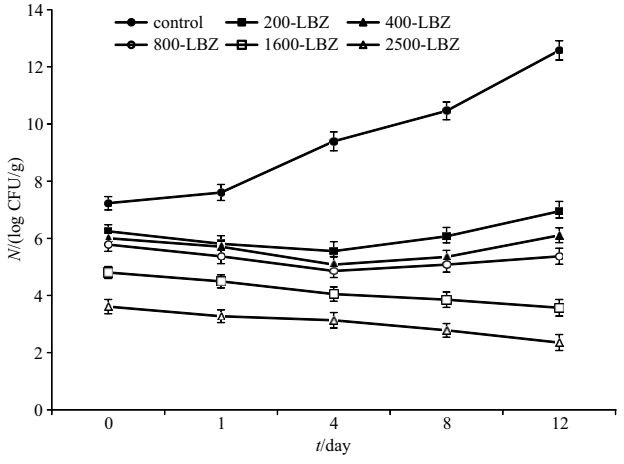

Fig. 2. Inhibitory effect of lactococcin BZ (LBZ) at 200, 400, 800, 1600 and 2500 AU/mL on the total count of aerobic mesophilic bacteria in raw meat stored at refrigeration temperature

Fiorentini *et al.* (*20*) reported that in the fresh meat treated with bacteriocins produced by *Lb. plantarum* BN, the initial content of mesophilic bacteria of 5.34 log did not decrease, but remained under control (bacteriostatic effect). Bacteriocins from *Lb. plantarum* BN, when applied to raw meat, inhibited the multiplication of aerobic mesophilic bacteria up to nine days. During the storage under refrigeration, mesophilic bacterial counts in the treated meat samples increased slightly, multiplying slowly when compared to the control samples without bacteriocin.

Effect of lactococcin BZ on total coliform and faecal coliform bacterial counts

The coliform (coli-aerogenes) bacteria are of faecal and non-faecal origin and they are found naturally in the intestinal tract of humans and animals. They may also be found in soil, on plant material, and on many types of food materials. The presence of coliform bacteria in food may indicate faecal contamination, presence of potential pathogens, food spoilage, and unsanitary food processing conditions. Faecal coliform is a subgroup of total coliforms that grows and ferments lactose at elevated incubation temperature; therefore, it is also referred to as thermotrophic coliforms. The faecal coliform group involves mostly *E. coli* but some other enteric bacteria such as *Klebsiella* (*22*).

Total coliform and faecal coliform contents in meat samples were determined by using the most probable number technique. Initial total coliform and faecal coliform counts in fresh meat samples were $1.90\cdot10^4$ and $1.04\cdot10^2$ CFU/g, respectively (Tables 1 and 2). Their counts increased slightly during the refrigerated storage ($p>0.05$) and reached $6.05\cdot10^5$ and $2.14\cdot10^2$ CFU/g at the end of storage. Total coliform bacteria in the meat samples were reduced from $1.90\cdot10^4$ to $0.90\cdot10^2$ CFU/mL at 200 AU/mL, to $0.68\cdot10^1$ CFU/mL at 400 AU/ mL, to undetectable value at 800, 1600 or 2500 AU/mL of lactococcin BZ treatment, respectively, $p<0.05$ (Table 1). The total count of coliform bacteria in the meat samples exposed to 200 and 400 AU/mL of lactococcin BZ decreased until the 12th day of storage. In meat samples containing 800, 1600 and 2500 AU/mL of lactococcin BZ, total coliform bacterial contents decreased to the undetectable level on the 8th, 8th and 4th day of storage, respectively. Lactococcin BZ at the level of 200 and 400–2500 AU/mL reduced the counts of faecal coli-

Table 1. Effect of lactococcin BZ (LBZ) at 200, 400, 800, 1600 and 2500 AU/mL on total count of coliform bacteria in meat samples

Sample	*t*/day				
	0	1	4	8	12
	N(CFU/g)				
Control	$1.90\cdot10^4$	$3.30\cdot10^4$	$8.40\cdot10^4$	$1.75\cdot10^5$	$6.05\cdot10^5$
200-LBZ	$1.35\cdot10^3$	$8.40\cdot10^2$	$5.35\cdot10^2$	$2.83\cdot10^2$	$0.90\cdot10^2$
400-LBZ	$7.85\cdot10^2$	$5.65\cdot10^2$	$1.46\cdot10^2$	$0.56\cdot10^2$	$0.68\cdot10^1$
800-LBZ	$2.20\cdot10^2$	$0.83\cdot10^2$	$0.49\cdot10^2$	<0.30	<0.30
1600-LBZ	$0.68\cdot10^2$	$0.93\cdot10^1$	$0.36\cdot10^1$	<0.30	<0.30
2500-LBZ	$0.77\cdot10^1$	$0.33\cdot10^1$	<0.30	<0.30	<0.30

form bacteria in the meat samples to undetectable level after 4 and 0 days of storage, respectively (Table 2). Similarly, Amin (*21*) reported that the addition of nisin (in the form of the chemical commercial product Nisaplin® (Danisco, Copenhagen, Denmark), at 200 ppm) to minced beef reduced total coliform and Enterobacteriaceae counts throughout the storage (at 4 °C for 6 days).

Table 2. Effect of lactococcin BZ (LBZ) at 200, 400, 800, 1600 and 2500 AU/mL on the count of faecal coliform bacteria in meat samples

Sample	*t*/day				
	0	1	4	8	12
	N/(CFU/g)				
Control	$1.04 \cdot 10^2$	$1.08 \cdot 10^2$	$1.52 \cdot 10^2$	$1.72 \cdot 10^2$	$2.14 \cdot 10^2$
200-LBZ	$0.38 \cdot 10^2$	$0.21 \cdot 10^2$	<0.30	<0.30	<0.30
400-LBZ	<0.30	<0.30	<0.30	<0.30	<0.30
800-LBZ	<0.30	<0.30	<0.30	<0.30	<0.30
1600-LBZ	<0.30	<0.30	<0.30	<0.30	<0.30
2500-LBZ	<0.30	<0.30	<0.30	<0.30	<0.30

Effect of lactococcin BZ on the content of lactic acid bacteria in meat

Initial count of lactic acid bacteria in fresh meat samples was 6.43 log CFU/g and their number increased during storage and reached up to 10.54 CFU/g at the end of storage (Fig. 3). Lactococcin BZ was effective in reducing the counts of lactic acid bacteria and its inhibitory effect depended on its amount. The contents of lactic acid bacteria in the meat samples exposed to 200, 400 or 800 AU/mL of lactococcin BZ were reduced by 1.43, 1.69 and 2.08 log units, respectively, on the 4th day of storage as compared to their initial numbers in the control sample and after that, their numbers slightly increased at the end of storage (up to 6.02, 5.52 and 4.79 log CFU/g, respectively), but these increases were not found statistically significant, $p>0.05$ (Fig. 3). However, the counts of lactic acid bacteria in meat samples treated with 1600 or 2500 AU/mL of lactococcin BZ decreased by 3.35 and 3.94 log cycles at the end of storage, respectively.

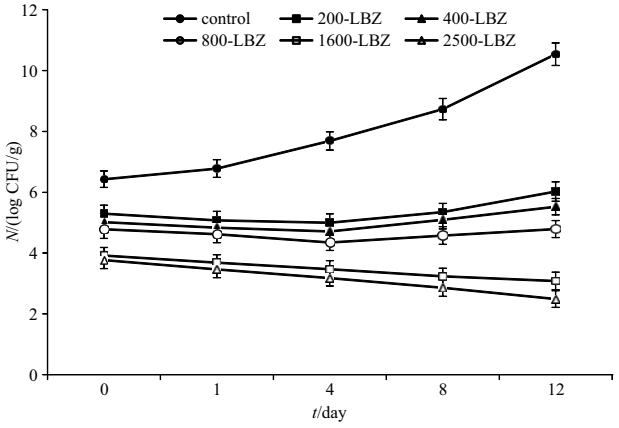

Fig. 3. Inhibitory effect of lactococcin BZ (LBZ) at 200, 400, 800, 1600 and 2500 AU/mL on the count of lactic acid bacteria in raw meat stored at refrigeration temperature

Inhibitory effect of lactococcin BZ on the survival of Listeria innocua

L. innocua is widely distributed in the environment and food sources such as meat and meat products. It can survive in extreme pH and temperature, and high salt concentration (*23*). A few atypical *L. innocua* strains have been reported to contain *L. monocytogenes*-specific genes and exhibit phenotypic characteristics similar to *L. monocytogenes* such as weak haemolysis (*23*,*24*).

The antilisterial effect of lactococcin BZ on *L. innocua* in fresh beef during refrigeration storage for 6 days is shown in Fig. 4. The cell count in the control samples inoculated only with *L. innocua* increased significantly from 6.04 to 7.28 log CFU/g during storage ($p<0.05$). Lactococcin BZ at 800 and 1600 AU/mL showed inhibitory effect against *L. innocua* in the meat samples, causing a decrease of 2.63–4.54 and 2.95–6.04 log units, respectively ($p<0.05$) from day 0 to day 6 (Fig. 4). Antilisterial effect of lactococcin BZ at 1600 AU/mL was more pronounced than at 800 AU/mL ($p<0.01$). Lactococcin BZ at 1600 AU/mL reduced the cell number of *L. innocua* to undetectable level in the meat samples on the 6th day of storage (detection limit of this analysis was 10 CFU/g).

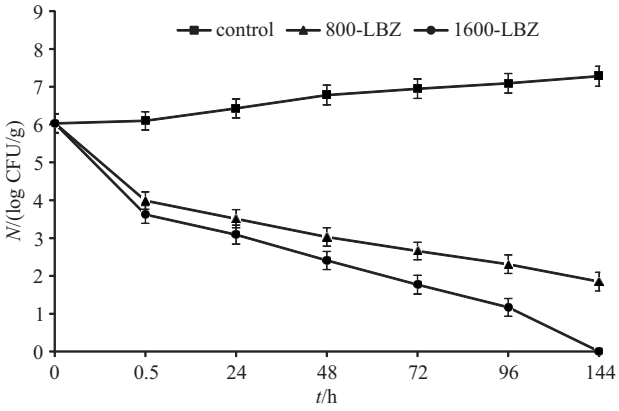

Fig. 4. Inhibitory activity of lactococcin BZ (LBZ) at 800 and 1600 AU/mL on the survival of *Listeria innocua* in raw meat at refrigeration temperature

Castellano and Vignolo (*25*) reported that lactocin AL 705 produced by *Lactobacillus curvatus* CRL705 when applied at 6400 AU/mL was effective in inhibiting *L. innocua* in refrigerated vacuum-packed fresh meat. Vignolo *et al.* (*26*) reported that lactocin 705 from *Lb. casei* CRL705 (17 000 AU/mL), enterocin CRL35 from *Enterococcus faecium* CRL35 (17 000 AU/mL), and nisin (2000 IU/mL) showed an initial decrease in viable counts of *L. monocytogenes* and *L. innocua* followed by the regrowth of the survivors after 1 h in the presence of each bacteriocin. They also observed a greater antilisterial effect when the bacteriocins were combined in pairs. When a mixture of three bacteriocins was used, no survivors were detected after 24 h of incubation.

The observed variation in antilisterial activities of bacteriocins could be due to the strains of *L. innocua* tested or due to the bacteriocin molecule composition itself. The sensitivity of *L. innocua* to LAB bacteriocins depends on the tested strain (*26*,*27*). In addition, the weak antilisterial activity might be caused by binding of bacteriocins to food constituents (fat or protein) or inactivation by glutathione S-transferase and proteases in raw meat (*11*,*28*, *29*). Our findings show that the binding of lactococcin BZ to meat surface and proteases found in meat did not affect its antilisterial activity.

In contrast to our findings, some researchers reported that the use of nisin in meat is limited due to its low solubility in meat pH, its interaction with lipids and proteins and loss of its inhibitory activity because of meat proteases (*11*,*30*,*31*).

Conclusion

The application of lactococcin BZ as a biopreservation agent to fresh, raw beef improved the microbiological quality, shelf life and safety of the meat samples. Lactococcin BZ at the level of 1600–2500 AU/mL was very effective in reducing the counts of psychrotrophic and mesophilic aerobic bacteria, lactic acid bacteria, and total coliform and faecal coliform bacteria. The counts of these bacteria in control samples without lactococcin BZ increased during storage. These results show that meat pH and meat components such as lipids, proteins and proteases do not affect inhibitory activity of lactococcin BZ. In addition, lactococcin BZ showed very strong antilisterial activity against *L. innocua* and inhibited its growth in the meat samples. Therefore, the results of this study show that the use of lactococcin BZ in the meat industry has the potential to reduce the counts of *L. innocua* and indigenous microorganisms and extend the shelf life of fresh meat.

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