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The inhibitory effect of lactoccocin BZ against *Escherichia coli* on fresh beef

Die hemmende Wirkung von Lactoccocin BZ gegen Escherichia coli auf frischem Rindfleisch

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Summary

During the processing of fresh meat, there is a risk of contamination with foodborne pathogenic *E. coli.* The use of bacteriocins is considered to ensure the safety of fresh meat. In this context, the impact of lactococcin BZ on the *E. coli* population in fresh beef has been investigated. The antibacterial activity of lactococcin BZ was observed in meat samples inoculated with *E. coli* both during and after attachment condition (10^3 and 10^6 CFU/mL), and over a 12 days storage period in refrigeration (4°C). Different amounts of lactococcin BZ (ranging from 400 to 3200 AU/mL) were applied to fresh meat for varying treatment durations (0–30 minutes). Following the application of lactococcin BZ at levels of 800, 1600, and 3200 AU/mL during attachment, *E. coli* counts were immediately reduced by 3.62 log units. At high inoculum dose, lactococcin BZ (3200 AU/mL) decreased the pathogen by approximately 6 log units in 5 minutes during attachment. *E. coli* exhibited sensitivity to lactococcin BZ (400, 1600, and 3200 AU/mL) both in low and high inoculum doses after attachment to fresh beef. Furthermore, the inhibitory effect of lactococcin BZ increased with its concentration over the 12 days of refrigerated. In conclusion, lactococcin BZ demonstrated inhibitory effect against *E. coli* in fresh beef, suggesting its potential use as a biopreservative in the meat industry.

Keywords: bacteriocin, biopreservation, meat, *Escherichia coli,* lactococcin BZ

Introduction

Meat plays a crucial role in human nutrition. According to the European Food Safety Authority (EFSA) guidelines, it is recommended to consume 0.83 grams of high quality protein per kilogram of body weight each day (EFSA, 2015). In Turkey, the average protein intake per person for the years 2011–2013 was 106.4 g, with 34 grams of this coming from animal sources (FAOSTAT, 2019). Meat must be handled and preserved carefully, from the point of production to its delivery to the consumer, to prevent food-borne diseases (Yılmaz and Gümüş, 2008; Yılmaz and Yılmaz, 2012).

Besides being a valuable source of protein, meat is abundant in essential nutrients such as B complex vitamins and iron, due to its high moisture content and favourable pH values (5.5–6.5), meat provides and ideal environment for microbial growth (WHO, 2023). The process of meat contamination with microorganisms initiates at the slaughterhouse through contact with the tools and equipment. After slaughtering process, microorganisms transferred to the meat from water, air, soil and workers. Poor conditions during other stages of the chain, including processing, cutting and storage, can lead to the proliferation of microorganisms (Yılmaz and Gümüş, 2008; Al-Mutairi, 2011; Mboto et al., 2012; Casaburi et al., 2015; Stellato et al., 2016). Fresh meat is particularly prone to microbiological spoilage, making it one of the potentially riskiest foods. While the quantity and types of initial microorganisms may vary based on animal health and production conditions, both mesophilic and psychrotrophic bacteria can be present in meat, serving as potential pathogens and factors contributing to spoilage factors (Fiorentini et al., 2001). According to the World Health Organization (WHO), 600 million people worldwide fall ill each year due to the consumption of contaminated food, with 420,000 fatalities (WHO, 2019).

E. coli is a bacterium commonly present in the intestinal tract of both warm-blooded animals and humans (FAO, 2011). According to the Foodborne Disease Outbreak Surveillance System of the Centers for Disease Control and Prevention (CDC), analysis of the data between 2008 and 2012 revealed that *E. coli* was linked to 30% of foodborne outbreaks and 24% of associated illness (IFSAC, 2015). The report also highlights, that 46% of disease causing foods are of meat origin. *E. coli* stands out as one of the most significant microbiological challenges faced by the meat industry.

The technique of utilizing natural antimicrobial compounds, specifically lactic acid bacteria (LAB) or inhibitory substances they produce, to enhance the shelf life and safety of foods is known as the bio-preservation technique (Soomra et al., 2002; Devlieghere et al., 2004). Lactic acid bacteria produce natural antimicrobial compounds known as bacteriocins. In recent years, bacteriocins have been extensively studied, due to their natural origin, easy disintegration in the human and animal intestinal tract, and their capability to degrade and inhibit disease-causing bacteria without causing any alteration in the physicochemical structures of the foods (Cleveland et al., 2001; O'Sullivan et al., 2002; And and Hoover, 2003; Cotter et al., 2005; Fimland et al., 2005; Deegan et al., 2006; Drider et al., 2006).

In earlier studies, *Lactococcus lactis* ssp. *lactis* BZ isolated from boza was identified as a bacteriocin producer, and its bacteriocin was characterized. Lactococcin BZ exhibited inhibitory activity against eleven Gram-positive and seven Gram-negative bacteria in the medium (Şahingil et al., 2011).

Due to the potent antimicrobial activity of lactococcin BZ against pathogenic microorganisms in the medium, the research progressed to the next stage. To assess the inhibitory effect of the mentioned bacteriocin on pathogenic bacteria in the food system, experiments conducted on milk, yoghurt and cheese products. The inhibitory activity of lactococcin BZ in milk and dairy products was successfully demonstrated (Öncül et al., 2015; Yıldırım et al., 2016a; Öncül and Yıldırım, 2019; Öncül and Yıldırım, 2020). It is crucial to evaluate lactococcin BZ, proposed for useas a bio-preservative, in food matrices with varying components. Previous studies have demonstrated, inhibitory effect against *Lis. monocytogenes* and *Lis. innocua* in fresh beef (Yıldırım et al., 2016b; Yıldırım et al., 2017).

In the current study, it was aimed to investigate the inhibitory effect of lactococcin BZ on *E. coli*. In this way, it was aimed to prolong the storage duration of meat and meat products by laveraging the bio-preservative properties of lactococcin BZ. The intention is to contribute to the economy by minimizing product losses and to safeguard human health by incorporating it within the framework of food safety. In this context, the inhibitory effect of Lactococcin BZ was examined on i) *E. coli* attached to meat, ii) *E. coli* during attachment to meat and iii) *E. coli* growth during its refrigerator storage.

Material and method

Material

Fresh beef

This study utilized freshly cut beef as the meat samples. The lean beef meat samples were obtained from a local butcher of known for maintaining high hygiene standards. The lean beef meat, purchased in 1 kg pieces for every replication, was transported to the laboratory in an icebox while still in its original packaging. The samples were kept at 4–5°C until analysis. The analyses were conducted immediately upon the meat's arrival at the research laboratory.

Microorganisms and media

As bacteriocin producing bacteria used in this study were *Lactococcus lactis* ssp. *lactis* BZ, which was previously isolated from boza in laboratory. *L. lactis* BZ and its inhibitory compound (lactococcin BZ) were characterized in a previous study through morphological, physiological, and biochemical analyses, including SDS-PAGE, fatty acid profiling, and sequence homology of the 16S rDNA gene (Şahingil et al., 2011). To facilitate the growth of *L. lactis* BZ and the production of bacteriocin, de Man Rogosa and Sharpe (MRS, Fluka, Germany) broth medium was utilized. *E. coli* served as test bacteria and *Lactiplantibacillus plantarum* DSM2601 was employed as the indicator bacteria to assess bacteriocin activity. Both strains were obtained from Refik Saydam Hıfzıssıhha Culture Collection in Turkey. *L. lactis* BZ and *Lb. plantarum* were cultured in MRS medium at 30°C for 18 hours and 24 hours, respectively. *E. coli* was grown in Brain Hearth Infusion (BHI, Merck, Germany) broth medium at 35–37°C for 24 hours. The bacteria used in the study were stored at –80°C in a medium containing 20% glycerol (Merck, Germany).

Method

This study is divided into four parts: acquiring the bacteriocin produced by the bacteriocinogenic strain, elucidating the inhibitory effect of bacteriocin on *E. coli* attached to meat, uncovering the inhibition of *E. coli* during attach-

ment, and revealing the bacteriocin's inhibitory effect on the growth of *E. coli* during refrigerated meat storage. The detailed methods of the work plan are presented below:

Preparation of bacteriocin

L. lactis BZ produces the lactococcin BZ bacteriocin (Şahingil et al., 2011). To produce bacteriocin, *L. lactis* BZ bacteriocinogenic strain was activated twice, then inoculated into 1% MRS medium and incubated at 30°C for 18 hours. After incubation, the bacterial culture was centrifuged at 7000 × g for 20 minutes. The filtrate was then collected and sterilized with a 0.45 µm pore diameter membrane filter. The cell-free supernatant was frozen and dried in a lyophilizer. The resulting lactococcin BZ obtained through lyophilization was then tested for bacteriocin activity test and stored at –80°C until use (Moreno et al., 2002; Öncül and Yıldırım, 2019).

Determination of bacteriocin activity

Bacteriocin production and activity were determined using the agar spot method. Prior to the analysis, the samples underwent heat treated at 75°C for 10 minutes, followed by the preparation of serial two-fold dilutions (1/2, 1/4, 1/8 etc.). 20 µl of each dilution was applied to soft agar MRS (0.8%) containing *Lb. plantarum* as the indicator microorganism, and the petri dishes were then incubated for 24 hours at 30°C. Inhibition zones of 2 mm or larger as a result of the incubation process were considered positive. Bacteriocin activity was quantified in arbitrary unit (AU), defined as the inverse of the highest dilution demonstrating inhibitory activity (Mayr-Harting et al., 1972).

Preparation of meat samples

To minimize surface contamination, all areas of the meat samples were cut to a thickness of 2 cm, and the outer portions were discarded. The remaining meat was aseptically cut in 1 cm^3 pieces (approximately 5 grams). These portions were then placed in polyethylene stomacher plastic bags and turned every 15 minutes for 2 hours in the laminar flow cabinet with UV light, effectively minimizing the risk of contamination (Yildirim et al., 2016).

Inhibitory effect of lactococcin BZ on E. coli attached to meat

E. coli was cultured in BHI medium at 35°C for 24 hours. At the end of the incubation period, the culture was centrifuged and the resulting pellet was collected. After washing the bacterial cell pellet twice with phosphate buffer, the cell concentration was diluted in the same buffer to achieve the initial concentrations of 10^3 and 10^6 CFU/mL. The initial counts were assessed on Violet Red Bile Agar (VRBA) at 35°C for 24 hours. In order for *E. coli* to attach to meat, meat samples prepared according to the above-given method were placed in a phosphate buffer (pH 7; composed of potassium dihydrogen phosphate and disodium hydrogen phosphate heptahydrate) containing approximately 10^3 CFU/mL and 10^6 CFU/mL *E. coli* and left there for 90 minutes. At the conclusion of this time period, the samples were collected and immersed in sterile lactococcin BZ solutions at concentrations of 400, 1600, 3200 AU/mL, with phosphate buffer serving as a control. The immersion times included 0, 5, 10 and 30 minutes. The samples were placed in a sterile stomacher bag, 20 mL of phosphate buffer was added. The mixture was then homogenized using in a stomacher. The enumeration of *E. coli* was carried out using the pour plate method in VRBA at 35–37°C for 24–48 hours. A total of 24 different samples were analyzed, comprising 2 different inoculation doses (CFU/mL) 3 different bacteriocin concentrations (AU/mL) at 4 different time points. This excludes positive and negative controls. In the study, UV treated meat samples and UV treated meat samples containing different concentrations of lactococcin BZ were designated as negative controls and no bacterial growth was observed. Samples prepared by adding low (10^3 CFU/mL) and high (10^6 CFU/mL) levels of *E. coli* to UV treated meat samples were considered as positive control (Yildirim et al., 2017).

Inhibitory effect of lactococcin BZ on E. coli during meat attachment

Meat samples, sterilized using UV light, were immersed in bacteriocin solutions prepared at concentrations of 400, 800, 1600 and 3200 AU/mL for duration of 10 minutes. The samples were subsequently immersed *E. coli* bacterial solutions at concentrations of 10^3 and 10^6 CFU/mL for 0, 5 and 10 minutes. At the conclusion of these time intervals, the samples were retrieved and placed in a sterile stomacher bag. Subsequently, 20 mL of phosphate buffer was added, and the samples were disintegrated in the stomacher for 1 minute. The enumeration of *E. coli* was conducted using pour plate method in VRBA at a temperature range of 35–37°C for a period of 24–48 hours. A total of 24 different samples were included in the analysis, comparing 2 different inoculation doses (CFU/mL) multiplied by 4 distinct bacteriocin concentrations (AU/mL) and observed at 3 different time points. This excludes the positive and negative controls. In the study, UV-treated meat samples and UV-treated meat samples containing various concentrations of lactococcin BZ were designated as negative controls and no bacterial growth was observed in these samples. Samples prepared by adding low levels (10^3 CFU/mL) and high level (10^6 CFU/mL) of *E. coli* to UV-treated meat samples, were considered as positive controls (Yildirim et al., 2017).

Inhibitory effect of lactococcin BZ on E. coli growth during refrigerated storage of meat

Meat samples, approximately 5 g each, sterilized under UV in the laminar flow cabinet, were inoculated with 1 mL of cultures containing various concentrations of *E. coli* (10^3, 10^5 and 10^7 CFU/mL). About 30 minutes post inoculation, 1 mL of bacteriocin solutions, prepared at concentrations of 400, 800, 1600 and 2500 AU/mL, was added to the samples. The samples were stored under refrigeration conditions for 12 days. At specific intervals during the storage period (0^{th}, 1^{st}, 4^{th}, 8^{th} and 12^{th} days), samples were collected and transferred to sterile stomacher bags. Afterward, 20 mL of phosphate buffer was added to the meat samples and dilutions were prepared following their disintegration in the stomacher for 1 minute. Subsequently, the mixture was plated using the pour plate method in VRBA medium and incubated for 24 hours at at 35–37°C. A total of 60 different samples were included in the analysis, comprising 3 distinct inoculation doses (CFU/mL) multiplied by 4 varied bacteriocin concentrations (AU/mL) and observed at 5 different time points. This excludes the positive and negative controls. In the study, UV-treated meat samples and UV-treated meat samples containing various concentrations of lactococcin BZ were designated as negative controls and no bacterial growth was observed in these samples. Samples prepared by separately adding 10^3 CFU/mL, 10^5 CFU/mL and 10^7 CFU/mL of *E. coli* cells

to UV-treated meat samples were considered as positive controls (Yildirim et al., 2017).

Statistical analysis

In the study, each analysis was conducted in triplicate, with three repetations and three parallels (3×3). In this study, microbiological analysis results are expressed as log CFU/g, and the detection limit is set at <2.00 log CFU/g. Duncan test was employed to compare the means, and data analyses were conducted using SPSS version 17 (17.0.3.2010, SPSS Inc., Chicago, USA) a statistical package program with 95% confidence interval.

Results

Inhibitory effect of lactococcin BZ on *E. coli* attached to meat

Table 1 illustrates the inhibitory effect of lactococcin BZ on *E. coli* attached to meat. It was observed that lactococcin BZ inhibited *E. coli* in meat samples with a low pathogen concentration (10^3 CFU/g). A reduction of 0.11 log units in pathogen count was noted after treatment with 400 AU/mL of lactococcin BZ, and a further decrease of 0.48 log units was observed within 30 minutes. At a bacteriocin concentration of 1600 AU/mL, 0.59 log inhibition was observed following the initial application. Additionally, after 30 minutes, it was determined that the bacterial count decreased to an undetectable level ($p<0.05$). At a lactococcin BZ concentration of 3200 AU/mL, it was observed that incubation consistently reduced the number of *E. coli* to an undetectable level across all treatment times. No significant change in the number of pathogenic microorganisms was observed in the control during the treatment times at low-level inoculation ($p>0.05$). There was no significant difference in the inhibitory effect of 400 AU/mL lactococcin BZ across the application times ($p>0.05$).

It was observed that the inhibitory effect of lactococcin BZ decreased with the increase in *E. coli* level (10^6 CFU/g). However, when used at a high concentration, it effectively controlled the growth of the tested pathogen. The control sample exhibited an increase to 6.50 log CFU/g after 30 minutes of application ($p<0.05$). After 30 minutes of high-level inoculation in meat samples, the pathogen count decreased from 6.50 log CFU/g to 5.59 log CFU/g and 3.41 log CFU/g in samples treated with 400 AU/mL and 1600 AU/mL lactococcin BZ, respectively ($p<0.05$). Samples with 3200 AU/mL lactococcin BZ effectively inhibited pathogenic microorganism at all application times, as shown in Table 1.

Inhibitory effect of lactococcin BZ on *E. coli* during meat attachment

The inhibitory effect of lactococcin BZ on *E. coli* was observed during the attachment of the pathogen to meat samples and the data are presented in Table 2. It was observed that the inhibitory effect increased with a rise in bacteriocin concentration and decreased with an increase in the amount of *E. coli*.

At low inoculation dose, bacteriocin exhibits effectiveness against the pathogen. While the number of detected pathogens (3.42 log CFU/g) did not show a statistically significant difference from the control ($p>0.05$) after the application of lactococcin BZ at a dose of 400 AU/mL, the pathogen count dropped below the detectable level prolonged treatment time. At levels of 800 AU/mL, 1600 AU/mL and 3200 AU/mL of lactococcin BZ, it effectively reduced the number of *E. coli* cells to undetectable level at all application times.

As the inoculation dose increased, the inhibitory effect also increased with longer application times and higher bacteriocin concentrations. The number of *E. coli* cells was found to be 5.31 log CFU/g after applying lactococcin BZ at a concentration of 400 AU/mL. A reduction of 2.05 log CFU/g was observed after a 10 minute application. After a 10 minute application, bacteriocin demonstrated inhibitory effects on the pathogenic microorganism, achieving reductions to 4.02 and 4.83 log CFU/g at doses of 800 and 1600 AU/mL, respectively. At a bacteriocin level of 3200 AU/mL, the number of *E. coli* decreased to an undetectable level within the 5^{th} and 10^{th} minutes of the application (Table 2).

Inhibitory effect of lactococcin BZ on *E. coli* growth during refrigerated storage of meat

The results pertaining to the impact of lactococcin BZ on *E. coli* during the refrigerated storage of the meat samples are outlined in Table 3. It was observed that the inhibitory effect of lactococcin BZ, applied at various concentrations, was directly proportional to both the bacteriocin concentration and application time. Conversely, it was inversely

TABLE 1: *The antimicrobial effect of lactococcin BZ against E. coli attached to raw meat (log CFU/g).*

Sample	Low Inoculation Dose (10^3 CFU/mL)				High Inoculation Dose (10^6 CFU/mL)			
	Treatment Time (min)				Treatment Time (min)			
	0.	5.	10.	30.	0.	5.	10.	30.
Control	3.17* (±0.01)^{Ac}	3.18 (±0.04)^{Ac}	3.24 (±0.03)^{Ac}	3.31 (±0.14)^{Ab}	6.04 (±0.09)^{Ac}	6.22 (±0.04)^{ABb}	6.32 (±0.04)^{BCc}	6.50 (±0.13)^{Cd}
Lac. BZ 400 AU/mL	3.06 (±0.29)^{Ac}	3.00 (±0.34)^{Ac}	2.89 (±0.39)^{Ac}	2.83 (±0.44)^{Ab}	6.00 (±0.16)^{Ac}	5.98 (±0.42)^{Ab}	5.86 (±0.28)^{Ac}	5.59 (±0.61)^{Ac}
Lac. BZ 1600 AU/mL	2.58 (±0.04)^{Cb}	2.52 (±0.05)^{Cb}	2.16 (±0.21)^{Bb}	<2 (±0.00)^{Aa}	5.09 (±0.10)^{Bb}	4.95 (±0.11)^{Bb}	4.77 (±0.08)^{Bb}	3.41 (±008)^{Ab}
Lac. BZ 3200 AU/mL	<2 (±0.00)^{Aa}	<2 (±0.00)^{Aa}	<2 (±0.00)^{Aa}	<2 (±0.00)^{Aa}	<2 (±0.00)^{Aa}	<2 (±0.00)^{Aa}	<2 (±0.00)^{Aa}	<2 (±0.00)^{Aa}

* n=9, (±standard deviation); Different lowercase letters indicate a significant difference among the average values in the rows and different capital letters a indicate significant difference among the average values in the columns ($p<0.05$)

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TABLE 2: *The antimicrobial effect of lactococcin BZ against E. coli during its attachment to raw meat (log CFU/g).*

Sample	Low Inoculation Dose (10^3 CFU/mL)			High Inoculation Dose (10^6 CFU/mL)		
	Treatment Time (min)			Treatment Time (min)		
	0.	5.	10.	0.	5.	10.
Control	3.62* (±0.01)^{Aa}	3.74 (±0.01)^{Aba}	3.90 (±0.11)^{Ba}	6.16 (±0.66)^{Aa}	6.29 (±0.62)^{Ba}	6.67 (±0.16)^{Ca}
Lac. BZ 400 AU/mL	3.42 (±0.17)^{Aa}	<2 (±0.00)^{Bb}	<2 (±0.00)^{Bb}	5.31 (±0.16)^{Aab}	5.06 (±0.09)^{Bb}	4.62 (±0.33)^{Cb}
Lac. BZ 800 AU/mL	<2 (±0.00)^{Ab}	<2 (±0.00) ^{Ab}	<2 (±0.00)^{Ab}	5.02 (±0.76)^{Aabc}	3.60 (±0.37)^{ABc}	2.65 (±0.08)^{Bc}
Lac. BZ 1600 AU/mL	<2 (±0.00)^{Ab}	<2 (±0.00)^{Ab}	<2 (±0.00)^{Ab}	4.43 (±0.18)^{Abc}	2.94 (±0.11)^{Bc}	1.84 (±0.07)^{Cd}
Lac. BZ 3200 AU/mL	<2 (±0.00)^{Ab}	<2 (±0.00)^{Ab}	<2 (±0.00)^{Ab}	3.72 (±0.33)^{Ac}	<2 (±0.00)^{Bd}	<2 (±0.00)^{Be}

* n=9, (±standard deviation); Different lowercase letters indicate a significant difference among the average values in the rows and different capital letters indicate a significant difference among the average values in the columns ($p<0.05$)

proportional to the inoculation dose of the pathogenic bacteria. At all inoculation levels, an increase in the bacterial count was observed during the storage for positive controls.

The viable cell number of *E. coli* was decreased from 3.82 log CFU/g to 3.27 log CFU/g by the end of storage, starting at the level of *E. coli* 10^3 CFU/mL after treatment with 400 AU/mL lactococcin BZ. And it was found to be different from the control ($p<0.05$). The lowest bacteriocin application dose, 400 AU/mL, exhibited an inhibitory effect during the 12-day storage period, resulting in a 0.87 log CFU/g reduction compared to the control by the end of the storage ($p<0.05$). As the concentration of bacteriocin increased, the inhibitory effect on the pathogen increased. Lactococcin BZ reduced the number of *E. coli* cells to undetectable levels of the 8th, 1st and day of the administration, at concentrations of 800 AU/mL, 1600 AU/mL and 2500 AU/mL, respectively (Table 3).

When applying 400 AU/mL lactococcin BZ to an *E. coli* inoculation dose of 10^5 CFU/mL, the pathogens count was 4.97 log CFU/g at the beginning of the storage and decreased to 4.39 log CFU/g by the last day of the storage. With the application of 800 AU/mL lactococcin BZ, the *E. coli* count was 4.91 log CFU/g on the initial day and decreased to 3.39 log CFU/g on the last day. The count of pathogenic bacteria dropped to undetectable levels with a bacteriocin concentration of 1600 AU/mL on the 8th day and 2500 AU/mL on the 4th day of the storage (Table 3).

The inhibition values at the start and end of the storage were statistically significant at all bacteriocin concentrations for an *E. coli* inoculation dose of 10^7 CFU/mL ($p<0.05$). On the final day of the storage, lactococcin BZ at the levels of 400 AU/mL, 800 AU/mL, 1600 AU/mL and 2500 AU/mL reduced the *E. coli* counts by 2.25 log CFU/g, 4.62 log CFU/g, 5.72 log CFU/g and, 7.99 CFU/g, respectively (Table 3).

Discussion

Inhibitory effect of lactococcin BZ on *E. coli* attached to meat

To assess the inhibitory effect of lactococcin BZ on *E. coli* contaminating and adhering to meat, *E. coli* at the level of approximately 10^3 and 10^6 CFU/g levels was introduced to meat samples. The samples were then kept at room temperature for 1.5 hours, allowing the pathogen cells to attach to meat. Subsequently, they were treated with varying concentrations of lactococcin BZ. The results of the analysis are presented in Table 1.

In the absence of lactococcin BZ, the *E. coli* population increased to 3.31 and 6.50 log CFU/g by the end of the treatment time. Although lactococcin BZ had an inhibitory effect on *E. coli*, there was no observed in the pathogen population throughout the 30 minute period. Nielsen et al., (1990) were tested a bacteriocin produced by *Pediococcus acidiactici* on *Lis. monocytogenes* attached to meat. After 2 minute application of bacteriocin, all bacteriocin concentrations (500, 1000 and 5000 AU/mL) produced 1 to 2 log unit reduction on pathogenic bacteria at low inoculum level (10^4 CFU/mL). In this study, lactococcin BZ inhibited *E. coli* cells between 0.18–3.18 log CFU/g at all bacteriocin concentration at low inoculum doses after 5 minutes. At a bacteriocin level of 500 AU/mL, there was more than 1 log unit inhibition on listeria cells. However, the inhibition was less than 1 log unit when the bacteriocin level was kept constant and the amount of the pathogen was increased to 10^7 CFU/mL. Lactococcin BZ at a concentration of 400 AU/mL resulted in a reduction of the *E. coli* by less than 1 log unit, both at low and high inoculum doses after 30 minutes. The application of bacteriocin at 5000 AU/mL resulted in a 2 log unit reduction within 10 minutes in the high quantity of listeria cells attached to meat. This result is significantly lower than the counts observed in current study. Despite the differences in bacteriocin type and pathogens, these findings consistently support the application of the bacteriocin for enhancing meat safety. In a previous study, the inhibitory effect of lactococcin BZ on *Lis. monocytogenes* attached to meat was investigated (Yıldırım et al., 2017). The antilisterial activity at a low inoculation level (4.71 log CFU/g) with a concentration of 400 AU/mL was observed to be 1.48 log units after 5 minutes and 2.62 log units after 30 minutes of application. At 1600 AU/mL, there occurred 2.58 log reductions in the 5th minute of the application. The test pathogen was below the detectable value at the 30th minute of 1600 AU/mL lactococcin BZ and at the all application times of 3200 AU/mL concentration. Compared to *E. coli*, lactococcin BZ produced an inhibition ranging from 0.11 to 0.48 log units compared to the positive control at the lowest bacteriocin concentration. An inhibition of 0.49 log CFU/g relative to the positive control was observed immediately after the application at 1600 AU/mL. It was observed that lactococcin BZ remained below the detectable level for 30 minutes at the concentration of 1600 AU/mL and at the highest concentration. This was consistent with the results obtained for *Lis. monocytogenes* in all the applications.

In the present study, lactococcin BZ inhibited the *E. coli* count 6.50 log CFU/g by 0.91 and 3.10 units at concentrations

of 400 and 1600 AU/mL after 30 minutes. This resulted in reductions to 5.59 log CFU/g and 3.41 log CFU/g, respectively similar to findings in the previous study (Yıldırım et al., 2017). As the bacteriocin concentration increased, it was observed that the test pathogen decreased to an undetectable level at 3200 AU/mL. Lactococcin BZ, similar to nisin, the first commercial bacteriocin, exhibits limited antimicrobial activity in meats at low concentration (Aesan et al., 2003; Stergiou et al., 2006). This can be resolved by increasing the bacteriocin concentration. Lactococcin BZ reduced the test pathogen to undetectable levels at both low and high inoculation levels with a concentration of 3200 AU/mL. The limited bacteriocin activity in meat can be attributed to its binding to fat or protein in meat and subsequently hydrolysis by meat proteases.

Inhibitory effect of lactococcin BZ on *E. coli* during meat attachment

To assess the inhibitory effect of lactococcin BZ during the attachment stage of *E. coli* to meat, meat samples were initially immersed in solutions with varying lactococcin BZ concentrations (400, 800, 1600, 3200 AU/mL), followed by exposure to phosphate buffer containing *E. coli* for brief periods (5 and 10 min). Results of the analysis are presented in Table 2.

Lactococcin BZ significantly reduced the counts of *E. coli* upon initial application, consistent with findings by Schneider et al. (2018) and Lu et al. (2020). In a different study, the inhibitory effect of bacteriocin produced by *P. acidiactici* was tested at concentrations 500, 1000 and 5000 AU/mL on *Lis. monocytogenes* in meat during the attachment at levels of 10^4 and 10^7 CFU/mL. At a high inoculum level (10^7 CFU/mL), bacteriocin resulted in a reduction of 1 log (500 AU/mL) to 2 log (1000 and 5000 AU/mL) units after 2 minute of application (Nielsen et al., 1990). In comparision, at high inoculum level, lactococcin BZ induced an inhibition of 1.23 to 6.29 log units on *E. coli* within 5 minutes of application, across concentrations ranging from 400 to 3200 AU/mL. Lactococcin BZ exhibited a stronger inhibitory effect against the target pathogen compared to the bacteriocin produced by *P. acidiactici.*

In previous study, lactococcin BZ decreased *Lis. monocytogenes* attached to meat (10^4 CFU/mL) to below detectable levels within 10 minutes at 800 AU/mL and within 5 minutes at 1600 and 3200 AU/mL. At a high inoculation level (10^7 CFU/mL), lactococcin demonstated this effect at 3200 AU/mL within the first 5 minutes of application (Yıldırım et al., 2017). The inhibitory effect of lactococcin BZ on *E. coli* was observed to be <2 log CFU/g at an inoculation level of 10^3 CFU/mL in the 5th minute with a concentration of 400 AU/mL and in the 10th minute at a concentration ranging from 800 to 3200 AU/mL. While, lactococcin BZ appears to be

TABLE 3: *The antimicrobial effect of lactococcin BZ against the growth of E. coli into raw meat during refrigeration (log CFU/g).*

Samples	10^3(CFU/mL) Treatment Time (day)					10^5(CFU/mL) Treatment Time (day)					10^7(CFU/mL) Treatment Time (day)				
	0.	1.	4.	8.	12.	0.	1.	4.	8.	12.	0.	1.	4.	8.	12.
Control	3.88 (±0.31)* Aa	3.95 (±0.09) Aa	3.95 (±0.37) Aa	4.04 (±0.08)A Aa	4.14 (±0.04) Aa	5.10 (±0.03)A a	5.45 (±0.02)B a	5.77 (±0.06)C a	6.01 (±0.04)D a	6.39 (±0.06)E a	7.24 (±0.07) Aa	7.29 (±0.14) Aa	7.49 (±0.06) Aa	7.78 (±0.14) Ba	7.99 (±0.05)B a
Lac. BZ 400 AU /mL	3.82 (±0.89) Aa	3.55 (±0.31) Ab	3.51 (±0.26) Aa	3.38 (±0.03)A b	3.27 (±0.21) Ab	4.97 (±0.03)A ab	4.92 (±0.06) Ab	4.75 (±0.06)A Bb	4.56 (±0.10)B Cb	4.39 (±0.22) Cb	7.13 (±0.30) Aa	7.01 (±0.16) Aa	6.94 (±0.07) Ab	5.89 (±0.06) Bb	5.74 (±0.10)B b
Lac. BZ 800 AU /mL	3.62 (±0.06) Aa	3.42 (±0.04)B b	3.32 (±0.02)C a	<2 (±0.00)D c	<2 (±0.00) Dc	4.91 (±0.06)A b	4.86 (±0.05) Ab	4.72 (±0.01)A b	3.76 (±0.11)B c	3.39 (±0.13) Cc	6.40 (±0 03) Ab	6.17 (±0.25) Ab	4.64 (±0.25)B c	3.57 (±0.08) Cc	3.37 (±0.30)C c
Lac. BZ 1600 AU /mL	3.28 (±0.11) Aa	<2 (±0.00)B c	<2 (±0.00)B b	<2 (±0.00)B c	<2 (±0.00)B c	3.63 (±0.12)A c	3.59 (±0.29) Ac	3.19 (±0.04)B c	<2 (±0.00)C d	<2 (±0.00) Cd	6.24 (±0.10) Ab	5.89 (±0.07) Ab	4.19 (±0.11)B d	2.78 (±0.07) Cd	2.27 (±0.34) Dd
Lac. BZ 2500 AU /mL	<2 (±0.00) Ab	<2 (±0.00) Ac	<2 (±0.00) Ab	<2 (±0.00)A c	<2 (±0.00) Ac	3.21 (±0.04)A d	3.04 (±0.06)B d	<2 (±0.00)C d	<2 (±0.00)C d	<2 (±0.00) Cd	5.42 (±0.09) Ac	4.74 (±0.12)B c	<2 (±0.00)C e	<2 (±0.00) Ce	<2 (±0.00)C e

* n=9, (±standard deviation); Different lowercase letters indicate a significant difference among the average values in the rows and different capital letters indicate a significant difference among the average values in the columns ($p<0.05$)

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more effective against *E. coli*, it is believed that this observation may influenced by the variation in the inoculation levels.

Inhibitory effect of lactococcin BZ on *E. coli* growth during refrigerated storage of meat

Meat samples were inoculated with pathogenic microorganism at three different inoculation levels in order to analyse the inhibitory effect of lactococcin BZ during the storage of the meat contaminated with *E. coli* in refrigerator conditions. The growth of *E. coli* was examined in samples with varying levels of lactococcin BZ and in samples without it during a 12 day storage period in the refrigerator. Results of the analysis are presented in Table 3.

In the present study, the initial counts of *E. coli* were 3.88 log CFU/g, 5.10 log CFU/g and 7.24 log CFU/g, and at the end of the storage period, they were recorded as, 4.14 log CFU/g, 6.39 log CFU/g and 7.99 log CFU/g. At a concentration of 400 AU/mL, lactococcin BZ was unable to reduce *E. coli* cells a detectable level. These results align with the finding reported by Yıldırım et al., (2017). Lactococcin BZ inhibited *E. coli* (7.29 log CFU/g) at concentrations of 400 AU/mL, 800 AU/mL, 1600 AU/mL and 2500 AU/mL by 0.28, 1.12, 1.40 and 2.55 logarithmic units on the first day of the storage, respectively. However, the results are considerably lower than the counts observed for *Lis. monocytogenes* at previous study.

In its previous assessments, lactococcin BZ at concentrations of 800 and 1600 AU/mL demonstrated effectiveness in inhibiting *Lis. innocua,* ranging between 2.63–4.54 log and 2.95–6.04 log under refrigerated conditions for 6 days (Yıldırım et al., 2016b). Interestingly, lactococcin BZ at concentrations of 800 and 1600 AU/mL exhibited a stronger antibacterial activity against *Lis. innocua* than *E. coli* during storage in meat.

Nisin (5000 IU/mL) was administered to *Lis. monocytogenes* Scott A and *E. coli* O157:H7 inoculated beef in vacuum packs. The meat samples were stored at 4°C for 30 days. On the initial day of the storage, *Lis. monocytogenes* count was 2.52 log CFU/cm^2 in the control sample, reduced to 0.56 log CFU/cm^2 in the nisin-treated sample. Similarly, the *E. coli* count was 3 log CFU/cm^2 in the control sample, which decreased to 2.74 log CFU/cm^2 in the nisin-treated sample (Zhang and Mustapha 1999). The *E. coli* count in the control sample on the first day of storage was 3.88 log CFU/g. Lactococcin BZ applications at various concentrations (400–2500 AU/mL) resulted in a reduction ranging from 0.06 to 3.88 log CFU/g.

Nisin (nisaplin) was tested on *Lis. monocytogenes* at two different storage temperatures on 3 logarithmic pathogens as 400 IU/g and 800 IU/g in minced beef. Samples were stored at 4°C for 16 days and at 37°C for 36 hours. At the end of the storage period, no effect of nisin on the pathogen was observed at 400 IU/g bacteriocin level at both temperatures. However, an inhibition of 2.4 log units at 800 IU/g at 4°C and 0.9 log unit at 37°C was detected (Pawar et al., 2000). Lactococcin BZ showed a significant difference from the control (4.14 log CFU/g) on the 12th day of the storage under refrigerator conditions at the level of 400 AU/mL. It resulted in a 0.87 log unit reduction on *E. coli* ($p<0.05$).

In the present study, while the impact of lactococcin BZ increased with rising concentration and decreased with an increasing dose of pathogen, the cell count remained significantly lower than the control group, consistent with findings from previous studies (Vignolo et al., 1996; Castellano and Vignolo, 2006; Abdollahzadeh et al., 2014). Bacteriocin BM1829 effectively decreased the counts of *E. coli* and *S. aureus* in meat samples over a 10-day period under refrigeration, as compared to the control (approximately 7–8 log). This effect heightened with the increase bacteriocin concentration, similar to lactococcin BZ (Yan et al., 2021). At two different storage temperatures (7°C and 26°C), 10% bacteriocin concentration was tested in chicken meat to regulate both the total viable count and the number of *E. coli*. The examined bacteriocin reduced both the total viable count and *E. coli* cell count compared to the control at both storage temperatures (Yuliana et al., 2020). The bacteriocin produced by *Lb. plantarum* SC01 inhibited the growth of the *S. aureus* pathogen in pork compared to the control sample during 48 hours storage at room temperature (Le et al., 2019). Nisin inhibited *Lis. monocyotegenes* (2×10^3 CFU/g) cells in chicken breast meat by 0.4 log units on the 0th day of the storage at 4°C (Halimi et al., 2010). Additionally, it has been reported that the bacteriocin produced by *P. acidilactici* inhibits listeria cells at a concentration of 5000 BU/mL for 21 days (Nieto-Lozano et al., 2006), a combination of bacteriocin (lactocin, enterocin, and nisin) inhibits *Lis. monocytogenes* on minced meat for 24 hours at 20°C (Vignolo et al., 2000), bacteriocin LFX101 reduced *S. aureus* and *E. coli* on fresh pork meat for 7 days at 4°C (Xin et al., 2023), the bacteriocin produced by *C. piscicola* L103 is effective for inhibiting *Lis. monocytogenes* on vacuum-packed meat for 14 days (Schöbitz et al., 1999).

As a result, bacteriocins isolated from various sources are tested against pathogenic microorganisms in food matrix under different conditions. Generally, the inhibitory activity of bacteriocin in meat and meat products are affected by components such lipid, protein, and proteases. In the current study, lactococcin BZ produced by *L. lactis* ssp. *lactis* BZ, demonstrated and potent antibacterial activity in the meat environment against *E. coli*, a significant pathogenic bacterium posing public health concern in the meat industry. These results indicate that the antibacterial activity of lactococcin BZ is not affected by meat components against *E. coli*. Therefore, lactococcin BZ holds potential as a biopreservative agent for meat industry. In future studies, research can explore topics such as different packaging materials, various application methods of bacteriocin, and the utilization of bacteriocin-producing bacteria as a protective culture.

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Conflict of interest

The authors declare that there is no conflict of interest.

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