THE IRAQI JOURNAL OF
VETERINARY MEDICINE

eISSN:2410-7409
pISSN: 1609-5693

OPEN ACCESS

Antibacterial Activity of *Lactiplantibacillus plantarum* from Dairy Products Against Some Foodborne Bacteria

Doaa A Qasim[1], Inam J Lafta[2*], Oluyinka A Iyiola[3]

[1]Market Research and Consumer Protection Center, University of Baghdad, Baghdad, Iraq, [2]Department of Microbiology, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq, [3]Department of Zoology, Cell Biology and Genetics Unit, Faculty of Life Sciences, University of Ilorin, Ilorin, Nigeria

***Correspondence:**
Inam.j@covm.uobaghdad.edu.iq

Received: 3 April 2023
Accepted: 15 May 2023
Published: 28 Jun 2023

DOI:
https://doi.org/10.30539/ijvm.v47i1.1500

Cite:
Qasim DA, Lafta IJ, Iyiola O. Antibacterial Activity of *Lactiplantibacillus plantarum* from Dairy Products Against Some Foodborne Bacteria. Iraqi J. Vet. Med. 2023;47(1):44-51.

ABSTRACT

Lactiplantibacillus plantarum, one of lactic acid bacteria (LAB), is found in various foods, including dairy products, meat, and vegetables, and most of these bacteria offer beneficial effects to humans and animals as potential probiotics with broad-spectrum antimicrobial activities. The aim of this study was evaluating the antibacterial efficacy of *L. plantarum* against some foodborne bacteria isolated from dairy products. This research involved 34 dairy products, including local and imported milk, cheese, and yogurt sold locally in Baghdad province, Iraq, during May 2022. For the isolation of *L. plantarum*, a special medium called MRS (de Man Rogosa and Sharpe) was applied. Colonies were purified and identified by routine bacteriological methods, Vitek2 system, and confirmed by the polymerase chain reaction (PCR) targeting the *16S rRNA* gene followed by the amplicon sequencing. Other aerobic bacteria contaminating dairy products were also isolated onto sterile selective media specific for each microorganism, and the isolates were identified by routine diagnostics tests followed by verification with Vitek2 system. Then, the culture supernatant of *L. plantarum* was tested for its antagonistic activity toward foodborne bacteria by the use of agar well diffusion assay. The findings showed the isolation of 2 *L. plantarum*, 3 *Pseudomonas aeruginosa*, 4 *Escherichia coli*, one isolate of *Bacillus subtilis*, and another *Staphylococcus hominis*. The filtered supernatant of *L. plantarum* was significantly efficient in inhibiting the growth of the above bacteria. Each of *E. coli* and *B. subtilis* revealed zones of inhibition of 36 and 38 mm in diameter, respectively, while *P. aeruginosa* and *S. hominis* had inhibition zones diameters of 27 and 29 mm, respectively. This suggests that the *L. plantarum* supernatant possesses a broad-spectrum activity against foodborne bacteria. To conclude, locally made dairy products can hold different contaminating bacteria, which can be eliminated by using probiotics, such as *L. plantarum*, to avoid foodborne diseases onset.

Keywords: favipiravir, amlodipine, pharmacokinetics, aldehyde oxidase, hypertension

INTRODUCTION

Lactic acid bacteria (LAB) are anaerobic to aerotolerant homo-fermentative bacteria that produce L-lactic acid and belong to the Gram-positive category of bacteria (1). An example of LAB is *Lactobacillus plantarum*, which was reclassified by Zheng and colleagues in 2020 into the newly proposed genus *Lactiplantibacillus* (2). *Lactiplantibacillus plantarum* is the most diverse LAB species, belonging to the heterofermentative group and producing both L- and D-lactic acid. It has been widely employed as a probiotic microorganism in the food industry (3-5).

According to the World Health Organization (WHO) and the International Scientific Association of Probiotics and Prebiotics (ISAPP), probiotics are live microorganisms that

can provide health benefits to their host when supplied in sufficient amounts (10^6-10^8 CFU/mL) (6). In addition to the health advantages, probiotics are also safe and cost-effective (7). They can serve as effective alternatives to traditional antibiotics, with many possible biomedical applications (5). They are also useful for achieving products with extended shelf life and harmless properties due to their ability to prevent or delay the growth of contaminating microorganisms (8).

During the fermentation process of some foods, LAB can be produced and isolated from numerous natural sources (9). These bacteria produce different types of inhibitory compounds, including metabolic end products, hydrogen peroxide, antimicrobial peptides with antibiotic activity (e.g., bacteriocins), and many organic acids (10). The antimicrobials produced by these bacteria are rather diverse and are divided into two major chemical classes: proteinaceous and non-proteinaceous materials (5). *L. plantarum* strains, in particular, have been shown to produce many enzyme systems (including lactase dehydrogenase, α-glucosidase, β-glucosidase, α-amylase, enolase, esterase, lipase, and phosphoketolase) and bioactive substances (such as dipeptides, bacteriocins, and other preservatives). Because of their significant effects against foodborne pathogens, the qualities of *L. plantarum* strains as probiotics can increase the shelf life and safety of fermented foods (3). The antimicrobial activity of *L. plantarum* toward opponent microorganisms has been shown to be facilitated by its production of a bacteriocin-like compound called plantaricin (11). Plantaricins of *L. plantarum* spp. are characterized by their small size, heat stability, potency, activity at very low concentrations, and their ability to exert their bactericidal influence via membrane permeability, formation of pores, followed by cytoplasmic compounds leak (5). Plantaricin has shown broad-spectrum action against Gram-negative bacteria, such as *Escherichia coli*, as well as Gram-positive bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA) (4, 11) and *Listeria monocytogenes* (12).

Antibiotic resistance among microorganisms linked with foodborne illness has become a serious therapeutic challenge for many clinicians (13). With the increasing concern over the distribution of multidrug-resistant microorganisms and the possibility of existing medications becoming ineffective, it is essential to obtain alternative strategies, such as the use of probiotics (10). Therefore, the purpose of this study was to investigate the influence of *L. plantarum* from dairy products as a probiotic on the growth inhibition of other foodborne bacteria that contaminate dairy products.

Materials and Methods

Ethics

The local Committee for Animal Care and Use at the College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq reviewed and approved all procedures involved in the current study.

Sample Collection

This study involved the random collection of 34 samples of dairy products, including local and imported milk, cheese, and yogurt using sterile containers from different supermarkets in Baghdad, Iraq, throughout May 2022. Within a few hours, the samples were transported via ice box to the Department of Microbiology, College of Veterinary Medicine, University of Baghdad, and stored in the refrigerator until evaluation (8).

Aerobic Bacteria Isolation and Identification

Aerobic bacteria were isolated from the dairy samples mentioned above, 1 g or 1 mL of each sample was serially diluted 10-fold in phosphate-buffered saline (PBS) as described by Harrigan and MacCance (1976) (14). Then, 1 mL from each dilution was poured on the surface of a solid medium selective for each suspected microorganism using the pour plate method, then the plates were incubated at 37 °C overnight. The isolates were maintained on Brain Heart Infusion agar (BHI, Eiken, Japan) slants (8). Afterward, the isolates identification was done by microscopic examination with the aid of biochemical tests (Oxidase, Catalase, Urease, Motility, Gelatinase, Indole, Methyl red, Voges-Proskauer, and Arginine Hydrolysis), followed by further confirmation with the Vitek2 system (bioMérieux, France). Before doing Vitek, the suspected bacterium was inoculated on MacConkey's agar then incubated at 37 °C for 24 to 48 h. After that, the bacterial suspension was made, and its turbidity was adjusted to a McFarland standard tube 0.5 (15).

Lactic Acid Bacteria Isolation and Identification

One gram or 1 mL of each dairy sample was diluted into 10-fold serial dilutions using PBS as described above. Then, the microorganisms were isolated by taking 1 mL from each dilution to be poured onto MRS (de Man Rogosa and Sharpe; Difco, USA) agar medium using the pour plate method. The plates were then anaerobically incubated in a jar at 30 °C for 48-72 h. Pure colonies were selected and stored in Brain Heart Infusion broth (8.5 mL) overlaid with sterile glycerol (1.5 mL) to be stored at -20 °C. For diagnosis, the pure colonies were first examined for lactic acid bacteria (LAB) by microscopic examination of the Gram's stained smears (8). All Gram-positive isolates were identified phenotypically based on the tests: Oxidase, Catalase, Urease, Motility, Indole, Gelatinase, Methyl red, Voges-Proskauer, Arginine Hydrolysis, and Carbohydrate Fermentations (16). Gas production by the suspected LAB was determined by the carbohydrate fermentation broth in presence of an inverted Durham tube, in which 5 g of either glucose, mannose, sucrose, maltose, or fructose was added to the fermentation medium. Subsequently, the suspected findings were confirmed with the Vitek2 system, as

illustrated above, and the ANC card specific for the Gram-positive bacterial species was used.

Molecular Detection of L. plantarum

DNA extraction

L. plantarum genomic DNA was isolated from its culture by ABIO pure™ Total DNA Extraction Kit (ABIOpure, USA). In short, 1 mL of overnight culture was spun at 13000 rpm for 2 min, and the supernatant was discarded. The cell sediment was then mixed with 100 µL of nuclease-free water and 100 µL of lysozyme solution and vortexed. The tube contents were incubated in a water bath at 37 °C for 30 min, followed by spinning at 13000 rpm for 2 min, with discarding the supernatant. Proteinase K (20 µL; 20 mg/mL) and Buffer BL (200 µL) were added to each sample and vigorously mixed before incubating at 56 °C for 30 min. Additional lysis was done by incubating the tube contents for 30 min at 70 °C inside the water bath. Then, 200 µL of Absolute ethanol was mixed thoroughly with the samples by vortexing. All of the sample mixes were carefully transferred to the mini-column and spun at >8000 rpm for 1 min before the collecting tube was replaced. Later, Buffer BW (600 µL) was introduced to the mini-column, which was spun for 1 min at >8000 rpm before the collecting tube was removed. After adding buffer TW (700 µL) and spinning for 1 min at >8000 rpm, the flowthrough was discarded and the mini-column was re-inserted into the collecting tube. To remove wash buffer, the mini-column was spun for 1 min at high speed (>13000 × g), after which it was placed in a new 1.5 mL tube. Finally, 100 µL of Buffer AE was added and incubated for 1 min at ambient temperature before spinning for 5 min at 5000 rpm. The final DNA extract was stored at -20 °C until it was used.

Polymerase chain reaction

L. plantarum molecular identification was carried out using Thermal cycler (Biosystems™ ProFlex™ PCR System, Fisher Scientific, USA) and universal primers (Macrogen, Korea) to amplify the isolates' *16S rRNA* gene. As a stock solution, the lyophilized primers were dissolved in nuclease-free water to a concentration of 100 pmol/µL, from which the working concentration of 10 pmol/µL was prepared. The nucleotides sequences of these primers were: 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-TACGGTTACCTTGTTACGACTT-3') that amplified a region of approximately 1500 bp. The PCR reaction used 25 µL of GoTaq® Green Master Mix, 1 µL of Forward primer at 10 pmols/µL, 1 µL of Reverse primer at 10 pmols/µL, 1.5 µL of DNA template, and 9 µL of nuclease-free water.

The PCR program was set to include initial denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 30 sec, annealing for 30 sec at 60 °C, and elongation for 1 min at 72 °C. The last elongation phase was performed at 72 °C for 7 min, followed by a 10-min hold step at 10 °C. Finally, electrophoresis was performed at 70 volts for 1 h on a 1% agarose gel stained with Ethidium Bromide (0.5 g/mL, Thermo Fisher Scientific, USA).

DNA sequencing and bioinformatic analysis

The Sanger sequencing technique was used to sequence the *16S rRNA* gene of *L. plantarum* that had been amplified by PCR using an automated DNA sequencer (ABI3730XL, Macrogen Corporation, Korea). Briefly, 20 µL from each PCR amplicon along with 50 µL of the Forward primer were sent to the above Company to determine the *16S rRNA* nucleotide sequences. Following obtaining the sequence through the e-mail, the Basic Local Alignment Search Tool (BLAST), which exists freely online at the website of National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov), was used to conduct a homology search along with the BioEdit program. Then, the local isolates' nucleotide sequences were submitted to GenBank/NCBI for registration in their online databases under a specific accession number.

Assessment of the Antibacterial Activity

The agar well diffusion test was used to assess the antibacterial activity of an *L. plantarum* (crude plantaricins) isolate against some foodborne bacterial isolates, including Pseudomonas aeruginosa, Escherichia coli, Bacillus subtilis, and Staphylococcus hominis. Firstly, *L. plantarum* was cultivated in 25 mL of BHI broth anaerobically with shaking at 30 °C for 18 h. The cells were then pelleted by spinning for 15 min at 8,000 rpm at 4 °C, and the supernatant was filtered through a 0.22-µm Millipore filter. The crude plantaricin was made from the cell-free supernatant, and its pH was adjusted to 7.0±0.2. Before testing the antimicrobial activity, each foodborne isolate was grown overnight in a nutrient broth.

The inhibitory activity of the crude plantaricin against the other foodborne bacteria was assayed on Mueller-Hinton agar by employing Benavides and his colleagues (17) method of agar well diffusion. Briefly, plates of Mueller-Hinton agar were inoculated with 1.5×10^8 CFU/mL, which is equivalent to 0.5 McFarland standards tube, in 250 µL of either isolate of the foodborne bacteria. A 6 mm well already made in the center of each agar plate was filled with 150 µL of crude plantaricin (cell-free culture supernatant). Afterward, the plates were placed in an incubator of 30 °C for 24 to 48 h, and the antibacterial activity was measured by estimating the diameter of the inhibitory zone around the wells. Inhibition halos more than 15 mm in diameter showed considerable inhibitory action. Three separate tests were conducted.

RESULTS

Isolation and Identification of Aerobic Bacteria

Among the 34 milk and milk product samples, nine bacterial isolates were obtained, including 3 (8.82%) isolates of *Pseudomonas aeruginosa*, 4 (11.76%) *Escherichia*

coli, one isolate (2.94%) of *Bacillus subtilis*, and another one (2.94%) for *Staphylococcus hominis* (Table 1, Figure 1).

Table 1. Numbers and percentages of the aerobic bacterial isolates from dairy products

Bacterial isolates	No. samples	Positive samples	
		No.	%
Pseudomonas aeruginosa	34	3	8.820
Escherichia coli		4	11.76
Bacillus subtilis		1	2.940
Staphylococcus hominis		1	2.940

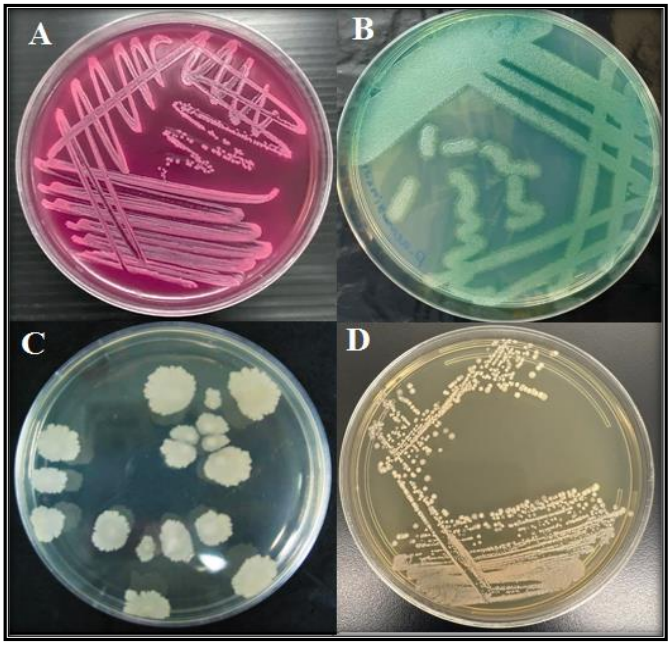

Figure 1. Colonial morphology of the isolated aerobic bacteria on selective agar media. **(A)** *E. coli* on MacConkey's agar, **(B)** *P. aeruginosa* on Cetrimide's agar, **(C)** *B. subtilis* on Nutrient's agar , **(D)** *S. hominis* on Tryptic Soy

Isolation and Identification of *L. plantarum*

Out of the 34 samples of milk and milk products, only two isolates (5.88%) were identified as *L. plantarum*. The identification of *Lactobacillus* was performed based on a combination of phenotypic, cultural, and biochemical tests (Figure 2). Microscopic examination of the Gram-stained smears revealed Gram-positive, elongated, rod-like bacilli with rounded ends that were non-spore-forming and arranged singly, in pairs, or short chains. Regarding cultural characteristics, the isolates were facultative anaerobes but grew better under microaerophilic conditions. Colonies on MRS agar were white to pale, round in shape, soft, and convex with an entire margin (Figure 2). They were non-hemolytic on the blood agar and chocolate agar. Biochemical tests showed that the isolates were negative for oxidase, Furthermore, *L. plantarum* was non-motile and produced acid/acid in Kligler iron agar (KIA), along with its ability to ferment carbohydrates, including glucose, mannose, sucrose, maltose, and fructose. The identification of *L. plantarum* was further confirmed using the Vitek 2 system, where it was detected with a probability of 89% (Figure 3).

Figure 2. *Lactiplantibacillus plantarum* grown on **(A)** MRS agar and **(B)** different biochemical tests: Citrate, Maltose, Mannose, Sucrose, Fructose, Glucose, Arginine, Urease, Motility, MR, VP, Gelatinase, Indole, and slide catalase.

bioMérieux Customer: **Laboratory Report**
System #: Printed by: Labadmin
Patient Name: **Doaa Adel** Patient ID:
Isolate: RESEARCH-1 (Qualified)
Card Type: ANC Bar Code: 2441978503652074 Testing Instrument: 00000B4E24D0 (1372)
Setup Technologist: Laboratory Administrator(Labadmin)
Bionumber: 3017711030641
Organism Quantity: **Selected Organism: Lactobacillus plantarum**

Comments:

Identification Information	**Card:** ANC	**Lot Number:** 2441978503	**Expires:** Apr 27, 2023 13:00 CDT		
	Status: Final	**Analysis Time:** 6.33 hours	**Completed:** May 21, 2022 13:51 CDT		
Organism Origin	VITEK 2				
Selected Organism	89 % Probability	**Lactobacillus plantarum**			
	Bionumber: 3017711030641	Confidence: Acceptable identification			
Analysis Organisms and Tests to Separate:					
Analysis Messages:					
Contraindicating Typical Biopattern(s)					
Lactobacillus plantarum	PheA(99),TyrA(88),lARA(7),dXYL(4),				

Biochemical Details

4	dGAL	+	5	LeuA	(+)	6	ELLM	-	7	PheA	-	8	ProA	-	10	PyrA	-
11	dCEL	+	13	TyrA	-	15	APPA	-	18	dGLU	+	20	dMNE	+	22	dMAL	+
28	SAC	+	30	ARB	+	33	NAG	+	34	BGLUi	+	36	URE	-	37	BGURi	-
39	BGALi	(+)	41	AARA	-	42	AGALi	-	43	BMAN	(-)	44	ARG	-	45	PVATE	-
51	MTE	+	53	ESC	+	54	BdFUC	-	55	BNAGi	-	56	AMANi	-	57	AIFUC	-
59	PHOS	-	60	lARA	+	61	dRIB2	+	62	OPS	-	63	AARAF	-	64	dXYL	+
	GRAM	+		MORPH	-		AERO	?									

Figure 3. The Vitek2 system reading of *L. plantarum*

Molecular Identification of the Isolates

For the molecular diagnosis, the DNA was successfully extracted from the 11 isolates of this study with a purity ranged 1.6-1.8. Then, all of the isolates were diagnosed by amplifying the *16S rRNA* gene as represented by bands of roughly 1500 bp seen on the agarose gel (Figure 4), followed by nucleotide sequencing. The BLAST analysis revealed the presence of homology between the *16S rRNA* gene of the local DI1 strain (OQ673824) of this study with that of *Lactiplantibacillus plantarum* deposited on the NCBI website.

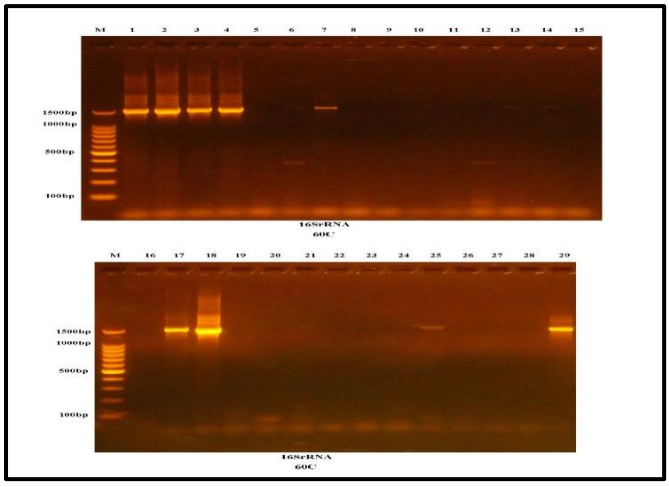

Figure 4. Agarose gel of 1% reveals bands of the expected size of roughly 1500 bp of the *16S rRNA* gene amplified by the PCR assay and electrophoresed at 70V for 1 h

Antibacterial Activity of L. plantarum

The *L. plantarum* cell-free supernatant formed large inhibition circles against all of the tested foodborne bacteria, both the Gram-positive and the Gram-negative isolates. That supernatant produced strong growth inhibition halos against *B. subtilis* (38 mm) followed by *E. coli* (36 mm) (Figure 5). Regarding *P. aeruginosa* and *S. hominis*, the zones of inhibition were 27 mm and 29 mm, respectively (Figure 5).

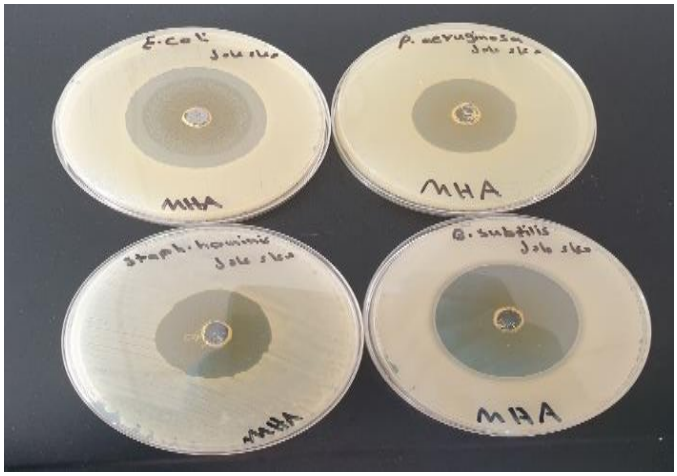

Figure 5. Antibacterial activity of *L. plantarum* cell-free supernatant against some foodborne bacteria

DISCUSSION

Recently, probiotic bacteria have obtained large interest as medicinal agents for treating different diseases and controlling pathogens (4, 18). It is well-known that most bacteria have acquired antibiotic resistance; thus, providing alternative therapies, for instance probiotics, may solve this problem (13). Probiotics can replace antibiotics, reduce new outbreaks of microbial resistance, and enhance responses to production diseases frequently treated with antibiotics or chemicals (18). Among probiotics is *L. plantarum*, which is recognized as safe with strong probiotic properties (5). Many investigations have been conducted to isolate and characterize lactic acid bacteria (LAB) from diverse sources with evaluating their antimicrobial activities towards other pathogens (19, 20). In this study, however, isolation and identification of *L. plantarum* and other contaminants from dairy products and investigation of its bacteriocins in supernatant against some foodborne bacteria have been given more attention. This would be important as a preservation method, particularly for locally-made dairy products.

The cow's raw milk and its products are good materials for the isolation of LAB, which possess probiotic properties and can produce novel bacteriocins (20). In the present research, *L. plantarum* was obtained from 2 out of 34 dairy products, and de Man Rogosa Sharpe (MRS) medium successfully isolated these bacteria. In addition, the related biochemical tests and the Vitek2 system were useful for bacterial detection. The definite confirmation of the isolates depending on the PCR amplification of the *16S rRNA* gene followed by its sequencing also succeeded in their diagnosis. This is partially consistent with the results of other researchers (21-23). In the same context, numerous researchers cultivated LAB from different sources, including dairy products, e.g., the study of Oldak et al. (2017) (24) where *L. plantarum* was isolated from cheese and its antibacterial activity was reported against pathogenic microorganisms. Similarly, *L. plantarum* was shown to produce bacteriocin antagonistic to Gram-negative and Gram-positive bacteria after being isolated from traditionally fermented dairy products (25). Strains with probiotic features have also been obtained from diverse niches that are dairy-related, for instance, camel milk (26), whey, and cheese of cow's or ewe's raw milk (24, 27, 28).

The antibacterial activity of *L. plantarum* supernatant containing crude bacteriocins was assessed in the current research against four foodborne bacteria. Bacteriocins produced by LAB can be formed in completely purified products, partially purified, or the supernatant (29). The supernatant prepared in the present study has a broad-spectrum efficacy antagonistic to Gram-positive and Gram-negative isolates. The inhibition zones were 38 mm, 36 mm, 29 mm, and 27 mm against each of *B. subtilis*, *E. coli*, *S. hominis*, and *P. aeruginosa*, respectively. Such broad-

spectrum antagonistic activity of *L. plantarum* plantaricin was reported by other researchers, such as Jandaik et al. (2013) (30) and Chaalel et al. (2015) (31) who observed a significant inhibitory effect of bacteriocin produced by *Lactobacillus* spp. against *E. coli*. Furthermore, *L. plantarum* showed a full inhibitory effect on *P. aeruginosa* growth (13, 32). Bacteriocins generated by LAB have also been proven to inhibit the development of harmful microbes as well as the bacteria implicated in decomposition, e.g., *Bacillus cereus* among others (33). Similarly, preliminary experiments performed by De Giani et al. (2019) (11) revealed that *L. plantarum* cell-free supernatant (CFS) had antagonistic consequences on *S. aureus* and *E. coli* growth. Other studies have reported that *L. plantarum* isolated from infants' feces showed growth inhibition to foodborne bacteria, including *B. cereus* and *E. coli* among others (34, 35).

This inhibitory activity of the *L. plantarum* supernatant used in this study against other microorganisms has been attributed to the release of broad-spectrum antibacterial agents, for instance bacteriocin-like compounds, H_2O_2, and extracellular organic acids (36). The organic acids created by *L. plantarum* strains involve lactic acid, the major one, as well as propionic acid, formic acid, acetic acid, succinic acid, and phenyl lactic acid (3). It has been demonstrated that the antibacterial action of *L. plantarum* is typically dependent on the production and release of several organic acids, primarily lactic and acetic acids, followed by citric, tartaric, succinic, malic, and oxalic acids (4). Nevertheless, culture conditions, such as density and ingredients of the medium, the species and strain used (4, 9), the medium pH, as well as time and temperature of incubation also have high impacts on the growth inhibitory activity of probiotic bacteria (37, 38).

The optimum pH for perfect bactericidal activity of bacteriocin was indicated by Sankar et al. (2012) (39) to be pH 7, which was used in this study. Thus, the results here suggest that the cell-free supernatant might have bactericidal effects on the tested food-contaminating bacteria. This suggestion was also stated by Hernández et al. (2005) (40), who found that the mode of action of plantaricins was through inducing of pore formation in the membrane of target cells causing intracellular ATP disruption, leakage of proton motive force, depletion of intracellular substances, and finally cell death. Nevertheless, plantaricin has also revealed bacteriostatic effects in many studies (40-42). Plantaricins have been shown to bind to specific sites on the cell membrane and influence its integrity and function, leading to a bacteriostatic influence on some species of bacteria and bactericidal impact on others (43).

To summarize, the preliminary inhibition findings of *L. plantarum* supernatant against some food-borne bacteria observed in this study are promising as the results indicated that the cell-free supernatant could be utilized as a possible food supplement that is safe, cheap, and effective, and this method might be used as a control strategy for these bacterial species contaminating foods, which in turn might cause food poisoning or other food-related diseases. However, this study has some limitations, such as a few bacterial species tested whose pathogenicity and antimicrobial resistance were not examined. Adding to the absence of in vivo experiments, the precise antimicrobial mechanism of action of the *L. plantarum* supernatant at the molecular level needs to be further illustrated.

In conclusion, the current results demonstrate that *L. plantarum* obtained from dairy products possesses a potential antagonistic effect against Gram-positive and Gram-negative foodborne bacteria responsible for human and animal diseases. The *L. plantarum* supernatant had significant effects on preventing each of *P. aeruginosa*, *E. coli*, *B. subtilis*, as well as *S. hominis* growth by producing certain antimicrobial substances. Thus, the probiotics field will bring uncountable modifications in this study area resulting in consumer health due to producing foods of higher quality.

ACKNOWLEDGEMENTS

The researchers would like to thank Market Research and Consumer Protection Center, University of Baghdad, for providing the essential equipment to perform this study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Liu B, Yang M, Qi B, Chen X, Su Z, Wan Y. Optimizing l-(+)-lactic acid production by thermophile *Lactobacillus plantarum* As. 1.3 using alternative nitrogen sources with response surface method. Biochem. Eng. J. 2010; 52(2): 212–219. DOI: 10.1016/j.bej.2010.08.013.
2. Zheng J, Wittouck S, Salvetti E, Franz C, Harris H, Mattarelli P, et al. A taxonomic note on the genus *Lactobacillus*: Description of 23 novel genera, emended description of the genus *Lactobacillus* beijerinck 1901, and union of *Lactobacillaceae* and *Leuconostocaceae*. Int. J. Syst. Evol. Microbiol. 2020;70(4):2782–858. DOI: 10.1099/ijsem.0.004107.
3. Behera S, Ray R, Zdolec N. *Lactobacillus plantarum* with functional properties: An approach to increase safety and shelf-life of fermented foods. BioMed. Res. Int. 2018; Article ID 9361614. DOI: 10.1155/2018/9361614.
4. Saito R, Sato N. Characterization of Lactiplantibacillus plantarum TO-A growth inhibition activity against pathogenic bacteria. J. Prob. Health. 2021;9(9): 253. Available at: https://www.longdom.org/open-access/characterization-of-lactiplantibacillus-plantarum-toa-growth-inhibition-activity-against-pathogenic-bacteria.pdf.
5. Rocchetti M, Russo P, Capozzi V, Drider D, Spano G, Fiocco D. Bioprospecting antimicrobials from *Lactiplantibacillus plantarum*: Key factors underlying its probiotic action. Int. J. Mol. Sci. 2021; 22(21):12076. DOI: 10.3390/ijms222112076.
6. WHO. Oral health information systems. 2014. Available at: http://www.who.int/oral_health/action/information/surveillance/en/.
7. Zhang F, Li Y, Wang X, Wang S, and Bi D. The impact of *Lactobacillus plantarum* on the gut microbiota of mice with DSS-induced colitis. BioMed. Res. Int. 2019;3921315. DOI: 10.1155/2019/3921315

8. Panda S, Kar N, Ray R, Montet D. Probiotic lactic acid bacteria: applications in food, feed and pharmaceutical industries. Biotechnol. Emerg. Trends. 2008;177–196. Available at: https://agritrop.cirad.fr/549776/.
9. Aritonang S, Roza E, Sandra A. Short Communication: Application of bacteriocin from *Lactobacillus plantarum* SRCM 1 004 34 strain isolated from okara as a natural preservative in beef sausage. Biodiversitas. 2020; 21(5): 2240-2245. DOI: 10.13057/biodiv/d210553.
10. Timothy B, Iliyasu AH, Anvikar AR. Bacteriocins of lactic acid bacteria and their industrial application. Curr. Top. Lact. Acid Bact. Probiotics. 2021; 7(1):1-13. DOI: 10.35732/ctlabp.2021.7.1.1.
11. De Giani A, Bovio F, Forcella M, Fusi P, Sello G, Di Gennaro P. Identification of a bacteriocin-like compound from *Lactobacillus plantarum* with antimicrobial activity and effects on normal and cancerogenic human intestinal cells. AMB Expr. 2019;9:88 DOI: 10.1186/s13568-019-0813-6.
12. Dong Q, Lu X, Gao B, Liu Y, Aslam MZ, Wang X, Li Z. *Lactiplantibacillus plantarum* subsp. *plantarum* and Fructooligosaccharides combination inhibits the growth, adhesion, invasion, and virulence of Listeria monocytogenes. Foods. 2022;11:170. DOI: 10.3390/foods11020170.
13. Soltan Dallal M, Davoodabadi A, Abdi M, Hajiabdolbaghi M, Sharifi Yazdi M, Douraghi M, Tabatabaei Bafghi S. Inhibitory effect of *Lactobacillus plantarum* and Lb. fermentum isolated from the faeces of healthy infants against nonfermentative bacteria causing nosocomial infections. New Microbe New Infect. 2017; 15: 9–13. DOI: 10.1016/j.nmni.2016.09.003.
14. Harrigan W, McCance M. Laboratory Methods in Food and Dairy Microbiology. Academic Press Inc. Limited, London. 1976. Available at: https://agris.fao.org/agris-search/search.do?recordID=US201300540912.
15. Fritsche T, Swoboda S, Olson J, Moore F, Meece J, Novicki T. Evaluation of the sensititre aris2x and vitek 2 automated systems for identification of bacterial pathogens recovered from veterinary specimens. Marshfield labs. 2011; University of Wisconsin, LA CROSSE. Available at: http://www.trekds.com/techinfo/posters_abstracts/files/3229posterF8.pdf.
16. Sneath P, Mair S, Sharpe E, Holt G. Bergey's Manual of Systematic Bacteriology. Baltimore: in Kleins and Wilkins. 2009.
17. Benavides A, Ulcuango M, Yépez L, Tenea G. Assessment of the in vitro bioactive properties of lactic acid bacteria isolated from native ecological niches of Ecuador. Rev. Argent. Microbiol. 2016; 48(3): 236–244. DOI: 10.1016/j.ram.2016.05.003.
18. Hassan AF, Muhsin SN. The protective effect of *Lactobacillus* against Ciprofloxacin and Levofloxacin associated diarrhea in sample of Iraqi patients. Iraqi J. Pharm. Sci. 2019; 28 (2):174-179. DOI: 10.31351/vol28iss2pp174-179.
19. Al-Qayim A, Abass D. Effects of probiotics (*Lactobacillus acidophilus*) on liver functions in experimental colitis in rats. Iraqi J. Vet. Med. 2014; 38(2):48-54. Available at: https://www.iasj.net/iasj/download/4f9cc22edf4d8345.
20. 20. Abd A, Ali T. Efficacy of bacteriocin extracted from *Lactobacillus acidophilus* (LAK) against *Bacillus cereus* in cow raw milk. Iraqi J. Vet. Med. 2015; 39(2):91-97. DOI: 10.30539/iraqijvm.v39i2.184.
21. Najim N, Daher A. The synergistic bactericidal effects of bacteriocin and pressurization against *E. coli* O157:H7 in raw milk. Iraqi J. Vet. Med. 2013; 38(1): 15 -23. DOI: 10.30539/iraqijvm.v38i1.249.
22. Khudhir Z. The synergistic effects of *Lactobacillus acidophillus* ROO52 and *Lactobacillus bulgaricus* LB-12 bacteriocins against E.coli O157:H7 in milk. Iraqi J. Vet. Med. 2014; 38(2):35-40. DOI: 10.30539/iraqijvm.v38i2.220
23. Mannan J, Rezwan R, Rahman S, Begum K. Isolation and biochemical characterization of *Lactobacillus* species from Yogurt and Cheese samples in Dhaka Metropolitan Area. Bangladesh Pharm. J. 2017; 20(1): 27-33. DOI: 10.3329/bpj.v20i1.32090.
24. Ołdak A, Zielin´ska D, Rzepkowska A, Kołozyn- Krajewska D. Comparison of antibacterial activity of *Lactobacillus plantarum* strains isolated from two different kinds of regional cheeses from Poland: oscypek and korycinski cheese. BioMed. Res. Int. 2017; Article ID 6820369, 10. DOI: 10.1155/2017/6820369.
25. Jia F, Zhang L, Pang X. Complete genome sequence of bacteriocin-producing *Lactobacillus plantarum* KLDS1. 0391, a probiotic strain with gastrointestinal tract resistance and adhesion to the intestinal epithelial cells. Genomics. 2017;109(5-6):432–437. DOI: 10.1016/j.ygeno.2017.06.008E.
26. Abushelaibi A, Al-Mahadin S, El-Tarabily K, Shah N, Ayyash M. Characterization of potential probiotic lactic acid bacteria isolated from camel milk. J. Food Sci. Technol. 2017; 79: 316–325. DOI: 10.1016/j.lwt.2017.01.041.
27. Pisano M, Viale S, Conti S. Preliminary evaluation of probiotic properties of *Lactobacillus* strains isolated from Sardinian dairy products. BioMed. Res. Int. 2014; Article ID 286390, 9. DOI: 10.1155/2014/286390.
28. Hulak N, Maksimovic Z, Kaic A, Skelin A, Fuka M. Indigenous strains of *Lactobacillus* isolated from the Istrian cheese as potential starter cultures. Mljekarstvo. 2016;66(4):282–292. DOI: 10.15567/mljekarstvo.2016.0404.
29. Woraprayote W, Malila Y, Sorapukdee S, Swetwiwathana A, Benjakul S, Visessanguan W. Bacteriocin from lactic acid bacteria and their applications in meat and meat products. Meat Sci. 2016. DOI: 10.1016/j.meatsci.2016.04.004.
30. Jandaik S, Sharma M, Kumar Singh R. Antimicrobial activity of bacteriocin produced by lactic acid bacteria isolated from milk products. J. Pure Appl. Microbiol. 2013;7(1): 603-608. Available at: http://www.amb-express.com/content/2/1/48.
31. Chaalel A, Riazi A, Dubois-Dauphin R, Thonart P. Screening of plantaricin EF and JK in an Algerian *Lactobacillus plantarum* isolate. Asian Pac. J. Trop. Dis. 2015; 5:474–482. DOI: 10.1016/S2222-1808(15)60819-2.
32. Jameel AA, Haider NH. Study the antimicrobial and antiadhesive activity of purified biosurfactant produced from *Lactobacillus plantarum* against pathogenic bacteria. Iraqi J. Agric. Sci. 2021; 52(5):1194-1206. DOI: 10.36103/ijas.v52i5.1457.
33. Diop M, Dubois-Dauphin BR, Tine E. Bacteriocin producers from traditional food products. Biotechnol. Agron. Soc. Environ. 2007; 11(4): 275-281. Available at: https://popups.uliege.be/1780-4507/index.php?id=17301&file=1&pid=1636.
34. Tsai C, Lin P, Hsieh M. Three Lactobacillus strains from healthy infant stool inhibit enterotoxigenic *Escherichia coli* grown in vitro. Anaerobe. 2008; 14:61-7. DOI: 10.1016/j.anaerobe.2007.11.003.
35. Jara S, Sanchez M, Vera R, Cofre J, Castro E. The inhibitory activity of *Lactobacillus* spp. isolated from breast milk on gastrointestinal pathogenic bacteria of nosocomial origin. Anaerobe. 2011; 17:474-7. DOI: 10.1016/j.anaerobe.2011.07.008.
36. Azizi F, Habibi M, Edalatian R. The biodiversity of *Lactobacillus* spp. from Iranian raw milk Motal cheese and antibacterial evaluation based on bacteriocin-encoding genes. AMB Expr. 2017; 7:176. DOI: 10.1186/s13568-017-0474-2.
37. Ouwehand A, Vesterlund S. Antimicrobial components from lactic acid bacteria. Lactic Acid Bacteria Microbiological and Functional Aspects. New York: Marcel Dekker Inc. 2004. Available at: https://www.routledge.com/Lactic-Acid-Bacteria-Microbiological-and-Functional-Aspects/Vinderola-Ouwehand-Salminen-Wright/p/book/9780815366485.
38. Mohsin ZA, Ali WS. Antagonistic activity of bacteriocin-producing Lactobacillus against Candida spp. Iraqi J. Sci. 2021; 62 (7): 2153-2162. DOI: 10.24996/ijs.2021.62.7.4.
39. Sankar R, Priyanka D, Reddy S, Rajanikanth P, Kumar K, Indira M. Purification and characterization of bacteriocin produced by *Lactobacillus plantarum* isolated from cow milk. Int. J. Microbiol. Res. 2012; 3:133–137. DOI: 10.5829/idosi.ijmr.2012.3.2.62182.
40. Hernández D, Cardell E, Zarate V. Antimicrobial activity of lactic acid bacteria isolated from Tenerife cheese: Initial characterization of plantaricin TF711, a bacteriocin-like substance produced by *Lactobacillus plantarum* TF711. J. Appl. Microbiol. 2005; 99: 77–84. DOI: 10.1111/j.1365-2672.2005.02576.x.

41. Milioni C, Martínez B, Degl'Innocenti S, Turchi B, Fratini F, Cerri D, Fischetti R. A novel bacteriocin produced by *Lactobacillus plantarum* LpU4 as a valuable candidate for biopreservation in artisanal raw milk cheese. Dairy Sci. Technol. 2015; 95: 479–494. DOI: 10.1007/s13594-015-0230-9.
42. Barbosa M, Todorov S, Ivanova, Belguesmia Y, Choiset Y, Rabesona H, Chobert J, Haertlé T, Franco B. Characterization of a two-peptide plantaricin produced by *Lactobacillus plantarum* MBSa4 isolated from Brazilian salami. Food Control. 2016; 60: 103–112. DOI: 10.1016/j.foodcont.2015.07.029
43. Arsi K, Donoghue M, Woo-Ming A, Blore P, Donoghue D. The efficacy of selected probiotic and prebiotic combinations in reducing *Campylobacter* colonization in broiler chickens. J. Appl. Poult. Res. 2015; 24(3): 327–334. DOI: 10.33 82/japr/pfv032.

الفعالية المضادة للجراثيم لجرثومة *Lactiplantibacillus plantarum* من منتجات الالبان ضد بعض الجراثيم المنقولة عن طريق الغذاء

دعاء عادل قاسم[١]، إنعام جاسم لفته[٢]،أولينكا أيولا[٣]

[١]مركزبحوث السوق وحماية المستهلك، جامعة بغداد، بغداد، العراق، [٢]فرع الاحياء المجهرية، كلية الطب البيطري، جامعة بغداد، بغداد، العراق، [٣]قسم علم الحيوان، وحدة بيولوجيا الخلية وعلم الوراثة، كلية علوم الحياة، جامعة ايلورين، ايلورين، نيجيريا

الخلاصة

تعد جراثيم حامض اللبن مثل *Lactiplantibacillus plantarum* من أهم الانواع المتواجدة في الأغذية، ومعظم هذه الأنواع تكون ذات تأثير ايجابي ومفيد للإنسان والحيوان باعتبارها من المعززات الحيوية. تنتج هذه الكائنات الحية المعزولة من الحليب مركبات مضادة للميكروبات واسعة الطيف تسمى بالبكتريوسينات. لذلك هدفت الدراسة الحالية إلى عزل وتقييم فاعلية جراثيم *L. plantarum* في تثبيط بعض أنواع الجراثيم التي تنتقل عن طريق الأغذية. اشتملت هذه الدراسة على فحص ٣٤ عينة من منتجات الألبان المحلية والمستوردة، مثل الحليب، الجبن واللبن، إذ جمعت هذه العينات عشوائيا" ومن مصادر متعددة ومن مختلف المحلات التجارية الصغيرة والكبيرة في مدينة بغداد، العراق، خلال شهر ايار لسنة ٢٠٢٢. أستعمل الوسط الخاص deMan, Rogosa, and Sharpe medium (MRS) في عزل هذه الجراثيم، وحضنت الأطباق في ظروف لاهوائية لمدة ٤٨-٧٢ ساعه بدرجة حرارة ٣٧ °م. فحصت العينات المشكوك فيها عن طريق الفحوصات الجرثومية الروتينية، بعدها جرى التأكد من العزلات باستعمال جهاز Vitek2 و متبوعا" بالتشخيص الجزيئي اعتمادا" على جين *16S rRNA* . كذلك جرى عزل والتعرف على أنواع أخرى من الجراثيم الهوائية الملوثة لمنتجات الالبان عن طريق استعمال الأوساط الزرعية الخاصة بكل منها واستخدام الفحوصات المجهرية والاختبارات الكيموحيوية، وجرى التأكد من العزلات باستخدام نظام Vitek2. أظهرت نتائج العزل الحصول على ٢ من جراثيم *L. plantarum*، 3 عزلات من الزوائف الزنجارية، 4 عزلات من الاشريكية القولونية، وعزلة واحدة من كل من العصوية الرقيقة والجراثيم العنقودية البشرية. استخدمت طريقة الانتشار بالحفر لغرض الكشف عن الفعالية التثبيطية لراشح *L. plantarum* ضد الجراثيم الاخرى المعزولة، وتبين تأثيرها الفعال في تثبيط نمو العزلات المدروسة. اظهرت النتائج تثبيطًا قويًا لنمو الاشريكية القولونية و العصوية الرقيقة مع منطقة تثبيط قطرها ٣٦ و ٣٨ ملم على التوالي، اما الزائفة الزنجارية والجراثيم العنقودية البشرية فكانت منطقة التثبيط بقطر ٢٧ و ٢٩ ملم على التوالي. وهذا يشير إلى امتلاك *L. plantarum* نشاط واسع الطيف ضد الجراثيم المنقولة عن طريق الأغذية. نستنتج من هذا، يمكن أن تحتوي منتجات الألبان المصنوعة محليًا على جراثيم ملوثة مختلفة، والتي يمكن القضاء عليها باستخدام المعززات الحيوية المنتجة من *L. plantarum* لتجنب ظهور الأمراض المنقولة عن طريق الغذاء.

الكلمات المفتاحية: منتجات الألبان، لاكتيبلانتيباسيلس بلانتاروم، البكتيريا المسببة للأمراض المنقولة عن طريق الأغذية، النشاط المضاد للميكروبات