ERCİYES ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ
Journal of Faculty of Veterinary Medicine, Erciyes University

Research Article / Araştırma Makalesi
21(3), 155-161, 2024
DOI: 10.32707/ercivet.1587311

Antimicrobial Effect of Partially Purified Bacteriocins on *Pseudomonas aeruginosa**

Eda HACIOMEROGLU[1,a], Gizem CUFAOGLU[1,b]

[1]Kırıkkale University, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology, Kırıkkale-TÜRKİYE
[2]Kırıkkale University, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology, Kırıkkale-TÜRKİYE
ORCID: [a]0000-0003-1859-4399; [b]0000-0001-8639-532X

Corresponding author: Gizem ÇUFAOĞLU; E-mail: gizemcufaoglu@kku.edu.tr
How to cite: Hacıömeroğlu E, Çufaoğlu G. Antimicrobial effect of partially purified bacteriocins on *Pseudomonas aeruginosa*. Erciyes Univ Vet Fak Derg 2024; 21(3):155-161

Abstract: Bacteriocins are commonly used in foods to inhibit the growth of spoilage and pathogenic bacteria, thus extending the shelf life of food products. Interest in bacteriocins is increasing because of the increasing tendency of consumers to use healthy, natural and additive-free products in foods. In this study, it was aimed to investigate the control of *P. aeruginosa* in milk by using partially purified bacteriocins produced from lactic acid bacteria (LAB) strains. Among the 13 reference LAB strains, four strains that showed the highest antimicrobial activity by the agar spot test were selected for bacteriocin production. The bacteriocins were partially purified with 40% ammonium sulfate. The antibacterial activity of bacteriocins on *P. aeruginosa* strains was determined in arbitrary unit by the well diffusion method. Then, UHT milk samples inoculated with *P. aeruginosa* and bacteriocin cocktail were stored at +4°C for a week and bacterial counts were performed on the 1st, 3rd, 5th, and 7th days. The LAB strains that displayed the widest clear zones on *P. aeruginosa* were determined as *L. plantarum*, *L. paraplantarum*, *L. fermentum* and *L. pentosus* and the antimicrobial activities of the partially purified bacteriocins of these strains were 640, 640, 160 and 80 AU/ml, respectively. Among tested *P. aeruginosa* strains, the highest antibacterial effect was observed against *P. aeruginosa* ATCC 15442 (>18mm). In the milk model, the bacteriocin cocktail caused a decrease of approximately 2 log cfu/ml in the number of bacteria for up to three days and the number remained constant until the end of the seventh day. However, the decrease in the number of bacteria was not statistically significant (P>0.05). As a result, bacteriocins obtained from *Lactobacillus* strains showed antibacterial effect on *P. aeruginosa* on agar medium but could not achieve a significant decrease on the milk. However, bacteriocins, which have generally been proven to efficient on Gram-positive bacteria, have been determined to be effective on *P. aeruginosa*, a Gram-negative bacterium. Moreover, this study emphasizes that in addition to *in-vitro* experiments, products to be used for biocontrol purposes in foods are also needed to complement with food models.
Keywords: Bacteriocin cocktail, lactic acid bacteria, milk, *P. aeruginosa*, storage

Kısmi Saflaştırılmış Bakteriyosinlerin *Pseudomonas aeruginosa* Üzerindeki Antimikrobiyal Etkisi

Öz: Bakteriyosinler, patojen ve/veya gıdalarda bozulmaya neden olan bakterilerin büyümesini engellemek ve gıdaların raf ömrünü uzatmak amacıyla yaygın olarak kullanılmaktadır. Tüketicilerin gıdalarda sağlıklı, doğal ve katkı maddesi içermeyen ürünleri kullanma eğiliminin artması nedeniyle bakteriyosinlere olan ilgi de artmaktadır. Bu çalışmada laktik asit bakterisi (LAB) suşlarından üretilen kısmi saflaştırılmış bakteriyosinler kullanılarak sütte *P. aeruginosa*'nın kontrolünün araştırılması amaçlanmıştır. On üç adet referans LAB suşu arasında agar spot testiyle en yüksek antimikrobiyal aktiviteyi gösteren dört suş, amonyum sülfatla kısmi olarak saflaştırılmıştır. Bakteriyosinlerin *P. aeruginosa* suşları üzerindeki antibakteriyel aktivitesi kuyu difüzyon yöntemiyle arbitrary unit (AU/ml) olarak belirlenmiştir. Ek olarak, *P. aeruginosa* ile kontamine edilen UHT süt örneklerine bakteriyosin kokteyli eklenmiş bir hafta boyunca +4°C'de inkübe edilerek 1., 3., 5. ve 7. günlerde bakteri sayımları yapılmıştır. *P. aeruginosa* üzerinde en geniş zon sergileyen LAB suşlarının *L. plantarum*, *L. paraplantarum, L. fermentum* ve *L. pentosus* olduğu ve bu suşlardan elde edilen kısmi saflaştırılmış bakteriyosinlerinin antimikrobiyal aktivitelerinin sırasıyla 640, 640, 160 ve 80 AU/ml olduğu belirlenmiştir. Test edilen *P. aeruginosa* suşları arasında en yüksek antibakteriyel etki *P. aeruginosa* ATCC 15442'ye (>18mm) karşı gözlenmiştir. Süt modelinde ise, bakteriyosin kokteyli bakteri sayısında üç güne kadar yaklaşık 2 log kob/ml azalmaya neden olmuş ve sayı yedinci günün sonuna kadar sabit kalmıştır. Bununla birlikte, bakteri sayısındaki azalmanın istatistiksel olarak anlamlı olmadığı tespit edilmiştir (P>0.05). Sonuç olarak çalışmada elde edilen bakteriyosinlerin *P. aeruginosa*'ya karşı *in-vitro* ortamda antibakteriyel etki gösterdiği tespit edilmesine karşın gıda modelinde anlamlı bir sonuç elde edilememiştir. Bu çalışmanın verileri gıdalarda biyokontrol amaçlı kullanılacak maddelerin *in-vitro* analizlerin yanı sıra gıda modelleri ile tamamlanmasının önemi vurgulamıştır.
Anahtar kelimeler: Bakteriyosin kokteyli, laktik asit bakterileri, muhafaza, *P. aeruginosa*, süt

Geliş Tarihi/Submission Date : 29.02.2024
Kabul Tarihi/Accepted Date : 13.06.2024

* This study was presented as an oral presentation and full text at the Erzurum II. International Health Sciences and Multidisciplinary Approaches Congress.

Introduction

Foodborne illnesses have been a global concern for years. Despite the use of modern food storage techniques, the rate of illness and death due to foodborne pathogens is still increasing, especially in countries without appropriate food safety monitoring systems. On the other hand, consumers' renewed interest in ready-to-eat foods that are minimally processed and contain additives is a challenge for the food industry. The search for alternative natural compounds to increase food safety and shelf life has become inevitable (Sharma et al., 2017). In this context, bacteriocins are an option that cannot be ignored among the alternatives. Bacteriocins are peptides or proteins synthesized in the ribosomes of Gram-positive and Gram-negative bacteria and released into the extracellular environment (Cotter et al., 2013). Bacteriocins are secreted by some types of bacteria to kill other bacteria. They generally vary in length between 30-60 amino acids and have been associated with many bacterial species such as *Lactobacillus, Pediococcus, Leuconostoc, Lactococcus*, *E. coli, Staphylococcus* and *Enterococcus* (Chen et al., 2003; Mills et al., 2011). Bacteriocins are potential alternatives for use in the food and pharmaceutical industries to prevent food spoilage and the growth of pathogenic bacteria (Verma et al., 2017). It is recognized by the US Food and Drug Administration (FDA) with the "Generally Recognized as Safe (GRAS)" status (Johnson et al., 2018).

Food spoilage is defined as loss of quality in terms of color, odor, texture and, in general, loss of sensory properties and is associated with a microbiological, chemical or physical source (Petruzzi et al., 2017). Psychotropic *Pseudomonas* species are among the most common bacteria that cause spoilage, especially in refrigerated foods (Tirloni et al., 2021). *Pseudomonas* species produce heat-stable lipolytic and proteolytic enzymes that play a very important role in reducing the quality and shelf life of raw and processed milk (Dogan and Boor, 2003). When raw milk is contaminated with *Pseudomonas*, lipolytic enzymes cause hydrolyzation of milk fat, leading to the development of off-flavors, rancidity, and texture changes. Proteolytic enzymes cause degradation of casein and the formation of bitter peptides, which further contribute to spoilage (Erkmen and Bozoglu, 2016). Because of these enzymes are heat stable, they do not vanish after pasteurization. On the other hand, *P. aeruginosa* attracts attention as a foodborne pathogen in various food groups such as water, milk, meat, fruits and vegetables (Chatterjee et al., 2016). On the light of these information, we aimed to investigate the antibacterial effect of partially purified LAB bacteriocins on *P. aeruginosa in-vitro* conditions and in milk model.

Materials and Methods

Strains

Thirteen reference LAB strains were included in the study following: *L. pentosus* ATCC 16366*, L. gasseri* ATCC 33323*, L. paraplantarum* ATCC 10641*, L. plantarum* ATCC 10241*, L. fermentum* ATCC 14931*, L. paracasei* ATCC 25302*, L. casei* ATCC 334, *L. brevis* ATCC 8287*, L. diolivorans* ATCC 4356*, L. acidophilus* ATCC 19435*, L. curvatus* ATCC 25601*, L. buncheri* ATCC 4005*, L. rhamnosus* ATTC 53103. Additionally, both multidrug-resistant strains with various virulence properties and commonly used laboratory reference strains with different virulence properties were used; *P. aeruginosa* ATCC 15442, *P. aeruginosa* ATCC 27853, *P. aeruginosa* PAO1, Vim-2, and Imp-13.

Determination of LAB strains effective against P. aeruginosa

Among 13 LAB strains, first it was intended to determine the ones which produce bacteriocins effective against *P. aeruginosa*. For this purpose, serial dilutions of LAB strains were prepared, and appropriate 0.1 ml was inoculated to MRS agar to obtain approximately 30 colonies on the petri dish. Also, *P. aeruginosa* strains were incubated individually at 37°C overnight. After incubation, 200 µl of each strain (10^9 cfu/ml) were inoculated in LB soft agar and overlaid on the MRS agar plates which had LAB colonies on it. Petri dishes were incubated at 37°C overnight. LAB strains that formed transparent zones around the colonies were determined as bacteriocin producers effective against *P. aeruginosa* and selected for further analyses (Kaya and Simsek, 2019).

Agar spot assay

LAB strains that formed clear zones on the *P. aeruginosa* mixed culture were checked once again. For this purpose, 200 µl of the active P*. aeruginosa* mix culture enriched the night before was inoculated into LB soft agar and poured onto LB agar and MRS agar as a second layer. Petri dishes were incubated at 37° C overnight in an aerobic environment to ensure that *P. aeruginosa* strains showed turbidity. The next day, each petri dish was divided into four and 20 µl of active LAB strains were added and incubated overnight at 30°C in an anaerobic environment. At the end of the incubation, four strains that formed the largest zone around the areas where LAB was dropped were selected to obtain bacteriocin (Elyass et al., 2015).

Partial purification of bacteriocins with ammonium sulfate

Selected LAB strains were enriched in 250 mL MRS broth medium and incubated at 30ºC for 24 hours. At the end of the incubation, the developing cultures

were centrifuged at 10,000 rpm for 30 minutes at 4° C. Then, the supernatant filtered through 0.22 µm microporous membrane filters (ISOLAB Laborgerate GmbH, Eschau, Germany). To eliminate the antimicrobial effect that may arise from organic acids or the pH of the MRS broth, the pH of the supernatants was adjusted to 6.5-7.0 with a pH meter (Ohaus, 3100, USA) using 5 N NaOH or 5 N HCl. Then, ammonium sulfate was slowly added to the final concentration of 40% and stirred until dissolved and incubated at +4ºC overnight. On the next day, the samples were centrifuged (Hermle Z326K, Germany) at 13,000 rpm for 45 minutes at +4°C. The upper phase was discarded, and the remaining precipitate was dissolved in 4 mL of sterile 0.05 M potassium phosphate buffer (pH 7.0). The suspended sediment mixtures were filtered through 0.22 µm filters again and stored at -20°C as crude extracts of bacteriocins (Zhao et al., 2020).

Antimicrobial activity of partially purified bacteriocins

Antimicrobial activity of partially purified bacteriocins was determined by well diffusion method. Double-layer LB agars were prepared with five *P. aeruginosa* strains, both separately and mixed. Wells were opened on the surface of the double-layered medium using a glass pasteur pipette. The bottom of the wells was covered by placing 10 µl of MRS agar at the bottom of the wells. After 100 µl of crude bacteriocins were added to the wells, they were incubated at 37°C for 24 hours. At the end of the incubation, bacteriocins that formed a zone around the well were evaluated as positive and the resulting zone diameters were measured and recorded (Lei et al., 2020; Parlindungan et al., 2021).

To determine the quantitative bacteriocin activity, crude bacteriocins were diluted twofold (1/2, 1/4, 1/8, 1/16, 1/32, 1/64) with phosphate buffer (20 mM, pH 7.0). One hundred µl of each dilution added to the wells opened in double-layer LB agar containing *P. aeruginosa* ATCC 15442, which gave the widest zone as a result of the well diffusion test. Petri dishes were left at room temperature for one hour to allow diffusion and then incubated at 37ºC for 24 hours. Inhibition zones around the wells were measured (mm) and recorded. Antimicrobial activity of bacteriocins were expressed in Arbitrary Unit (AU/ml) using the formula as follows: $AU/ml = 2^n \times (1000/x)$ [n: the last dilution showing any inhibition zone; x: the volume of the bacterium added to each hole (Zhao et al., 2020; Parlindungan et al., 2021).

Determination of antibacterial effect in milk model

Bacteriocin cocktail consisted of four bacteriocins which formed the widest zones were selected to examine the antibacterial effect on *P. aeruginosa* in milk. The reason for using a bacteriocin cocktail is that cocktails have a greater potential to lead to synergistic antimicrobial effects, so the overall activity of the cocktail would be bigger than the sum of the individual bacteriocins. The bacteriocin cocktail was prepared by mixing equal amounts of each bacteriocin. Then, UHT milk was added to the bacteriocin cocktail at a final concentration of 190 AU/ml and the test *P. aeruginosa* strain to a final concentration of 10^4 cfu/ml. For the control group, the samples were prepared with milk and bacterial culture only. The milk samples were stored at +4°C for 7 days. In addition to the initial day counts, *P. aeruginosa* counts were performed by planting on LB agar on the 1st, 3rd, 5th, and 7th days of storage. The experiment was carried out in three parallel repetitions (Kaya and Sımsek, 2019). The data was analyzed using the student t-test, considering the P value less than 0.05 as statistically significant.

Results

LAB strains with antimicrobial effect against P. aeruginosa

Among the thirteen ATCC strains, only transparent zones around *L. plantarum* and *L. fermentum* colonies were observed with the spread method, which was described in “Determination of LAB strains effective against *P. aeruginosa*” section. In addition to these strains, with the agar spot test *L. pentosus* and *L. paraplantarum* also displayed wide clear zones on *P. aeruginosa* mixed culture. Therefore *L. plantarum, L. fermentum, L. pentosus* and *L. paraplantarum* were selected to obtain bacteriocins.

Antimicrobial activity of partially purified bacteriocins

Based on the agar spot test results, *L. pentosus*, *L. plantarum, L. paraplantarum* and *L. fermentum* were selected for further bacteriocin production. The inhibition zone diameters formed by the partially purified bacteriocins by the well diffusion method against each of the *P. aeruginosa* strains and their mixtures are given in Table 1. Four bacteriocins formed the largest zones for *P. aeruginosa* ATCC 15442 strain. The antimicrobial activities of these four strains on *P. aeruginosa* ATCC 15442 were determined as 640 AU/ml for Bc-Pla and Bc-Para (from *L. plantarum* and *L. paraplantarum*), followed by Bc-Fer (from *L. fermentum*) with 160 AU/ml and Bc-Pen (from *L. pentosus*) with 80 AU/ml.

Table 1. The inhibition zone diameters of partially purified bacteriocins on *P. aeruginosa* ATCC 15442

Strain	Bacteriocin	Zone
***P. aeruginosa* ATCC 15442**	Bc-Pen	18 mm
	Bc-Fer	20 mm
	Bc-Para	20 mm
	Bc-Pla	21 mm
***P. aeruginosa* ATCC 27853**	Bc-Pen	17 mm
	Bc-Fer	19 mm
	Bc-Para	20 mm
	Bc-Pla	20 mm
***P. aeruginosa* PAO1**	Bc-Pen	14 mm
	Bc-Fer	16 mm
	Bc-Para	14 mm
	Bc-Pla	16 mm
***P. aeruginosa* VIM-2**	Bc-Pen	14 mm
	Bc-Fer	15 mm
	Bc-Para	16 mm
	Bc-Pla	16 mm
***P. aeruginosa* Imp-13**	Bc-Pen	14 mm
	Bc-Fer	16 mm
	Bc-Para	17 mm
	Bc-Pla	17 mm
***P. aeruginosa* mix**	Bc-Pen	14 mm
	Bc-Fer	13 mm
	Bc-Para	14 mm
	Bc-Pla	14 mm

Bc-Pen: bacteriocin of L. pentosus, Bc-Pla: bacteriocin of L. plantarum,
Bc-Para: bacteriocin of L. paraplantarum, Bc-Fer: bacteriocin of L. fermentum.

Antimicrobial effect of the bacteriocins in milk model

Milk samples with bacteriocin cocktail and *P. aeruginosa* were stored at +4°C for a week and bacterial counts were made on the 1st, 3rd, 5th and 7th days. Accordingly, while a decrease of 1.71 log cfu/ml was observed in the control group on the 1st day, 0.72 log cfu/ml reduction was recorded in the experimental group. However, on the 3rd day, while no logarithmic decrease was observed in the control group, there was a 1.01 log cfu/ml decrease in the experimental group. By the fifth and seventh days, the bacterial count remained at approximately 3 log cfu/ml in both groups. As a result, it was determined that the bacteriocin cocktail used in this study provided a 1.73 log cfu/ml reduction at refrigerator temperature (4°C) for up to three days, and the number of bacteria remained constant after three days. However, the decrease in the experimental group was not statistically significant (P>0.05). The average results of three parallel repeats are shown in Figure 1.

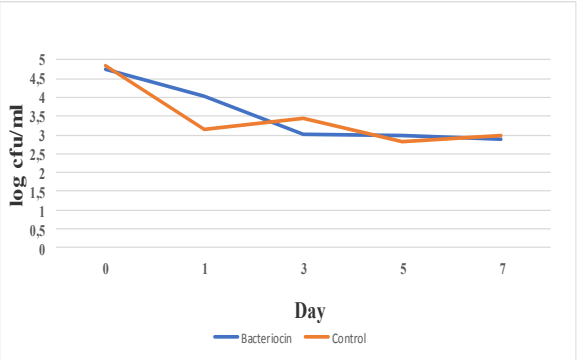

Figure 1. Antibacterial effect of bacteriocin cocktail in UHT milk against *P. aeruginosa* ATCC 15442.

Discussion and Conclusion

In this study, the antimicrobial effect of bacteriocins obtained from LAB strains on *P. aeruginosa* was investigated *in-vitro* and in milk model. Firstly, LAB strains with antibacterial effects on *P. aeruginosa* mix culture were identified. Afterwards, LAB strains that formed the largest clear zones against *P. aeruginosa* strains were distinguished. In this context, *L. pentosus, L. fermentum*, *L. paraplantarum* and *L. plantarum* strains were selected to obtain bacteriocins and further used in milk model.

The inhibitory effect of various LAB strains against Gram-positive and Gram-negative pathogens has been reported by many researchers. Shokri et al. (2017) isolated a total of 57 *Lactobacillus* strains from local yogurt and milk samples and examined their antibacterial effects against *P. aeruginosa* strains. Among the *Lactobacillus* strains, cell-free supernatants of two *Lactobacillus* strains (L1 and L2) were reported to show inhibition ranging from 12-20 mm in diameter against all 80 *P. aeruginosa* strains in the well diffusion method. Elyass et al. (2015) examined the antibacterial activity of *Lb. curvatus* M3 and *P. pentosaceus* N2 bacteriocins using the agar well test. While *Lb. curvatus* M3 showed a zone of 13-19 mm against *S. aureus*, *B. subtilis*, *E. coli* and 6-12 mm against *E. faecalis*, *P. pentosaceus* N2 showed a zone of over 20 mm against *S. aureus* and 13 -19 mm against *B. sublitis*, 6-12 mm zone against *E. coli* and *E. faecalis*. As a result of screening the inhibitor activity spectrum, it was reported that 7 out of 10 test bacteria were inhibited by both bacteriocins but could not inhibit *P. aeruginosa* and *Salmonella Typhi* isolates. In another study, the antibacterial activity of *Pediococcus acidilactici* BAMA 15 was investigated against *E. coli* and *S. aureus* and the zones were measured as 6.44 mm and 7.53 m, respectively. Researchers reported that of Gram-negative bacteria was more resistant than Gram-positive bacteria to the bacteriocin (Nasution et al., 2023). In the research conducted to isolate probiotic lactic acid bacteria from kiwi fruit pulp, a total of eight isolates were found and two of them were stated to be probiotic LAB strains. The antibacterial effect of isolates A2 and A5 against pathogenic bacteria such as *Staphylococcus*, *Pseudomonas* and *E. coli* was examined. The isolates exhibited inhibition against Gram-positive bacteria but not against Gram-negative bacteria such as *E. coli*. It was concluded that lactic acid bacteria have inhibitory properties primarily against Gram-positive bacteria. Overall, it has been stated that lactic acid bacterial strains are generally ineffective against Gram-negative bacteria due to the resistance provided by the outer membrane (Kamaliya et al., 2023).

In this study, it was observed that the inhibition activity of the bacteriocins differed on *P. aeruginosa* ATCC 15442. The highest bacteriocin inhibition activities were detected in the bacteriocins of *L. plantarum* and *L. paraplantarum* strains, with 640 AU/ml. In a study by Elhag et al. (2014), lactic acid bacteria were isolated from fresh sausages, intestines of different animals, saliva, cheese and cucumber. *Salmonella* spp., *S. Typhi, S. aureus*, *B. subtilis*, *B. cereus*, *B. stearothermophilus*, *B. pantotheticus*, *E. coli* and *Pediococcus* BFE 2306 strains were included in the study. Pellets of lactic acid bacteria (including *E. faecalis* (3 isolates), *E. avium*, *P. pentosaceus* (3 isolates), *P. domanosus*, *Lb. murinus* (2 isolates), *L. gasseri* (2 isolates), *Lb. acidophilus*, *L. plantarum*, *Lb. alimentarius* and *L. rhamnosus*, *E. faecalis*, *P. pentosaceus* and *Lb. murinus* were examined and it has been reported that the majority of partially isolated bacteriocins show either weak or no antimicrobial activity against the mentioned microorganisms (0.00-640 AU/ml).

It is possible to use more than one bacteriocin as a cocktail to increase the effect in the control of foodborne pathogens. In this study, the effect of the bacteriocin cocktail against *P. aeruginosa* in milk was investigated at refrigerator temperature. Although a decrease of approximately 2 log cfu/ml was observed in the number of bacteria for up to three days and no increase or decrease in the number of bacteria was observed until the seventh day, the results were not statistically significant when compared to the control group ($P>0.05$). The fact that the bacteriocin cocktail did not show a significant antibacterial activity may be associated with the fact that the pathogenic bacteria used were Gram-negative. As mentioned above, studies report that Gram-positive bacteria are generally more sensitive to bacteriocin (Nasution et al. 2023, Kamaliya et al. 2023, Elyass et al., 2015). The cell wall structure of Gram-positive bacteria has a lower lipopolysaccharide, lipoprotein and phospholipid composition than Gram-negative bacteria (Cao-Hoang et al., 2010). It is reported that the simpler cell wall structure of Gram-positive bacteria plays an important role on bacteriocin activity (Kusharyati et al., 2021). Additionally, although there are many characterized bacteriocins with potential for use in foods, biocontrol effectiveness in foods depends on various factors such as pH, temperature, food composition, and target pathogen/strain. Therefore, it is necessary to establish standardized conditions for the use of each bacteriocin in each food matrix (Prudencio et al., 2015). On the other hand, taking into account the pathogenic bacterial load in foods in a real-life scenario, each log decrease in the number of bacteria can be considered promising in terms of food safety.

There are studies on the application of bacteriocins in milk. However, up to our knowledge this is the first study focused on antimicrobial effect of bacteriocins on *P. aeruginosa* on this food model. In a study, a new broad-spectrum bacteriocin from fermented foods, Garviecin LG34, was obtained. While the numbers of *S. aureus* and *L. monocytogenes* in the control group increased over time during the 12-day incubation period at 4°C, significant differences were observed in the number of bacteria in the milk samples containing bacteriocin. It was reported that the number of *S. aureus* bacteria in whole milk, low-fat milk and skim milk in the experimental group decreased by 1.0, 2.9 and 4.1 log cfu/ml, respectively. In the same experimental setup, at the end of 12 days of incubation, a decrease of 2.0, 4.1 and 4.9 log cfu/ml was recorded in *L. monocytogenes* numbers, respectively. Additionally, the antimicrobial effect of

Garviecin LG34 bacteriocin against these two pathogens has been reported to be strongest in skim milk and weakest in whole milk. It has been stated that whole milk contains a large amount of fat globules, and its surface can be absorbed by garviecin LG34, which may cause a decrease in inhibition (Gao et al., 2023). In a study conducted by Verma et al. (2017) to increase the shelf life of raw buffalo milk, pediocin PA -1 was added to raw buffalo milk at 1%, 5% and 10% (v/v) concentrations contaminated with 10^5 cfu/ml *S. aureus*. It was stated that the number of *S. aureus* in milk was counted as 5, 4 and 3 log cfu/ml, respectively, depending on the percentage of Pediocin PA-1 addition, while it increased to 9 log cfu/ml in the control group. In another study, it was reported that 10,000 IU/mL nisin managed to reduce the number of *S. aureus* in milk by 4.68 log cfu/ml after 4 hours of incubation at 37°C, but the surviving cells again caused an increase in the number of bacteria at the end of 24 hours. It has been stated that this situation indicates the presence of nisin-resistant bacteria (Arques et al., 2011).

As a result, in this study, it was determined that bacteriocins obtained from *Lactobacillus* strains showed an antibacterial effect on *P. aeruginosa* on agar medium but could not achieve a significant decrease on the milk. However, bacteriocins, which have generally been proven to efficient on Gram-positive bacteria, have been determined to be effective on *P. aeruginosa*, a Gram-negative bacterium. Moreover, this study shows that in addition to in-vitro experiments, products to be used for biocontrol purposes in foods are also complemented with food models. In this regard, it is thought that detailed studies to understand how bacteria can survive and adapt in complex environments such as food matrix will be effective in increasing the success in biocontrol with bacteriocins. It is believed that as when the need for food safety increases day by day, characterizing target-specific bacteriocins in detail, standardizing their use and commercializing them with new generation strategies become important.

Acknowledgements

This article contains the findings of Eda Hacıömeroğlu's Master thesis of Kırıkkale University Institute of Health Sciences, Department of Food Hygiene and Technology.

Conflict of Interest

The authors state that there is no conflict of interest.

References

Arqués JL, Rodríguez E, Nuñez M, Medina M. Combined effect of reuterin and lactic acid bacteria bacteriocins on the inactivation of foodborne pathogens in milk. Food Contr 2011; 22: 457-61.

Cao-Hoang L, Chaine A, Gregoire L, Wache Y. Potential of nisin-incorporated sodium caseinate films to control *Listeria* in artificially contaminated cheese. Food Microbiol 2010; 27: 940-4.

Chatterjee M, Anju C, Biswas L, Kumar VA, Mohan CG, Biswas R. Antibiotic resistance in *Pseudomonas aeruginosa* and alternative therapeutic options. Int J Med Microbiol 2016; 306: 48-58.

Chen H, Hoover DG. Bacteriocins and their food applications. Compr Rev Food Sci Food Saf 2003; 2 (3): 82-100.

Cotter PD, Ros RP, Hill C. Bacteriocins-a viable alternative to antibiotics? Nat Rev Microbiol 2013; 11: 95-105.

Dogan B, Boor KJ. Genetic diversity and spoilage potentials among *Pseudomonas* spp. isolated from fluid milk products and dairy processing plants. Appl Environ Microbiol 2003; 69: 130-8.

Elhag NB, Babiker ERB, Mahdi AA. Effect of bacteriocins-producing lactic acid bacteria on target microorganisms. J Agri-Food Appl Sci 2014; 2 (5):155-62.

Elyass ME, Altayar MA, Mahdi AA, Abdelrawaf SS, Shigidi MT, Attitalla IH. Characterization and evaluation of antimicrobial activity of bacteriocins from *Lactobacillus curvatus* and *Pediococcus pentosaceus*. J Infect Non Infect Dis 2015; 1: 001.

Erkmen O, Bozoglu TF. Food microbiology: Principles into practice. Volume 1, New York: Wiley, 2016 p.311.

Gao Y. Antibacterial mechanism of garviecin LG34 against *S. aureus* and *L. monocytogenes* and its application in dairy products preservation. Research Square 2023; 2-18.

Johnson EM, Jung Y, YuJin Y, Jayabalan R, Yang SH, Suh JW. Bacteriocins as food preservatives: Challenges and emerging horizons. Crit Rev Food Sci Nutr 2018; 58(16): 2743-67.

Kamaliya DB, Javia BM, Gadhvi MS, Dudhagara DR. Isolation and characterization of probiotic bacteria from fruit pulp, screening of probiotic properties and production of bacteriocin and probiotic curd. Open J Biol Sci 2023; 8(1): 33-42.

Kaya HI, Simsek O. Characterization of pathogen-specific bacteriocins from lactic acid bacteria and their application within cocktail against pathogens in milk. LWT-Food Sci Technol 2019; 115: 108464.

Kusharyati DF, Satwika TD, Mariana A, Rovik A. Potential screening of bacteriocinogenic-lactic acid bacteria rom mangrove sediment of Logending

Beach for fisheries product preservation. J Trop Biodivers Biotechnol 2021; 6(1): 61927.

Lei S, Zhao R, Sun J, Ra J, Ruan X, Zhu Y. Partial purification and characterization of a broad-spectrum bacteriocin produced by a *Lactobacillus plantarum* zrx03 isolated from infant's feces. Food Sci Nutr 2020; 8(5): 2214-22.

Mills S, Serrano L, Griffin C, O'connor, PM, Schaad G, Bruining, C. Inhibitory activity of *Lactobacillus plantarum* LMG P-26358 against *Listeria innocua* when used as an adjunct starter in the manufacture of cheese. Microb Cell Fact 2011; 10: S7.

Nasution SA, Fachrial E, Ginting CN, Liena Piska F. Bacteriocin-producing *Pediococcus acidilactici* BAMA 15 isolated from "naniura" traditional foods in North Sumatra, Indonesia. Biodiversitas 2023; 24: 2830-5.

Parlindungan E, Dekiwadia C, Jones OA. Factors that influence growth and bacteriocin production in *Lactiplantibacillus plantarum* B21. Process Biochem 2021; 107: 18-26.

Petruzzi L, Corbo MR, Sinigaglia M, Bevilacqua A. Chapter 1-Microbial spoilage of foods: Fundamentals. In The MicroBiological Quality of Food-Foodborne Spoilers; Bevilacqua A, Corbo MR, Sinigaglia M, eds. Sawston, Cambridge, UK; Woodhead Publishing, 2017; pp. 1-21.

Prudêncio CV, dos Santos MT, Vanetti MCD. Strategies for the use of bacteriocins in Gram-negative bacteria: relevance in food microbiology. J Food Sci Technol 2015; 52(9): 5408-17.

Sharma C, Singh BP, Thakur N, Gulati S, Gupta S, Mishra SK, Panwar H. Antibacterial effects of *Lactobacillus* isolates of curd and human milk origin against food-borne and human pathogens. Biotech 2017; 7: 31.

Shokri D, Khorasgani, MR, Mohkam M, Fatemi SM, Ghasemi Y, TaheriKafrani A. The inhibition effect of *Lactobacilli* against growth and biofilm formation of *Pseudomonas aeruginosa*. Probiotics Antimicrob Proteins 2017; 10(1): 34-42.

Tirloni E, Bernardi C, Stella S. *Pseudomonas* spp. are food grade organic acids efficient against these spoilage microorganisms in fresh cheeses? Foods 2021; 10: 891.

Verma SK, Sood SK, Saini RK and Saini N. Pediocin PA-1 containing fermented cheese whey reduces total viable count of raw buffalo (*Bubalis bubalus*) milk. LWT-Food Sci Technol 2017; 83: 193-200.

Zhao R, Lu Y, Ran J, Li G, Lei S, Zhu Y and Xu B. Purification and characterization of bacteriocin produced by *Lactobacillus rhamnosus* zrx01. Food Biosci 2020; 38: 100754.