

# Antagonistic Activity of Bacteriocin-Producing *Lactobacillus* Local Isolates Against Select Pathogenic Bacterial Strains

Doaa Mahmood Mahdy<sup>1</sup> , Nibras Nazar Mahmood<sup>1</sup> , Wala'a Shawkat Ali<sup>2</sup> 

1. Department of Biology, College of Science, Mustansiriyah University, Baghdad, Iraq

2. Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq

## ABSTRACT

**Background and Aim:** *Lactobacilli* are known for their probiotic activities and potential to combat pathogens. This study aimed to investigate the antimicrobial activity of *Lactobacillus* strains isolated from various sources against pathogenic bacteria, to identify potential bacteriocin producers.

**Materials and Methods:** A total of 140 samples were collected from different sources. Samples were cultured on selective media such as MRS and SL agar for isolation of *Lactobacillus* spp. under anaerobic conditions. The suspected bacteria appeared Gram-positive, diploid bacilli or coccobacilli. The isolates were subjected to various biochemical tests such as catalase, and oxidase, also hydrolysis test was done by growing on MRS containing 1% calcium carbonate. The antimicrobial activity of bacteriocin was screened by using three methods including (Agar-plug diffusion method, Filter paper disc method, and agar-wells diffusion method). Molecular identification of the best bacteriocin producer was achieved using 16S rRNA sequencing.

**Results:** The study found that *L. helveticus* DF isolated from feces exhibited the highest bacteriocin production efficiency. Concentrated bacteriocin from *L. helveticus* DF demonstrated potent antibacterial activity against multi-drug resistant bacteria, with 80 AU/ml against *Pseudomonas aeruginosa* and 40 AU/ml against *Enterococcus faecalis*.

**Conclusion:** The study highlights the potential of *Lactobacillus* bacteriocins as effective antibacterial agents, offering a promising alternative to combat antibiotic resistance. Further research is warranted to elucidate the mechanisms and assess the safety and efficacy of bacteriocins for their use as bio-therapeutic agents.

**Keywords:** *Lactobacillus* Species, Antibacterial Activity, Bacteriocin, Multi-drug Resistance Bacteria

Received: 2023/06/21;

Accepted: 2023/09/01;

Published Online: 2023/09/27

## Corresponding Information:

Doaa Mahmood Mahdy, Department of Biology, College of Science, Mustansiriyah University, Baghdad, Iran Email: [doaamh19@gmail.com](mailto:doaamh19@gmail.com)



Copyright © 2023, This is an original open-access article distributed under the terms of the Creative Commons Attribution-noncommercial 4.0 International License which permits copy and redistribution of the material just in noncommercial usage with proper citation.

Use a device to scan and read the article online



Mahmood Mahdy D, Nazar Mahmood N, Shawkat Ali W. Antagonistic Activity of Bacteriocin-Producing *Lactobacillus* Local Isolates Against Select Pathogenic Bacterial Strains. Iran J Med Microbiol. 2023;17(4):432-40.

Download citation: [BibTeX](#) | [RIS](#) | [EndNote](#) | [Medlars](#) | [ProCite](#) | [Reference Manager](#) | [RefWorks](#)

Send citation to:  [Mendeley](#)  [Zotero](#)  [RefWorks](#)

## 1. Introduction

Antibiotic-resistant infections are widespread worldwide. The escalating resistance of pathogenic bacteria to a wide array of antibiotics has emerged as a significant global concern, primarily attributed to the extensive use of antibiotics in combating various bacterial infections (1, 2). Consequently, the pressing need to develop novel antimicrobial agents produced naturally by bacteria can combat pathogenic organisms with multi-drug resistance (3, 4).

Lactic acid bacteria (LAB), a group of generally recognized as safe microorganisms, have found widespread application in both the food and pharmaceutical industries. One of the notable attributes of LAB is their ability to confer health benefits by demonstrating antagonistic activity against numerous pathogens (5, 6). A pivotal class of metabolites produced by LAB, known as bacteriocins, plays a significant role in their antimicrobial action. Although bacteriocins can be broadly categorized as

antimicrobial agents, they diverge from traditional antibiotics in crucial ways (7, 8). Notably, bacteriocins exhibit a restricted spectrum of activity, targeting strains related to their producing species and specifically strains within the same species. Moreover, bacteriocins are ribosomally synthesized antimicrobial peptides or complex proteins produced during the primary phase of bacterial growth, in contrast to antibiotics that are typically secondary metabolites (7, 9).

Bacteriocins have garnered considerable attention from researchers and have been extensively studied due to their potential as alternatives to conventional antibiotics. These bioactive compounds boast several advantages, including physical stability, non-toxicity, and susceptibility to degradation by proteolytic enzymes, particularly those present in the mammalian gastrointestinal tract, rendering them safe for human consumption (10). The production of bacteriocins serves as a critical criterion in the selection of probiotic strains, and the quantity of bacteriocins generated by *Lactobacillus* is contingent upon the environmental conditions in which these bacteria thrive (11).

Prior research has demonstrated that supernatants of *Lactobacillus spp.* isolates exhibit varying degrees of inhibitory activity against several pathogenic bacteria, such as *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, and *Vibrio cholera* (12). The emergence of antibiotic resistance has spurred researchers to explore innovative strategies to combat pathogens effectively. As a result, this study aims to investigate the bioactivity of active bacteriocins produced by *Lactobacillus spp.* isolates and evaluates their antimicrobial effects against different pathogenic bacteria, with the ultimate goal of offering potential alternatives to traditional antibiotics.

## 2. Materials and Methods

*Samples Collection and Isolation of Lactobacillus spp.:*

A total of 140 samples were collected from various sources, including feces, high vaginal wall of healthy women, mouths of healthy infants, breast milk, and commercially available yogurt. The samples were obtained from the Microbiology Unit at Al-Batool Teaching Hospital, from private clinics, and from yogurt stores. For isolation of *Lactobacillus spp.* from clinical samples, the swabs were immersed in 9 ml of De Man, Rogosa, and Sharpe Broth (MRS) broth (Merck, Germany), while for the isolation of *Lactobacillus* from liquid and solid samples, each tube containing 9 ml of MRS broth was inoculated with 1 ml

of liquid sample or 1 g of solid sample and mixed gently to get a uniform sample, separately. All inoculated tubes were incubated at 37°C for 48h under anaerobic conditions, followed by tenfold serial dilution and sub-culture on solid MRS (Merck, Germany) supplemented with 1% calcium carbonate incubated at 37°C in 5% CO<sub>2</sub> conditions. After incubation, large white colonies were selected and purified by streaking on MRS agar plates (13). Purified isolates were stored in glycerol stock at -20°C for subsequent analysis (14). Identification tests were performed based on Gram staining, growth on selective MRS and SL agar, and biochemical tests, including catalase, and oxidase. Also, all isolates were tested for acid production ability on MRS supplemented with calcium carbonate by forming a lysis zone around a colony of tested bacteria (15). Moreover, only the Gram-positive, catalase, and oxidase-negative isolates that could be candidates as *Lactobacillus spp.*

Molecular identification was achieved by sequencing the 16S ribosomal RNA (16S-rRNA) for producer *Lactobacillus* isolate. Genomic DNA was extracted using the Mini Kit Promega. PCR amplification was carried out with the primers 27F (AGAGTTTGATCCTGGCTCA) and 1492R (GGTTACCTGTTACGACTT) (16) were supplied by MacroGen Company (Korea) in a lyophilized form. The PCR products were sequenced following the ruction manuals of the sequencing company (MacroGen Inc. Geumchen, Seoul, South Korea) using an ABI (Applied Biosystem) automated DNA sequencer. The sequencing results were edited, aligned, and analyzed along with the respective sequences in the reference database using BioEdit Sequence Alignment Editor Software Version 7.1. sequenced following the ruction manuals of the sequencing company.

### List of Clinical Isolates

Clinical isolates used in the study including *Pseudomonas. aeruginosa*, *klebsiellia.pneumoniae*, *Acinetobacter.baumannii*, *Kocuria. Kristinae*, *E. feacalis* and *Strep. agalactiae*.

### Screening of *Lactobacillus spp.* for Bacteriocin Production

*Lactobacillus* isolates were subjected to screening to identify the most potent bacteriocin-producing isolate to be used for further experiments in this study:

#### A. Agar-plug Diffusion Method

After overnight growth in MRS broth, A volume of 100 µL of 1.5×10<sup>8</sup> CFU/mL (McFarland tube No. 0.5) of fresh *Lactobacillus* isolates were transferred and spread on MRS agar plates and incubated anaerobically at 37°C for 48 hours. Plugs o about 0.5

cm in diameter were made with a sterile cork borer from each isolate which then placed on plates surface of Mullar-Hinton agar streaked with 100 µL of  $1.5 \times 10^8$  CFU/mL of pure overnight growth culture of the indicator bacteria, the plates were incubated at 37°C for 24 hours. After incubation, the diameter of inhibition zones around each plug was measured (15).

### B. Agar-Wells Diffusion Method

Well diffusion assay was used to estimate the production of bacteriocin by isolates as follows: *Lactobacillus* isolates were inoculated in MRS broth under anaerobic conditions at 37°C for 48 hours. The cultures were centrifuged at 6000 rpm for 15 minutes. The cell-free supernatant (CFS) of each isolate was assayed for the presence of bacteriocin using agar well diffusion assay as follows: A 100 µL of  $1.5 \times 10^8$  of indicator bacteria suspension was transferred and spread on Mullar-Hinton agar. Wells cut into the pour plates with 5mm sterile cork borer were filled with 100 µL of the CFS. The plates were kept at room temperature for 2 hours and then incubated at 37°C for 18-24 hours (17, 18). Finally, the inhibition zones formed around the wells were measured in mm, compared with that of the control which contained MRS broth only. The method was done with 2 replicates.

### C. Filter Paper Disc Method and Agar-Wells Diffusion Method

*Lactobacillus* isolates that recoded antimicrobial activity in screening were inoculated in MRS broth and incubated anaerobically at 37°C for 48 hours and then CFS (crude bacteriocin) was obtained by centrifuging at 6000 rpm for 15 minutes. The CFS was neutralized to pH 6.5 with 1N NaOH and then treated with catalase and sterilized by filtration through 0.45µm membranes. 100 µL of  $1.5 \times 10^8$  CFU/mL of pure overnight growth culture of indicator bacteria was transferred and spread on Mullar-Hinton agar. In the filter paper disc method; sterile filter paper discs measuring 5 mm diameter, saturated with 100 µL of CFS were placed on the Mullar-Hinton agar, and inhibition zones formed around the paper discs were recorded (17, 18).

To confirm the bacteriocin effect and the inhibitory activity of the neutralized CFS with 1N NaOH and treated with catalase (to neutralize the effect of organic acid and H<sub>2</sub>O<sub>2</sub> activity respectively) were screened by agar wells diffusion method, wells cut into the pour plates with 5mm sterile cork borer were filled with 100 µL of the CFS (18-20). All the plates were left in laboratory temperature for 2 hours and then incubated aerobically at 37°C for 18-24 hours. The inhibition zones formed around wells were measured in mm and recorded. One *Lactobacillus* isolate was

selected as a bacteriocin producer. According to the results of screening, the most efficient isolate of *Lactobacillus* spp. in the bacteriocin production. And the most sensitive isolates of pathogenic bacteria were selected as indicator and used in the subsequent experiments.

### Cell-Free Supernatant Concentration

Supernatants of producer isolate were concentrated twice by incubated at 40°C for two days or by evaporation, the antibacterial activity of concentrated bacteriocin was determined against indicator bacteria (21).

### Bacteriocin Activity Assay

The concentrated crude bacteriocin was serially diluted two-fold with physiological saline solution. The dilutions were used to assess the bacteriocin's antagonistic activity against the indicator bacteria using agar-well diffusion assay. The highest dilution producing a zone of inhibition growth was calculated as arbitrary units (AU) using the formula (20):

$$\frac{1}{DF} \times \frac{1000}{\text{Volums spotted in } \mu\text{L}}$$

### Statistical analysis

All data were analyzed using SPSS 26(SPSS Inc., Chicago, Ill., USA) and Graph Prism (Ver.8) program. The statistical analyses were performed using the analysis of variance independent (t-test) and Chi-square test. Data were presented as a percentage, mean, and SE. A P-value<0.05 was considered significant.

## 3. Results

### Isolation and Identification of *Lactobacillus* spp.

A total of 140 samples were collected from various sources, including the vagina, feces, mouth, breast milk, and yogurt. Only Gram-positive, catalase, and oxidase negative were isolated, from the total number of the samples, only 60 *Lactobacillus* spp. were isolated, with the highest number of isolates obtained from the vagina (28 isolates, 47%), followed by the mouth (7 isolates, 11.6%), breast milk (3 isolates, 5%), feces (3 isolates, 5%), and yogurt (19 isolates, 31.6%). All isolates were surrounded by clear zones after being cultured on MRS agar containing calcium carbonate as a result of dissolving by the acid produced by the bacteria which was described as high acid-producing ability.

### Screening for Bacteriocin Production

All isolates that were confirmed through the identification process were subjected to screening techniques in order to select the most efficient isolate as bacteriocin producer, As well as, the most sensitive bacterial isolates were selected as indicator.

The *Lactobacillus* isolates were screened for their ability to produce bacteriocins by different methods, the results showed that the agar-plug diffusion method did not appear any antibacterial activity. While by agar well diffusion assay, the number of *Lactobacillus spp.* showed strong antibacterial activity against Gram-positive bacteria (two isolates of *Enterococcus faecalis*) and 3 isolates of Gram-negative bacteria (three isolates of *P. aeruginosa*, one isolate of *Acinetobacter baumannii*) with the range of inhibition zone diameters between ( $7.1 \pm 0.14$ ) and ( $17.75 \pm 0.49$ ), also *Lactobacillus spp.* showed no inhibition zone against other indicator bacteria such as *Kocuria. Kristinae* and *Strep. agalactiae*. The highest effective strains against pathogenic bacteria were coded Lh2 DF, La1DV, Lp3DV, Lh1DF, La2 DV, Lp10DV, and Lp14DV (Table 1). There were no significant relationships between the source of *Lactobacillus* isolation and the production of bacteriocin (Figure 1). Thus, the selection of isolates for screening by filter paper disc method relied on the results of this method.

The size of the inhibitory zone for seven selected isolates against the four indicator bacteria (more sensitive indicator bacteria) by filter paper disc method is shown in (Table 2). However, the strains coded La1DV isolated from the vagina, Lh1DF and Lh2DF isolated from

feces showed high inhibitory activity against the indicator bacteria including two isolates of *P.aeruginosa* and two isolates of *Enterococcus faecalis* with inhibition zone ranging from ( $7.16 \pm 0.17$ ) to ( $15.4 \pm 0.43$ ).

In our study, to confirm the bacteriocin effect neutralized CFS were screened for their inhibition activity by agar well diffusion method. The result obtained with neutral CFS showed that the zone of inhibition became lower compared with the activity of CFS before neutralization by agar well diffusion method (Table 3). Therefore, the result shows that the inhibition activity was not related to lactic acid or H<sub>2</sub>O<sub>2</sub> but might be due to other antimicrobial substances such as bacteriocin. Thus, depending on the results of the screening (Table 3), the most efficient *Lactobacillus* isolates (Lh2DF) were selected as bacteriocin producers. Also, the most sensitive bacterial isolates including two isolates of Gram-negative bacteria such as *P. aeruginosa* (A) and *P. aeruginosa* (B), and two isolates of Gram-positive bacteria such as *E. faecalis* (A) and *E. faecalis* (B) were selected as indicator bacteria due to their higher sensitivity toward *Lactobacillus* isolates. The inhibition zones of *L. helveticus* DF against *P. aeruginosa* (A) and *P. aeruginosa* (B) were ( $14.1 \pm 0.40$ ) and ( $15.4 \pm 0.43$ ), respectively while the inhibition zones against *E. faecalis* (A) *E. faecalis* (B) were ( $10.3 \pm 0.27$ ) and ( $9.9 \pm 0.121$ ), respectively. The result of this experiment showed that both the agar well diffusion method and filter paper disc method showed antibacterial activity against more sensitive indicator bacteria (*P. aeruginosa* (B) and *E. faecalis* (A)) (Figure 2).

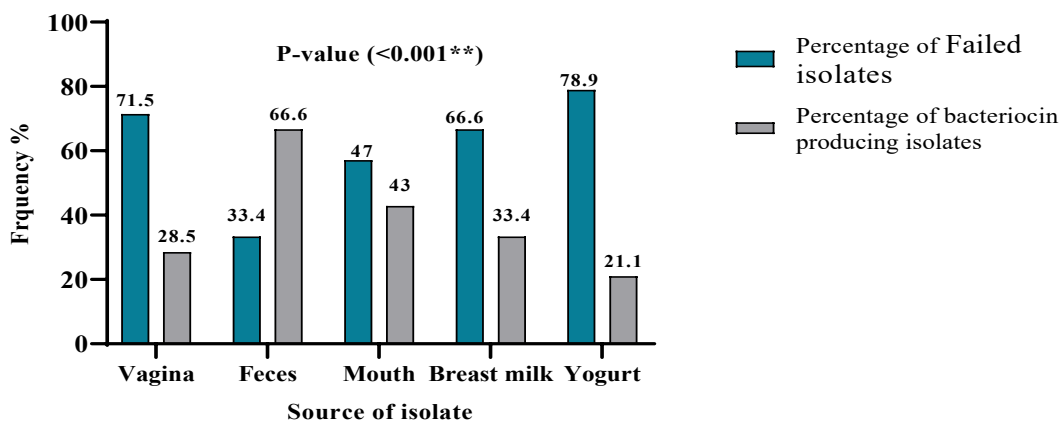


Figure 1. Percentage of bacteriocin producing and non-producing *Lactobacillus* isolates from different source.

**Table 1.** Antibacterial activity of *Lactobacillus* isolates against pathogenic bacteria by agar-wells diffusion method.

Lactobacillus Isolates	<i>P. aeruginosa</i> (A)	<i>P. aeruginosa</i> (B)	<i>P. aeruginosa</i> (C)	<i>A.baumannii</i>	<i>E. feacalis</i> (A)	<i>E. feacalis</i> (B)
Lp3DV	10.1 ± 0.125	11.9 ± 0.20	10 ± 0.122	9.6 ± 0.142	7.78 ± 0.09	0
Lp5DV	8.21 ± 0.20	8.2 ± 0.23	0	0	0	9.8 ± 0.30
Lp6DB	8.95 ± 0.08	0	0	0	8.3 ± 0.11	11.1 ± 0.21
Lp10DV	10.12 ± 0.2	12.5 ± 0.24	10.89 ± 0.25	8.8 ± 0.13	9.33 ± 0.13	0
LP13DV	10.87 ± 0.21	7.1 ± 0.14	0	0	-	8.2 ± 0.143
Lp14DV	8.17 ± 0.172	7.9 ± 0.11	7.45 ± 0.34	10.1 ± 0.123	10 ± 0.28	0
Lp17DV	9.4 ± 0.412	10.5 ± 0.27	0	0	-	0
La1DV	13.65 ± 0.40	15.9 ± 0.11	15.12 ± 0.20	10.7 ± 0.244	9.31 ± 0.122	10 ± 0.271
Lh1DF	13.32 ± 0.31	12.8 ± 0.07	10.6 ± 0.16	10.9 ± 0.21	9.12 ± 0.121	0
Lp20DY	13.6 ± 0.20	7.76 ± 0.21	0	6.8 ± 0.131	7.7 ± 0.088	0
Lh2 DF	8.4 ± 0.30	17.75 ± 0.49	13.67 ± 0.23	9.34 ± 0.15	12.17 ± 0.31	10.85 ± 0.29
La2 DV	7.3 ± 0.12	10.7 ± 0.13	8.2 ± 0.12	0	9.9 ± 0.132	10 ± 0.28
Lp22DY	7.55 ± 0.14	9.03 ± 0.145	0	10.2 ± 0.102	-	7.6 ± 0.09
LP30DY	8.1 ± 0.18	9.3 ± 0.11	0	0	9.7 ± 0.129	0
LP38DY	8.01 ± 0.10	11.8 ± 0.19	0	8.4 ± 0.43	0	0

Antibacterial activity of CFS of 15 probiotic bacteria against pathogenic bacteria inhibitory zone diameters expressed in mean±SD.

**Table 2.** Antibacterial activity of *Lactobacillus* isolates against pathogenic bacterial isolate by filter paper disc method.

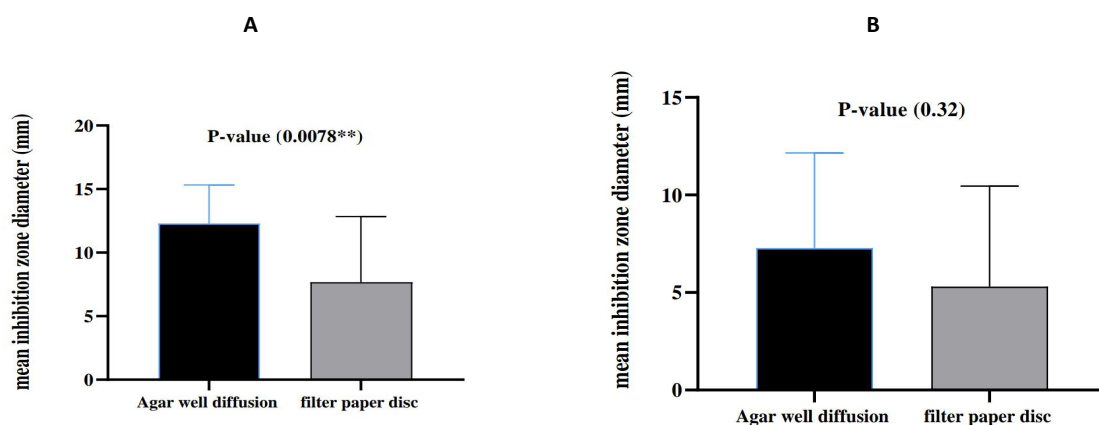
Lactobacillus no.	<i>P. aeruginosa</i> (A)	<i>P. aeruginosa</i> (B)	<i>E. feacalis</i> (A)	<i>E. feacalis</i> (B)
Lp3DV	10.92 ± 0.30	7.55 ± 0.23	0	0
Lp10DV	8.76 ± 0.35	11.17 ± 0.31	8.02 ± 0.09	0
Lp14DV	0	7.86 ± 0.25	8.86 ± 0.11	0
La1DV	12.68 ± 0.38	11.2 ± 0.33	10.1 ± 0.251	8.1 ± 0.141
Lh1DF	10 ± 0.26	13.08 ± 0.41	7.16 ± 0.17	0
Lh2 DF	14.1 ± 0.40	15.4 ± 0.43	10.3 ± 0.27	9.9 ± 0.121
La2 DV	7.84 ± 0.24	10.1 ± 0.24	7.52 ± 0.13	10.95 ± 0.29

Antibacterial activity of CFS of probiotic bacteria against pathogenic bacteria (inhibitory zone diameters expressed in mean ± SD).

**Table 3.** Antibacterial activity of neutralized CFS of *lactobacillus* isolates against pathogenic bacterial isolate by agar well diffusion method.

Lactobacillus no.	<i>P. aeruginosa</i> (A)	<i>P. aeruginosa</i> (B)	<i>E. feacalis</i> (A)	<i>E. feacalis</i> (B)
Lp3DV	10.92 ± 0.30	7.55 ± 0.23	0	0
Lp10DV	8.76 ± 0.35	11.17 ± 0.31	8.02 ± 0.09	0
Lp14DV	0	7.86 ± 0.25	8.86 ± 0.11	0
La1DV	12.68 ± 0.38	11.2 ± 0.33	10.1 ± 0.251	8.1 ± 0.141
Lh1DF	10 ± 0.26	13.08 ± 0.41	7.16 ± 0.17	0
Lh2 DF	14.1 ± 0.40	15.4 ± 0.43	10.3 ± 0.27	9.9 ± 0.121
La2 DV	7.84 ± 0.24	10.1 ± 0.24	7.52 ± 0.13	10.95 ± 0.29

Antibacterial activity of CFS of probiotic bacteria against pathogenic bacteria (inhibitory zone diameters expressed in mean ± SD).



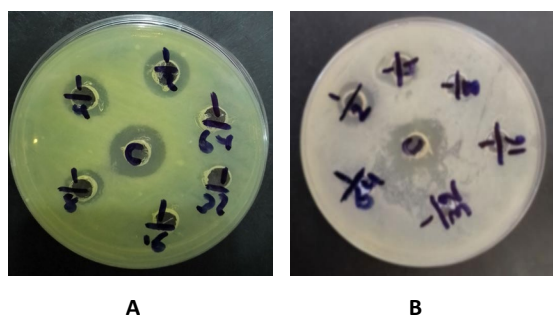
**Figure 2.** Compare between Agar well diffusion and Filter paper disc method A: *P. aeruginosa* B: *E. faecalis*.

### Molecular identification

Lh2DF isolate was subjected to molecular identification using 16S rRNA gene sequencing. The sequencing reactions of the Lh2 DF isolate indicated the exact identity after performing NCBI blasts for these PCR amplicons. The NCBI BLASTn engine also showed 98% sequence similarities between the sequenced sample and *Lactobacillus helveticus* reference sequences. By comparing the observed nucleic acid sequences of these investigated Lh2 DF isolates with the retrieved nucleic acid sequences (GenBank acc. KX247766.1).

### Bacteriocin Activity Assay

The bacteriocin activity of Lh2DF, identified as *Lactobacillus helveticus*, was further assessed as the inverse of the last dilution at which growth inhibition was still detectable following the agar well diffusion assay against multidrug-resistant bacteria (*Pseudomonas aeruginosa* and *Enterococcus faecalis*). Bacteriocin activity was 40 and 80 AU/mL against *E. faecalis*(A) and *P. aeruginosa* (B), respectively (Figure 3).



**Figure 3.** Bacteriocin activity of CFS from *L. helveticus* DF concentrated twice. (a) 80 AU/mL against *P. aeruginosa*(B), (b) 40 AU/mL against *E. faecalis*(A).

## 4. Discussion

The rise of antibiotic resistance has become a major global concern, therefore the use of probiotics has

attracted a lot of interest recently. In this study, we focused on isolating *Lactobacillus spp* from various sources to achieve suitable strains. In fact *Lactobacillus spp* dispersed in nature such as dairy, vegetables, and grains, they are also found in specific parts of the body such as the vagina, oral cavity, gastrointestinal and urogenital tract, and play an important role in the exclusion of pathogens (22, 23), the quality of bacteriocin produce from *Lactobacillus spp*. may well differ according to their source. Antimicrobial activity of *Lactobacillus* strains against bacterial pathogens emerges to be multi-factorial and attributed to the production of active metabolites such as lactic acid, acetic acid, propionic acid, hydrogen peroxide, and bacteriocins, bacteriocin has a wide range of activity and is generated by both Gram-positive and Gram-negative bacteria (24). The antibacterial mechanisms of bacteriocin can be classified into two categories: Bacteriocin act in sensitive cells by forming pores in the cytoplasmic membrane, and disrupt cell membrane permeability and integrity when bacteriocin exert their antimicrobial effects through intracellular action, they disrupt normal cellular metabolism which causes an efflux of intracellular contents such as potassium, ATP and lactic dehydrogenase resulting in depolarization of the membrane and consequently cell death. The mechanism of action of bacteriocin is complicated each type of bacteriocin can employ diverse antibacterial mechanisms to exert its antibacterial action (25).

The first part of this study was about the morphological, microscopic, and biochemical identification of *Lactobacillus spp* and its isolation from different sources, then molecular identification was used to confirm biochemical tests (26). According to our findings, gram-positive, rod-shaped bacteria, catalase, and oxidase negative, belong to the genus *Lactobacillus*. The second part of this study was about screening *Lactobacillus spp*. isolates for bacteriocin production, Searching for the best producer isolate to

be used in the subsequent step in this study, clinically isolated *L. helveticus* DF has the potential to produce bacteriocin that can be used as an alternative to antibiotics in the treatment of many nosocomial infection. The well diffusion assay revealed that *Lactobacillus spp* isolates reproducibly exhibited antibacterial effects against Gram-positive and Gram-negative indicator strains. This method allowed for diffusion of the bacteriocin into the agar medium before indicator strains began to grow (27, 28). In 2022 study by Champiri *et al.* (29) reported that the well diffusion method had better and more positive results than the disk diffusion method, additionally, we observed that the inhibitory activity of *lactobacilli* could vary between liquid and solid media, with liquid media demonstrating superior performance due to better diffusion of the bacteriocin substance secreted by *Lactobacillus* (30). In previous study by Ghorani (31) reported that probiotic metabolites can also be used in medical applications as antiviral, probiotic metabolites are active against various viruses like membrane and non-membrane viruses. The most significant antiviral compounds of probiotic metabolites are hydrogen peroxide, organic acids, and protein molecules. *P.aeruginosa* main cause of nosocomial infections due to its resistance to the broad spectrum antibiotics, Exposure to antimicrobial drugs for long periods is the most common cause widespread of resistance among Gram-negative bacteria (32, 33).

Moreover, we investigated the impact of concentrated bacteriocin on its antibacterial activity. Consistent with findings by Aziz *et al.* (34) in *lactococcus spp.*, we observed that the concentration of *L. helveticus* DF's bacteriocin led to enhanced inhibitory activity against multi-drug resistant bacteria which include Gram-negative bacteria (*P. aeruginosa* (B)) are highly sensitive in compared to Gram-positive bacteria *E. faecalis* (A), Bacteriocins are cationic peptides interact with negatively charge outer membrane of Gram-negative bacteria and the outer membrane became leaky, which allow bacteriocin to permeate, leading to cell lysis. Similar results were obtained by Jamalifar (35). Bacteriocin of *Lactobacillus* isolated from feces showed strong antibacterial activity against *Pseudomonas aeruginosa*.

This observation highlights the potential for using *L. helveticus* DF's bacteriocin as an effective antibacterial agent against a broad range of pathogenic microorganisms and underscores its significance as a bio-therapeutic microorganism. Utilizing friendly bacteria like *Lactobacillus* species as antimicrobial agents offers a promising strategy to address the escalating antibiotic resistance problem (36).

While our study provides valuable insights into the antibacterial potential of *L. helveticus* DF's bacteriocin,

it is essential to acknowledge some limitations. First, the study focused on a limited number of *Lactobacillus* isolates from specific sources, which may impact the generalizability of the findings. Further investigation with a larger and more diverse sample set would strengthen the study's conclusions. Additionally, we only assessed the *in vitro* antibacterial activity of the bacteriocin. Future studies should explore its efficacy *in vivo* and evaluate its safety profile for potential clinical applications.

## 5. Conclusion

In the present study, *L. helveticus* DF emerged as the most efficient isolate for bacteriocin production. *L. helveticus* DF exhibited antagonistic activity against indicator bacteria including *E. faecalis* and *P.aeruginosa*. The result showed that agar well diffusion showed a strong antibacterial activity against *P.aeruginosa* while there was no significant difference between agar well diffusion method and filter paper disc method against *E. faecalis*. The antagonistic activity of its bacteriocin suggests its potential as an alternative to antibiotics for combating bacterial infections. However, further investigations are warranted to comprehensively evaluate the antibacterial activities of *L. helveticus* DF and its base.

## Acknowledgment

None.

## Ethical Approval

The current study is derived from the thesis of a medical student and approved by the Ethical Approval Committee of the Mustansiriyah University, Baghdad, Iraq (Ref.:BCSMU/1221/0008M).

## Authors' Contribution

All authors contributed to the conception and design of the study, analysis of data, statistical analysis, preparation, editing, and reviewing of the article.

## Funding

None.

## Conflict of Interest

The authors declared no conflict of interest.

## Reference

- Kumar A, Schweizer HP. Bacterial resistance to antibiotics: active efflux and reduced uptake. *Adv Drug Deliv Rev.* 2005;57(10):1486-513. [PMID] [DOI:10.1016/j.addr.2005.04.004]
- Hosien B, Belhaj H, Atia A. Characteristics of antibiotic-resistant bacteria in Libya based on different source of infections. *Libyan Int Med Univ J.* 2022;7(02):039-044. [DOI:10.1055/s-0042-1759621]
- Ramzah NHHL, Yenn TW, Lee WH, Loo CY, Tan WN, Ring LC. Antimicrobial Peptides, An Alternative Antimicrobial Agent Against Multi-drug-Resistant Microbes: Source, Application, and Potential. In: Ismail A, Nur Zulkipli F, Husin HS, Öchsner A. (eds) *Advancements in Materials Science and Technology Led by Women.* Advanced Structured Materials, vol 165. Springer, Cham. [DOI:10.1007/978-3-031-21959-7\_17]
- Joni FH. Isolation and diagnosis of bacteria causing urinary tract infection in pregnant and non-pregnant females with diabetes mellitus type2. *Al-Mustansiriyah J Sci.* 2018;29(4):23-6. [DOI:10.23851/mjs.v29i4.465]
- Stoyanova LG, Ustyugova EA, Netrusov AI. Antibacterial metabolites of lactic acid bacteria: their diversity and properties. *Appl Biochem Microbiol.* 2012;48:229-43. [DOI:10.1134/S0003683812030143]
- Sharifi Yazdi MK, Davoodabadi A, Khesht Zarin HR, Tajabadi Ebrahimi M, Soltan Dallal MM. Characterisation and probiotic potential of lactic acid bacteria isolated from Iranian traditional yogurts. *Ital J Anim Sci.* 2017;16(2):185-8. [DOI:10.1080/1828051X.2016.1222888]
- Zacharof MP, Lovitt R. Bacteriocins produced by lactic acid bacteria a review article. *APCBEE Procedia.* 2012;2:50-6. [DOI:10.1016/j.apcbee.2012.06.010]
- Gutiérrez-Cortés C, Suarez H, Buitrago G, Nero LA, Todorov SD. Characterization of bacteriocins produced by strains of *Pediococcus pentosaceus* isolated from Minas cheese. *Ann Microbiol.* 2018; 68(6):383-98. [DOI:10.1007/s13213-018-1345-z]
- Hassan MU, Nayab H, Rehman TU, Williamson MP, Haq K U, Shafi N, et al. Characterisation of bacteriocins produced by *Lactobacillus spp.* isolated from the traditional Pakistani yoghurt and their antimicrobial activity against common foodborne pathogens. *Biomed Res Int.* 2020;2020. [DOI:10.1155/2020/8281623] [PMID] [PMCID]
- Manoharan M, Balasubramaniam TS. An Extensive Review on Production, Purification, and Bioactive Application of Different Classes of Bacteriocin. *J Trop Biotechnol Biodivers.* 2022;7(3):72735. [DOI:10.22146/jtbb.72735]
- Corr SC, Li Y, Riedel CU, O'Toole PW, Hill C, Gahan CG. Bacteriocin production as a mechanism for the anti-infective activity of *Lactobacillus salivarius* UCC118. *Proc Natl Acad Sci.* 2007;104(18):7617-21. [DOI:10.1073/pnas.0700440104] [PMID] [PMCID]
- Thirabunyanon M, Thongwittaya N. Protection activity of a novel probiotic strain of *Bacillus subtilis* against *Salmonella Enteritidis* infection. *Res vet Sci.* 2012;93(1):74-81. [DOI:10.1016/j.rvsc.2011.08.008] [PMID]
- Karami S, Roayaei M, Hamzavi H, Bahmani M, Hassanzad-Azar H, Leila M, et al. Isolation and identification of probiotic *Lactobacillus* from local dairy and evaluating their antagonistic effect on pathogens. *Int J Pharm Investig.* 2017;7(3):137-41. [DOI:10.4103/jphi.JPHI\_8\_17] [PMID] [PMCID]
- Mirzaei H, Barzgar A. Isolation and molecular study of potentially probiotic lactobacilli in traditional white cheese of Tabriz in Iran. *Ann Biological Res.* 2012;3:2213-6.
- Atassi F, Brassart D, Grob P, Graf F, Servin AL. *Lactobacillus* strains isolated from the vaginal microbiota of healthy women inhibit *Prevotella bivia* and *Gardnerella vaginalis* in coculture and cell culture. *FEMS Immunol Med Microbiol.* 2006; 48(3):424-32. [PMID] [DOI:10.1111/j.1574-695X.2006.00162.x]
- Loy A, Lehner A, Lee N, Adamczyk J, Meier H, Ernst J, et al. Oligonucleotide microarray for 16S rRNA gene-based detection of all recognized lineages of sulfate-reducing prokaryotes in the environment. *Appl Environ Microbiol.* 2002;68(10):5064-81. [DOI:10.1128/AEM.68.10.5064-5081.2002] [PMID] [PMCID]
- Rahman M, Nahidul Islam MD, Nurul Islam M, Shahnoor HM. Isolation and Identification of Oral Bacteria and Characterization for Bacteriocin Production and Antimicrobial Sensitivity. *J Pharm Sci Dhaka Univ.* 2015;14(1):103-9. [DOI:10.3329/dujps.v14i1.23742]
- Dyae NA, Luti KJK. Classical and Statistical Optimization by Response Surface Methodology For Enhancing Biomass and Bacteriocin Production by *Lactobacillus Plantarum*. *Iraqi J Sci.* 2019;60(3): 494-508.
- Biyari S, Fozouni L. The inhibitory effect of probiotic bacteria against drug-resistant *Candida* species isolated from the oral cavity of the elderly.



- Shiraz E-Med J. 2018;19(6):2-7.  
[DOI:10.5812/semj.62026]
20. Balouiri M, Sadiki M, Ibsouda SK. Methods for in vitro evaluating antimicrobial activity: a review. J Pharm Anal. 2016;6(2):71-9.  
[DOI:10.1016/j.jpha.2015.11.005] [PMID] [PMCID]
21. Hussain MA. Antibacterial effect of probiotic lactic acid bacteria on some pathogens. J Genes Environ Resour Conserv. 2022;10(1):1-6.
22. Dai J, Fang L, Zhang M, Deng H, Cheng X, Yao M, et al. Isolation and identification of new source of bacteriocin-producing *Lactobacillus plantarum* C010 and growth kinetics of its batch fermentation. World J Microbiol Biotechnol. 2022; 38(4):67. [DOI:10.1007/s11274-022-03244-1] [PMID]
23. Antonio MAD, Hawes SE, Hillier SL. The identification of vaginal *Lactobacillus* species and the demographic and microbiologic characteristics of women colonized by these species. J Infect Dis. 1999;180(6):1950-6. [DOI:10.1086/315109] [PMID]
24. Ibrahim SA, Ayivi R D, Zimmerman T, Siddiqui S A, Altemimi AB, Fidan H. et al. Lactic acid bacteria as antimicrobial agents: Food safety and microbial food spoilage prevention. Foods. 2021;10(12): 3131. [DOI:10.3390/foods10123131] [PMID] [PMCID]
25. Gu Q. Bacteriocinogenic Lactic Acid Bacteria and Antibacterial Mechanisms. In: Bacteriocins. Singapore: Springer Nature Singapore. 2023. p. 39-61. [DOI:10.1007/978-981-99-2661-9\_3]
26. Dubernet S, Desmasures N, Guéguen M. A PCRbased method for identification of lactobacilli at the genus level. FEMS Microbiol Lett. 2002; 214(2):271-5. [PMID] [DOI:10.1111/j.1574-6968.2002.tb11358.x]
27. Tagg JR, Read RSD, McGiven AR. Bacteriocine production by group A streptococci. Pathology. 1971;3(4):277-8.  
[DOI:10.3109/00313027109073746]
28. Rogers AM, Montville TJ. Improved agar diffusion assay for nisin quantification. Food Biotechnol. 1991;5(2):161-8.  
[DOI:10.1080/08905439109549799]
29. Champiri ID, Bamzadeh Z, Rahimi E, Rouhi L. Isolation and identification of *Lactobacillus brevis* from local cheese of bazoft and evaluation of antimicrobial activity against some pathogenic microorganisms. Iran J Med Microbiol. 2022;16(1): 17-34. [DOI:10.30699/ijmm.16.1.17]
30. Mahmoud Farhan S, Mahmoud Abd El-Baky R, Abdalla M, Osama El-Gendy A, Farag Azmy A. Efficacy of Amikacin and Imipenem Against Multi-Drug Resistant Gram-Negative Bacteria Isolated from Wound Infections, Egypt. Iran J Med Microbiol. 2023;17(2):218-29.  
[DOI:10.30699/ijmm.17.2.218]
31. Ghorani M. Antiviral Effects of Probiotic Metabolites. Iran J Med Microbiol. 2022;16(2):83-97. [DOI:10.30699/ijmm.16.2.83]
32. Ghorbani Z, Koupaei M, Sadari H, Amin Marashi SM, DehghanZadeh Z, & Owlia P. Inhibitory Effect of Supernatant and Lysate of *Saccharomyces cerevisiae* on Expression of *exoA* Gene of *Pseudomonas aeruginosa*. Iran J Med Microbiol. 2023;17(3):288-93. [DOI:10.30699/ijmm.17.3.288]
33. Altalhi AA. Antilisterial activity of plantaricin UG1 during manufacture of Zabady and Kareesh cheese: Two Arabian dairy products. Int J Biomed Sci. 2008;4(4):319.
34. Aziz RA, Salman, JAS, Hamel AH. Study of the Synergistic effect between purified and produced bacteriocin from bacteria *Lactococcus lactis* ssp. *lactis* Against Species of Pathogenic bacteria. J Coll Basic Educ. 2020;26:107.
35. Jamalifar H, Rahimi H, Samadi N, Shaverdi A, Sharifian Z, Hosseini F, et al. Antimicrobial activity of different *Lactobacillus* species against multi-drug resistant clinical isolates of *Pseudomonas aeruginosa*. Iran J Microbiol. 2011;3(1):21-5.
36. Zohreh-Mirbagheri S, Meshkat Z, Naderinasab M, Rostami S, Sadat-Nabavinia M, Rahmati M. Study on imipenem resistance and prevalence of blaVIM1 and blaVIM2 metallo-beta lactamases among clinical isolates of *Pseudomonas aeruginosa* from Mashhad, Northeast of Iran. Iran J Microbiol. 2015;7(2):72-8.