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Original Article

Antibacterial Activity of Bacteriocin - Isolated from Lactobacillus Spp. against Some Pathogenic Bacteria

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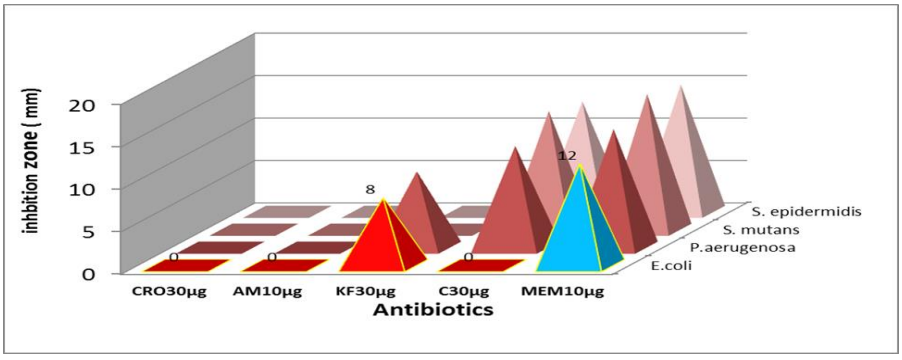
ABSTRACT

Objective: A member of the lactic acid bacteria (LAB) group, lactobacilli are well-known for their probiotic properties. They also possess several unique properties, including the ability to produce lactic acid, enzymes like –galactosidase, secretory proteinaceous toxins, and bacteriocins, which are naturally occurring antimicrobials. Bacteriocin is a bio-preservative substance with the potential (when employed as a natural food preservative) to inhibit the growth of some pathogenic bacteria in the food business, although it is not commercially available, little used, and expensive.
Methods: The study aimed to isolate and identify *Lactobacillus* spp. from milk and yogurt, and then some isolates were selected with the potential to produce bacteriocins to suppress the growth of some pathogenic bacteria isolated from clinical infection as MDR bacteria.
Results: 35 samples were collected from milk and yogurt, and then 12 isolates of *Lactobacillus* spp. were isolated. one isolate was selected to extract the bacteriocins to evaluate their activity against some pathogenic bacteria. The findings of this study showed all bacteria under study are MDR and bacteriocins have an effective action as antibacterial.
Conclusion: This research showed the potential of bacteriocin as a food bio-preservative to prevent food spoiling and harmful bacterial growth.

GRAPHICAL ABSTRACT



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Introduction

Organic acids, diacetyl, ammonia, reuterin, hydrogen peroxide, free fatty acids, and bacteriocin are just a few of the antimicrobial compounds that create lactic acid bacteria (LAB). These compounds can stop the growth of pathogenic and food-spoiled organisms [1]. When particular LAB manufacture lactic acid during fermentations, they produce bacteriocins, which are proteinaceous antibacterial chemicals with a ribosomal synthesis that have a bactericidal effect against closely related species [2, 3]. Bacteriocins of both Gram-positive and Gram-negative bacteria have been studied, isolated, and purified in recent years as a result of resurgence in interest in bacteriocin-like properties. They are now being investigated for a range of antibacterial applications in the food industries and medications [4]. Some bacteriocins generated by bacteria of lactic acids, such as nisin, do not only inhibit the growth of closely related species, but also work well against a variety of Gram-positive food-borne infections [5].

Microorganisms battle for survival in a range of ecological niches, and through evolution, they create distinctive flora. Lactic acid bacteria are the predominant microflora in some food habitats. These organisms can create antimicrobial chemicals to combat competing flora, such as food-borne spoilage and pathogenic bacteria [6]. The most efficient technique to produce cultures that are valuable for scientific and commercial reasons has always been to isolate and filter microbes from naturally occurring processes. Since over ten thousand years ago, man has recognized the antibacterial properties of lactic acid bacteria, which have allowed him to use fermentation techniques to increase the shelf-life of several foods. Raw milk represents an ideal growth medium for microorganisms. Many kinds of bacteria that may be detected in milk vary widely and rely on the unique circumstances of each batch. Bacteria are generally such as *streptococci*, *Lactobacillus*, *Micrococci*, *Microbacteria*, *Coliform*, *Bacillus*, etc.). Molds and yeasts, including types like Torula and Penicillium, are frequently seen [7]. The bulk of individuals living in the rural areas and those with low incomes depend heavily on raw milk for their diets [8].

Probiotics can be made from bacteriocin-producing bacteria. It is possible to employ bacteriocin as a food additive, cure pathogen-induced disorders, and treat cancer [9]. Bacteriocins can limit the spread of several infectious disorders brought on by pathogenic isolates of Gram-positive bacteria (such as staphylococci, streptococci, and micrococci) [10]. In addition, a variety of Gram-negative bacteria, including *Shigella, Salmonella*, Vibrio, *Listeria*, and *E. coli*, have been utilized as test organisms to examine the antagonistic action of recently identified antimicrobial peptides. Even though bacteriocin has been isolated from several strains of *Lactobacillus bulgaricus, Lactobacillus acidophilus, Lactobacillus Plantarum*, and *Lactobacillus lactis*, it has shown a large amount of antimicrobial activity. However, to address the problem of the most prevalent food-related diseases, researchers are looking at new strains from various food sources [11].

Researchers concentrate on identifying new natural inhibitors because of the rise in customer demand for natural preservatives. To prevent harmful microbes from colonizing several food items, bacteriocin-producing lactic acid bacteria were used in various starting cultures. Bacteriocin and bacteriocin-producing lactic acid bacteria were important not only in food preservation, but also for inhibiting some infections that were drug-resistant because of their promising properties [12].

Thus, in this present study, different samples of milk and yogurt were collected, and then isolation and identification of *Lactobacillus* spp., and extraction of bacteriocin was done to evaluate the antibacterial activity against some MDR bacteria.

Materials and Methods

Isolation and identifications of bacterial isolates

35 samples of milk and yogurt collected were cultured on MRS agar and MRS broth under the anaerobic conditions at 37 °C for 24-48 hours. If a colony appeared, it was recognized based on its

morphology (colony form, shape, color, boundaries, type of pigment, elevations, and textures), and after it was stained with Gram's stains, it was inspected by using a light microscope. Isolates of *Lactobacillus* spp. were stored and preserved by using 20% glycerol at -80 °C [13, 14].

Preparations of bacterial inoculum

MRS broth was used to activate the isolate for 18 hours at 37 °C, and proliferation was regulated at 0.5 McFarland.

Antibiotic susceptibility test

The Kirby-Bauer disk diffusion methods were employed to assess antibiotic susceptibility by the Clinical Laboratory Standard Institute (CLSI) guidelines 2021. In this study, 5 antibiotics Different antibiotic disks ceftriaxone (CRO-3Oμg), ampicillin (AM-10 μg), cephalothin (KF-30 μg), chloramphenicol (C-30 μg), and meropenem (MEM-10 μg) were purchased from (Bioanalyse, Turkey) against some isolates to assess the susceptibility of these isolates [15].

Preparation of bacteriocin

Cell-free lactobacilli supernatant was grown in MRS medium for 24 hours at 37 ºC. After that, the cultures have been centrifuged for 30 minutes at 4 ºC and 4000 rpm. Filtering the supernatants via a 0.22 mm filter, they were sterilized (Millipore, Billerica, MA, United States).

Antibacterial activity of bacteriocin against pathogenic bacteria

The antimicrobial activity of *Lactobacillus* spp. bacteriocin isolated against some pathogenic bacteria was evaluated by using the good diffusion method [16]. Pathogenic isolates, including (*E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis,* and *Streptococcus mutans*) were inoculated and preserved in nutrient broth. After incubation at 37 °C overnight, the microbial concentration was adjusted to 0.5 McFarland standard and sub-cultured on Muller Hinton agar when made a 6 mm diameter well placed in Muller Hinton agar plates, followed by incubation at 37 °C for 18-24 hours. Then, the growth-free inhibition zone surrounding the disks was measured as the antimicrobial activity of bacteriocins.

Results and Discussion

From 35 samples, 12 isolates of Gram-positive *Lactobacillus* spp. bacteria from milk and yogurt were isolated and identified by biochemical tests with different morphological, physiological, and biochemical characteristics, as presented in Table 1.

All 12 isolates understudy were found to be Gram-positive, and rod shapes were arranged in chains or pairs. Their colonies on the MRS medium were found to be circular, rough surface, low convex, and white colored. These isolates were determined as representative of the genus Lactobacillus [17]. Also, the isolates were tested for fermentation of glucose, fructose, lactose, manitol, sucrose, and maltose. It is clear from the table that the study of isolates reveals diversity in the fermentation of sugars used (Table 1), the isolates (1, 2, 3, 4, 5, 6, 7, 9, 10, 11, and 12) [11 isolates] gave the positive test results for glucose, fructose, and lactose, while (5, 6, and 9) [3 isolates] gave the positive test results with glucose, fructose, lactose, sucrose, and maltose. In addition (1, 2, 4, and 10) [4 isolates] gave the positive result for glucose, fructose, lactose, and mannitol, while (4) gave the positive results for glucose, fructose, lactose, mannitol, and sucrose. The isolates (5, 6, 7, 9, 10, and 11) [6 isolates] gave the positive results for glucose, fructose, lactose, and maltose. All isolates grew at 37°C. All isolates were catalase negative test ability to produce indole, they were observed unable to produce indole and all isolates gave a positive result for methyl red except one and finally, all of them could not liquefy gelatin [18, 19].

Table 1: Morphological and biochemical characteristics of *Lactobacillus* spp. were found to be Gram-positive, rod shapes, and chemical test

Morphological Biochemical	Milk and yogurt											
	1	2	3	4	5	6	7	8	9	10	11	12
Cell shape	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Colony elevation	Convex	Convex	Convex	Convex	Convex	Convex	Convex	Convex	Convex	Convex	Convex	Convex
Colony edge	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Colony surface	Rough	Rough	Rough cottony	Rough	Rough	Rough cottony	Rough cottony	Rough	Rough	Rough cottony	Rough cottony	Rough cottony
Gram stain	+	+	+	+	+	+	+	+	+	+	+	+
Colony color	White	White	White	White	White	White	White	White	White	White	White	White
Catalase test	-	-	-	-	-	-	-	-	-	-	-	-
Fermentation												
Glucose	+	+	+	+	+	+	+	+	+	+	+	+
Fructose	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	-	+	+	+	+
Mannitol	+	+	-	+	-	-	-	+	-	+	-	-
Sucrose	-	-	-	+	+	+	-	+	+	-	-	-
Maltose	-	+	-	-	+	+	+	-	+	+	+	-
Indol production	-	-	-	-	-	-	-	-	-	-	-	-
Methyl red	+	+	+	+	+	+	+	-	+	+	+	+
Urease test	-	-	-	-	-	-	-	-	-	-	-	-
Gelatin Liquidation	-	-	-	-	-	-	-	-	-	-	-	-

Susceptibility profiles of pathogenic bacteria isolates

Some antibiotics were used to show their effects on some pathogenic bacteria isolated from different locations in human body.In this study, 5 antibiotics were used as follows: ceftriaxone (CRO-30 µg), ampicillin (AM-10 µg), cephalothin (KF-30 µg), chloramphenicol (C-30 µg), and meropenem (MEM-10 µg), as displayed in Figure 1.

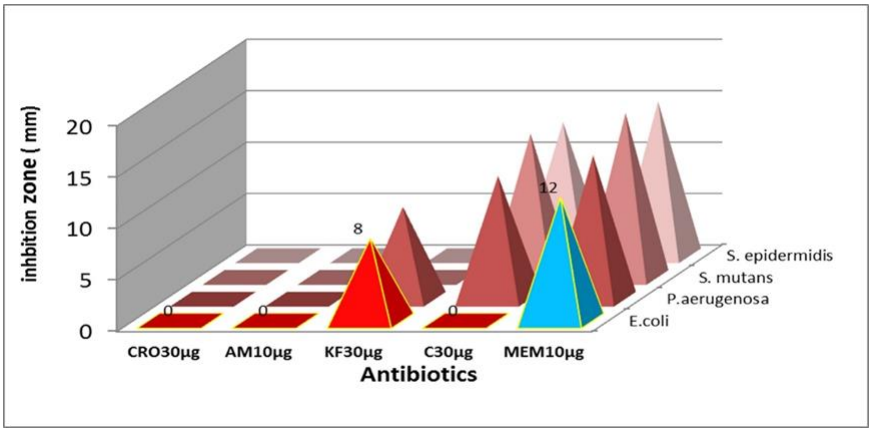

Figure 1: Antibacterial action of on *E. coli, Pseudomonas aeruginosa, Staphylococcus epidermidis,* and *Streptococcus mutans* indicating the inhibition zone diameter

According to the findings of the present study, it was illustrated that all bacterial isolates are resistant to antibiotics under study. All isolates are resistant (100%) to ceftriaxone and ampicillin, 50% to cephalothin, 75% to chloramphenicol, and 0% to meropenem, that means all bacteria in the study are resistant to all antibiotic disc used in the test (MDR) and that the selected antibiotic disc was not effective against all isolated bacteria except meropenem according to [20].

Antibacterial activity of bacteriocin isolated from Lactobacillus spp. against pathogenic bacteria

The antibacterial activity of bacteriocin was estimated against various pathogenic bacteria (*E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, and *Streptococcus mutans*). The inhibition zone is obtained in different diameters, As shown in Table 2 and Figures 2.

Table 2: Antibacterial activity of bacteriocin from *Lactobacillus* spp. indicating the inhibition zone diameter

Pathogenic bacteria	Diameter of inhibition zone (mm)
E. coli	14
P. aeruginosa	15
S. epidermidis	18
S. mutans	20

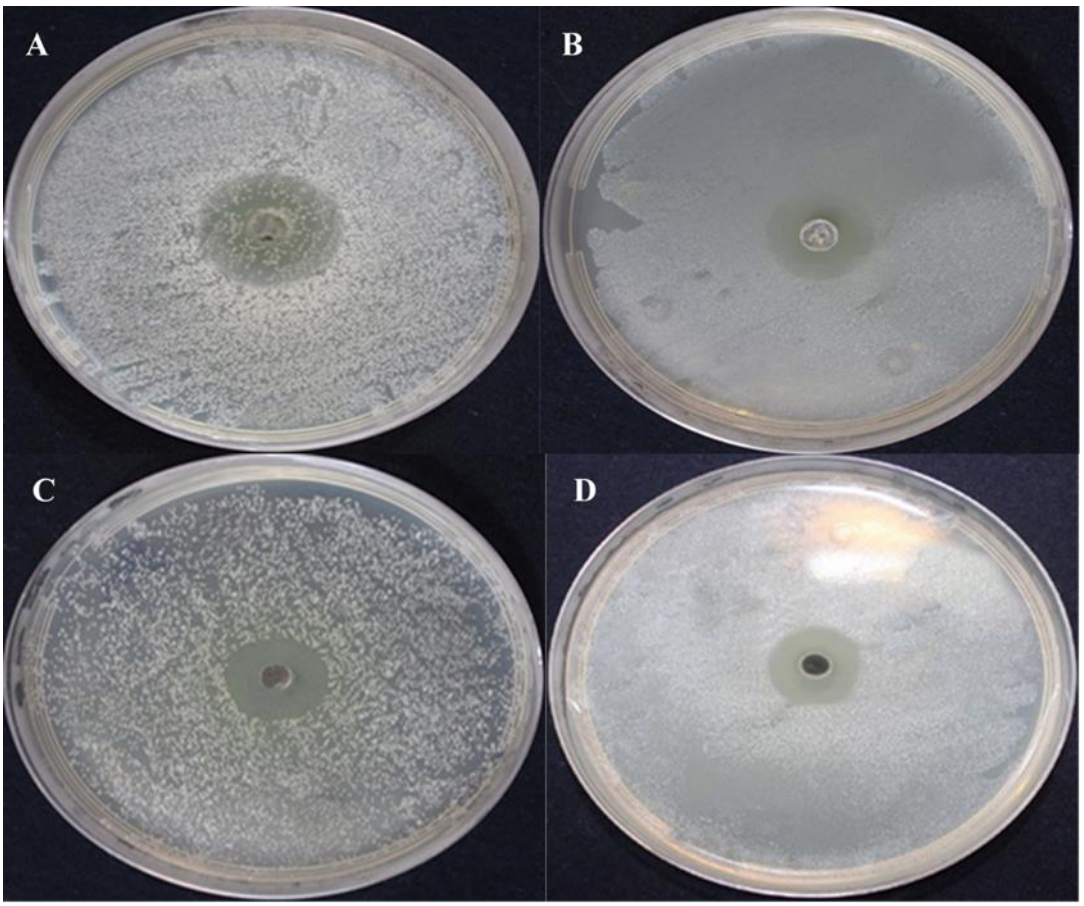

Figure 2: *Lactobacillus* spp. bactriocin against A: *E. coli* B*: P. aeruginosa* C: *S. epidermidis* D: *S. mutas* show the inhibition zone diameter

It depends on the processes by which *Lactobacillus* species control the colonization of other bacterial species in the culture, and whether or not probiotics are used in conjunction with other relevant pertinent pathogenetic or aetiological treatments [21, 23].

The results mentioned by [23] who reported that the normal gut of humans is primarily colonized

with *Lactobacillus* spp., which produce bacteriocins, hydrogen peroxide, and lactic acid, which can lower the glycemic index, support the idea that *Lactobacillus* spp. play an important role in maintaining human health by producing defense factors. Some of these defense factors have an inhibitory effect on some opportunistic pathogens. A decrease in *Lactobacillus* spp. leads to a rise in pH, which encourages the overgrowth of facultative and anaerobic bacteria that can lead to the infection [24].

Conclusion

Screening of *Lactobacillus* spp. isolated and identified from milk and yogurt for antimicrobial substances showed that they presented bacteriocin-like substances against Gram-positive and Gram-negative bacteria. This is an important finding since bacteriocins from *Lactobacillus* spp. are not usually effective against Gram-negative bacteria and rather present a narrow antimicrobial spectrum. Studies are conducted to fully characterize the bacteriocin-like substances produced by *Lactobacillus* spp. and evaluate their antimicrobial activity in dairy foods.

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Authors' contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

Conflict of Interest

We have no conflicts of interest to disclose.

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