

THE POTENTIAL PROTECTIVE ROLE OF PROBIOTICS (*Lactobacillus plantarum*) IN INHIBITING SOME MULTIDRUG-RESISTANT PATHOGENIC BACTERIA

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Received 28/ 10/ 2024, Accepted 26/ 12/ 2024, Published 30/ 9/ 2026

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ABSTRACT

Local dairy products were used to isolate *Lactobacillus plantarum*, which were then examined for their antibacterial and anti-biofilm properties against antibiotic-resistant clinical isolates of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Also, the qualitative chemical screening of lactobacillus extract was done to assess the presence of phenols, and flavonoid components. The results showed a high antibacterial activity of *L. plantarum* by inhibiting the tested antibiotic-resistant bacterial isolates. The results also showed that the virulence factor of biofilms could be effectively inhibited. In addition, the total phenolic and flavonoid content of the crude Lactobacillus extract was measured. The results showed that the crude Lactobacillus extract contained 98.5 mg rutin/mg flavonoids and 232.5 mg total phenolics., *L. plantarum* can be used as a potent probiotic to treat pathogenic bacteria such as *S. aureus* and *P. aeruginosa* that produce biofilms, which supports the concept of treating antibiotic-resistant pathogenic bacteria with probiotics instead of antibiotics.

Keywords: Probiotics, *Lactobacillus plantarum*; *Pseudomonas aeruginosa*; *Staphylococcus aureus*; Multidrug-resistant bacteria

الدور الوقائي المحتمل للبروبيوتيك (*Lactobacillus Planetarium*) في تثبيط بعض أنواع البكتيريا الممرضة المقاومة للأدوية المتعددة

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الخلاصة

تم استخدام منتجات الألبان المحلية لعزل *Lactobacillus plantarum* ، والتي تم فحصها بعد ذلك لمعرفة خصائصها المضادة للبكتيريا والمضادة للأغشية الحيوية ضد العزلات السريرية المقاومة للمضادات الحيوية من *Staphylococcus aureus* و *Pseudomonas aeruginosa*. كما تم إجراء الفحص الكيميائي النوعي للمستخلص البكتيري لتقييم وجود الفينولات ومكونات الفلافونويد. أظهرت النتائج نشاطاً مضاداً للبكتيريا عالياً لبكتيريا *Lactobacillus plantarum* عن طريق تثبيط العزلات البكتيرية المقاومة للمضادات الحيوية المختبرة. أظهرت النتائج أيضاً أنه يمكن تثبيط عامل ضراوة الأغشية الحيوية بشكل فعال. بالإضافة إلى ذلك، تم قياس إجمالي محتوى الفينول والفلافونويد في مستخلص *Lactobacillus* الخام. أظهرت النتائج أن مستخلص *Lactobacillus* الخام يحتوي على 98.5 ملغم روتين/ ملغم فلافونويد و 232.5 ملغم إجمالي فينول. يمكن استخدام *Lactobacillus plantarum* مغمزراً حيوياً قوياً لعلاج البكتيريا الممرضة مثل *S. aureus* و *P. aeruginosa* التي تنتج الأغشية الحيوية، مما يدعم مفهوم علاج البكتيريا الممرضة المقاومة للمضادات الحيوية بالبروبيوتيك بدلاً من المضادات الحيوية.

الكلمات المفتاحية: المعززات الحيوية، بكتريا اللاكتوباسيلس بلانتاريوم، الزائفة الزنجارية، المكونة العنقودية الذهبية، البكتيريا المقاومة للأدوية المتعددة.



INTRODUCTION

Antimicrobial agent resistance has become alarming in recent years and represents a serious threat to public health worldwide. Multidrug-resistant (MDR) bacteria have been linked to an increase in morbidity and mortality from microbial infections, and superbug bacteria resistant to nearly all antibiotics have made matters worse. This is generally linked to the overuse of antibiotics, which in turn promotes the emergence and development of strains with diverse genotypes and phenotypes (Al-Hayanni & El-Shora 2021; Al-Lami *et al.*, 2022). The prevalence of (MDR) strains has resulted in significant challenges in the management of infections. The ability of some bacterial strains to form biofilms, which is linked to 65–80% of human infections, is contributing to the worldwide rise in multidrug-resistant bacterial infections as a severe concern. This highlights the urgent need to look for alternatives.

Probiotics were first conceptualized by Ukrainian bacteriologist Elie Metchnikoff, who observed that microorganisms present in fermented milk engaged in competition with illnesses. A probiotic, as defined by (Bahaddad *et al.* 2023), is a live microbial food supplement that improves intestinal microbiota balance and positively affects the animal host. Probiotics, as defined by the World Health Organization, are living microbes that confer health advantages on animal hosts when consumed sufficiently. According to certain reports, probiotics can effectively treat a variety of conditions, including gastrointestinal disorders, urogenital infections, HIV, obesity, autoimmune diseases, cancer, asthma, lung diseases, allergies, diabetes, and *Helicobacter pylori* infections (Manzoor *et al.*, 2022). One of the many different lactic acid bacteria (LAB) species that are widely used in food operations is *L. plantarum*. It has been observed that some *L. plantarum* strains produce a variety of antibacterial chemicals. Additional examples of diverse activities include bacteriocins, hydrogen peroxide, diacetyl, and organic acids (Muhammad *et al.*, 2019). *S. aureus*, a highly recognized and prevalent bacterial pathogen, is responsible for a significant number of severe invasive infections globally each year, in addition to numerous minor skin infections (Rasigade *et al.*, 2014). *S. aureus* generates a remarkable variety of virulence factors that enable it to endure harsh environmental conditions in humans, facilitate tissue colonization and damage, and ultimately lead to potentially fatal systemic infections. Among them is a wide range of protein and non-protein components, as well as toxins and immune evasion mechanisms, which facilitate host colonization during infection (Cheung *et al.*, 2021).

The pathogenic bacteria *P. aeruginosa* are opportunistic and can infect humans, plants, animals, and immunocompromised patients. They are toxic and can develop antibiotic resistance quickly. These bacteria also have different virulence factors, such as hemolysin, and pyocyanin, and the ability to form biofilms (Jeong *et al.*, 2023). Therefore, the current study aimed to determine the effectiveness of the lactic acid bacterium *L. planetarum* extract against two isolates of multidrug-resistant and biofilm-producing pathogenic bacteria Gram-positive *S. aureus*, and Gram-negative *P. aeruginosa*.



MATERIALS AND METHODS

Isolation and Identification of Bacterial Isolates

A total of one hundred and fifty samples from burn injuries and wounds were collected between September 2023 and February 2024 from male and female Iraqi patients of different ages admitted to hospitals in Baghdad (Medical City Teaching Hospitals, Al-Karkh General Hospital, Al-Kindy Teaching Hospital, and Al-Nu'man Teaching Hospital). All samples were cultured on MacConkey agar, Blood agar, Cetrimide agar, and Mannitol salt agar and then incubated for 24 hrs. at 37°C. In addition, four samples, including yogurt and Arab cheese, were taken from the local supermarkets. After culturing on suitable selective media and incubation for 24 to 48 hours at 37 °C under anaerobic conditions, on rice medium *Lactobacillus plantarum* was cultivated. The rice medium were shaken at 120 rpm for three days while being incubated at 35 °C for seven days. The broth medium were centrifuged for 10 minutes at 10,000 rpm following incubation. Sterilized 0.22 µm pore-size filters were used to filter the cell-free supernatant at 4 °C. After giving the cell-free supernatant and rice media a good shake for a few minutes, ethyl acetate was added at a ratio of 1:1 (v/v). In order to separate the organic and aqueous phases, the ethyl acetate mixtures were put into separating funnels and let to stand. To produce ethyl secondary metabolite extract, the organic phase was obtained, dried, and evaporated using a rotary evaporator (Nisa *et al.*, 2019; Rajaofera *et al.*, 2019). All necessary tests for preliminary diagnosis of the bacterial isolates were carried out (Gram stain, Shape, Capsule, Spore, Motility, Hemolysis, Catalase, Coagulase, Indole, Oxidase, and Urease) according to (Holt *et al.* 1994). Finally, the isolates were subjected to confirmatory diagnosis using the Vitek-2 compact system.

Antibiotics Susceptibility Test (AST)

An antibiotic susceptibility test for various classes of antibiotics was carried out on *P. aeruginosa*, and *S. aureus* isolates by the Kirby-Bauer method (the disc diffusion method) according to Bauer (1966), and recommendations of the CLSI (2023), using commonly prescribed antibiotics, namely: Amikacin, Ciprofloxacin, Piperacillin/ Tazobactam, Aztreonam, Gentamicin, Imipenem, Levofloxacin, Cefepime, Ceftazidime, and Colistin for *P. aeruginosa*; and Penicillin G, Tetracycline, Nitrofurantoin, Gentamicin, Cefoxitin, Ciprofloxacin, Levofloxacin, Azithromycin, Chloramphenicol, and Rifampicin for *S. aureus*.

Detection of Biofilm Formation

The biofilm formation assay was done according to (Shinde *et al.* 2021). The biofilm formation ability of MDR bacterial isolates (*S. aureus* and *P. aeruginosa*) was established using a 96-well microtiter plate experiment that used the crystal violet staining technique.

Determination of Minimum Inhibitory Concentration (MIC) of *Lactobacillus plantarum* Extract Against Bacterial Isolates

The MICs refer to the smallest amounts of an antibiotic treatment that effectively prevent the visible growth of a bacterium after an incubation period of one night. MIC can be accurately determined by evaluating an antimicrobial agent's capacity to visibly limit bacterial growth. This technique is commonly employed in microbiological research. The experiment



was conducted according to (Elshikh *et al.* 2016) by 96-well microtiter plate assay using the resazurin-assisted microdilution technique in Mueller-Hinton broth (MHB) using serial dilutions of *L. plantarum* extract with concentrations of (250, 125, 62.5, 31.25, 15.625, 7.8125, 3.90625, and 1.953125) mg/mL. These concentrations were used to determine their effectiveness against bacteria.

Anti-biofilm Activity of *Lactobacillus plantarum*

The *Lactobacillus* extract's anti-biofilm activity was assessed according to (Shinde *et al.* 2021), with some modifications as follows: In microtiter plates with 180 µL of Mueller-Hinton broth supplemented with 1% glucose, 20 µL of suspended bacteria and 10 µL of *Lactobacillus* extract at MIC concentration were added as inoculants. Microplates were incubated at 37°C for a 24hr. The adhering cells were cleaned with D.W., the liquid medium was disposed of, and the wells were dried for no more than an hour at 37°C. Subsequently, it was dyed for 15 minutes with 200 µL of 1% crystal violet. Subsequently, D.W. rinsed the microplate wells stained with crystal violet to remove the stain. Following the process of air-drying the microplate wells, 200 µL of 95% ethanol is used to re-solubilize the biofilm dye that was lining the walls of the microplate. An ELISA reader was used to measure the optical density (OD) at 580 nm after five to ten minutes. The following formula was utilized to determine the biofilm inhibition percentage:

$$\text{Biofilm reduction (\%)} = \left(\frac{OD_{\text{control}}}{OD_{\text{sample}}} \right) \times 100 \%$$

Determination of Total Phenolic and Total Flavonoid Contents

According to (Saidulu *et al.* 2014). The quantification of phenolic compounds in the *Lactobacillus* extract was conducted using a standard Folin-Ciocalteu reagent. The total concentration of phenolic components was expressed in mg of gallic acid equivalent (GAE) per gram of dry weight. The total flavonoid concentration of the crude extract was determined using the aluminum chloride colorimetric method (Kuthi & Basar, 2019). The result was quantified as mg of Rutin equivalent per gram of dry weight.

Statistical Analysis

R is a programming language for statistical computing and data visualization. It has been adopted in the fields of data mining, bioinformatics, and data analysis. The R statistical programming package was used to analyze probability under the hypothesis of no difference or no effect in p-value for antibiotics (Venables & Smith, 2023).

RESULTS

Isolation and Identification of Bacterial Isolates

There were 63 (42%) pure isolates belonging to various bacterial species. The 63 pure isolates were subjected to confirmatory diagnosis using the Vitek-2 compact system. Then, antibiotic sensitivity and biofilm production tests were performed for these pure isolates.



Among them, 30 isolates were found to be multidrug-resistant and capable of producing biofilms. The strongest biofilm-producing isolates and the most antibiotic-resistant ones were selected for this study. Two isolates were chosen from Gram-positive (*Staphylococcus aureus*) and two Gram-negative (*Pseudomonas aeruginosa*).

Table (1): The results of biofilm formation for isolates under study.

Biofilm production	<i>S. aureus</i> isolates No. (%)	<i>P. aeruginosa</i> isolates No. (%)	Percentage (%)
Strong	10 (83.33)	14 (77.77)	80.0
Moderate	2 (16.66)	3 (16.66)	16.7
Weak	0 (0)	1 (5.55)	3.3
Total	12	18	30 (100)

Antimicrobial Susceptibility Test

Two isolates of *S. aureus* (S1 and S10) and two isolates of *P. aeruginosa* (P5 and P7) were selected as multidrug-resistant bacteria. The results showed that both of these isolates were resistant to used antimicrobial agents, chosen according to CLSI (2023) as shown in Table (2).

Table (2): Antibiotic Susceptibility profile of two *Staphylococcus aureus* and two *Pseudomonas aeruginosa* isolates.

Antibiotics group	Antibiotics	<i>Pseudomonas aeruginosa</i>		Antibiotics group	Antibiotics	<i>Staphylococcus aureus</i>	
		P5	P7			S1	S10
β -lactam combination agents	Piperacillin /Tazobactam	R	R	Penicillinase	Cefoxitin	R	R
Cephems	Cefepime	R	R		Oxacillin	R	R
	Ceftazidime	R	R		Penicillin G	R	R
carbapenems	Imipenem	R	R	Nitrofurantoin	Nitrofurantoin	S	S
Aminoglycoside	Amikacin	R	R	Lincosamides	Clindamycin	S	S
	Gentamicin	R	R	Aminoglycoside	Gentamicin	R	R
monobactams	Aztreonam	R	R	Macrolides	Azithromycin	R	R
fluoroquinolones	Ciprofloxacin	R	R	Fluoroquinolones	Ciprofloxacin	R	R
	Levofloxacin	R	R		Levofloxacin	R	R
lipopeptides	Colistin	S	S	Tetracycline	Tetracycline	I	S
	Nalidixic acid	R	R	Ansamycins	Rifampicin	R	R
				Phenicol	Chloramphenicol	S	S

S = Sensitive , I = Intermediate , R = Resistant

Determination of Minimum Inhibitory Concentration (MIC)

The experiment was conducted using serial dilutions of *L. plantarum* extract with concentrations of (250, 125, 62.5, 31.25, 15.625, 7.8125, 3.90625, and 1.953125) mg/mL. The results showed that the *L. plantarum* extract has antibacterial activity against chosen isolates of

MDR bacteria compared with untreated control as shown in Table (2) and Figure (1). Whereas, the MIC of *L. plantarum* extract against *S. aureus* was (7.8125mg/mL) and the Sub-MIC was (3.90625mg/mL), while the MIC and Sub-MIC were 15.625 mg/mL, and 7.8125 mg/mL respectively against *P. aeruginosa*.

Table (3): The MIC and sub-MIC results for *Lactobacillus plantarum* extract against MDR *Staphylococcus aureus* and *Pseudomonas aeruginosa* isolates.

Bacterial isolate	Concentrations of <i>Lactobacillus plantarum</i> extract (mg /ml)	
	MIC	Sub-MIC
S 1	7.8125	3.90625
S10	7.8125	3.90625
P 5	15.625	7.8125
P7	15.625	7.8125

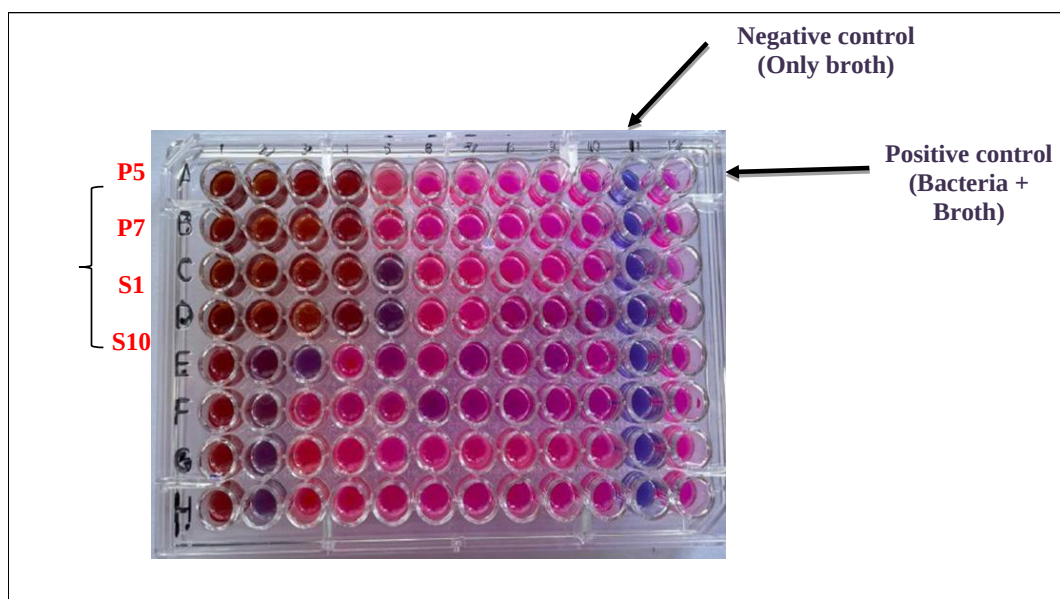


Figure (1): Determination of MIC and sub-MIC for *Lactobacillus plantarum* extract against MDR *S. aureus* (S1 and S10) and *P. aeruginosa* (P5 and P7) isolates.

Anti-biofilm Activity of *Lactobacillus plantarum*

It was important to investigate *L. plantarum* inhibitory activity on the capacity to produce biofilms since biofilm is the most potent virulence factor in pathogenic bacteria isolated from clinical sources. The inhibitory effect of *L. plantarum* was tested against chosen biofilm-producing bacterial isolates by microtiter plate assay using the Sub-MIC concentration of the antibacterial effect study of *L. plantarum* extract which was (3.90625 mg/mL) against *S.*



aureus and was (7.8125 mg/mL) against *P. aeruginosa*. Comparing the treated bacterial isolates to the control sample (untreated bacteria with quantities of extract), the results demonstrated inhibitory efficacy against the biofilm virulence factor as shown in Table (3).

Table (4): Anti-biofilm activity of *Lactobacillus plantarum* extract against the bacterial isolates.

Bacterial isolates	Mean $\pm$ SE of biofilm formation inhibition		P-value
	Control	<i>L. plantarum</i> extract	
<i>S. aureus</i> 1	1.432 $\pm$ 0.393	0.188 $\pm$ 0.075	3.485e-10***
<i>S. aureus</i> 10	0.327 $\pm$ 0.029	0.158 $\pm$ 0.019	0.856
<i>P. aeruginosa</i> 5	0.412 $\pm$ 0.047	0.167 $\pm$ 0.010	0.410
<i>P. aeruginosa</i> 7	0.346 $\pm$ 0.123	0.168 $\pm$ 0.013	0.809
*** (P $\leq$ 0.001).			

Determination of Total Phenolic and Total Flavonoid Contents

The crude *Lactobacillus* extract's total phenolic component content was ascertained using a standard Folin-Ciocalteu reagent. Gallic acid equivalents per gram of dry weight were used to express it. The aluminum chloride colorimetric method was used to determine the crude extract's total flavonoid concentration. Rutin equivalents per gram of the dry weight of the crude extract were used to express it. The results revealed that the total content of phenols was 232.5 mg Gallic /gm. and flavonoids for the crude *Lactobacillus* extract was 98.5 mg Rutin/mg.

DISCUSSION

Antimicrobial Susceptibility Test

The overuse of antibiotics has led to a rise in resistance to several antibiotics, which has in turn increased the prevalence of MDR strains of the bacteria. MDR bacteria exhibit resistance to three distinct categories of antimicrobials, posing challenges in identifying the most effective antimicrobial treatment (Yayan *et al.*, 2015). The study results agree with the report of the World Health Organization (WHO), which declared that the most dangerous bacteria, including *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Staphylococcus aureus*, *Klebsiella pneumonia*, and *Enterobacter species*, are immune to the effects of antibacterial medications (Gulick, 2017). Since *S. aureus* was resistant to the antibiotics Cefoxitin and Oxacillin, it is considered a methicillin-resistant bacteria (MRSA), as mentioned in the study by (Khalil & Al-Hayanni, 2024). Gram-positive bacteria can acquire antibiotic resistance through two primary mechanisms: enzymatically breaking down antibiotics by producing β -lactamases, or reducing the antibiotics' effectiveness at their target site, the penicillin-binding protein (PBP), by acquiring external DNA or modifying the native PBP genes (Munita *et al.*, 2015). Gram-negative bacterial (GNB) pathogens comprise most of the WHO list. Due to their unique structure, compared to Gram-positive bacteria, GNBs are more resistant, and they are a major global source of sickness and mortality. Gram-negative bacteria is resistant to antibiotics because it makes enzymes and other things that stop antibiotics from working. This can happen by moving around genetic elements that carry resistance genes, like



plasmids that code for β -lactamases. It can also happen when genetic genes change and make the organism more resistant. For example, antibiotic-inactivating enzymes, efflux pumps, permeability, or changes in the target can become more active (Breijyeh *et al.*, 2020).

Biofilm Formation of Bacterial Isolates

Biofilm formation has been described as an important virulence factor in various pathogenic bacteria. It helps them stick to hard surfaces, causing infections and nosocomial infections. It resists unsuitable conditions such as encounters with antibiotics and the host's immune response while providing suitable conditions for slow growth (Alaamery & Al-Hayanni, 2022).

Microtiter plate assay results in numerous investigations demonstrated that all *S. aureus* isolates were capable of forming a biofilm, with 38.7% of them being able to do so strongly. According to several studies, the presence of ica ABCD genes is linked to the ability of *S. aureus* isolates to build biofilm. Also, biofilm formation was seen in *P. aeruginosa* isolates, Nouraldin's study (2016) found that 47.9% produced strong biofilm. Moreover, 27 out of 100 *P. aeruginosa* isolates in the (Abdelraheem *et al.* study 2020) produced positive biofilms, representing 27% of the total. According to certain research, *P. aeruginosa* develops greater antibiotic resistance when biofilms. Biofilms indirectly contribute to bacterial antibiotic resistance by improving the anatomical region's pharmacokinetic properties, or the drugs' capacity to infiltrate and reach microorganisms in sufficient quantities (Verderosa *et al.*, 2019).

Determination of Minimum Inhibitory Concentration (MIC)

The results showed highly significant antibacterial activity of *L. plantarum* by inhibiting tested MDR bacterial isolates. Probiotics' competitive characteristics rely on their capacity to manufacture and release antimicrobial agents, contend with pathogens for surface adhesion sites, and fight for scarce resources. Therefore, antibacterial and anti-adhesive qualities may be linked to *L. plantarum*'s antagonistic impact. The bacterium's ability to make lactic acid, lower pH, and produce antimicrobial substances such as hydrogen peroxide, bacteriocins, ethanol, antibiotics, and other chemicals may be the cause of this action, one of lactobacilli's main antibacterial strategies is the production of organic acids like lactic and acetic acids (Carvalho *et al.*, 2021). It has been demonstrated that the use of organic acids to lower pH can successfully suppress microbial growth and kill Gram-negative bacteria, such as *Escherichia coli*, by inducing disruption to cell membranes and the subsequent release of intracellular substances. High amounts of lactic and acetic acid can be produced by Lactobacilli, depending on their fermentative routes and growth circumstances (Kaur *et al.*, 2018).

The co-incubation of three *P. aeruginosa* strains and *L. plantarum* in an MRS medium revealed an inhibitory action of *L. plantarum* against *P. aeruginosa*, according to the findings of (Layus *et al.* 2020). Furthermore, bacteriocin derived from *L. plantarum* exhibits inhibitory activity against a wide range of harmful microorganisms. Furthermore, bacteriocin can influence yeast and inhibit strains of Lactobacillus that are closely related (Lei *et al.*, 2020). The mechanism of Lactobacilli's antibacterial action can be linked to the strain and kind of biosurfactants produced by the bacteria, as well as to their synthesis of biosurfactants. The



cell-free extract (CFE) of the *L. plantarum* strain was demonstrated to suppress the growth of all strains of pathogens examined, including *S. aureus* and *P. aeruginosa*, by the study established by (Onbas *et al.* 2019).

Three *L. plantarum* strains (P1, S11, and M7) with potent antimicrobial activity against three pathogenic bacteria (*S. aureus*, *E. coli*, and *Salmonella*) were isolated from traditional dairy products in Xinjiang, and five common organic acids (lactic acid, citric acid, tartaric acid, and malic acid) produced by *Lactobacillus* strains were crucial in inhibiting these pathogenic bacteria, according to a study by (Hu *et al.* 2019), the low-molecular-weight fraction of the *L. plantarum* supernatant inhibited the growth of *E. coli*, *P. aeruginosa*, and *S. aureus*, but not the high-molecular-weight fraction. Conversely, *Streptococcus* growth was three times reduced by the high-molecular-weight fraction. Additionally, it was shown that the methicillin-resistant *S. aureus* and *P. aeruginosa* growth was inhibited by the *L. plantarum* CRL 759 cell-free supernatant (SLp759) (Layus *et al.*, 2020). The CFS of *Lactobacilli* species, such as *L. plantarum*, showed varying degrees of antibacterial activity against all investigated strains of *P. aeruginosa* and *Klebsiella pneumoniae* that produced extended-spectrum β -lactamase (El-Mokhtar *et al.*, 2020).

Anti-biofilm Activity of *Lactobacillus plantarum*

One virulence component that is crucial to the persistence and resistance of bacteria in infections is biofilm. Biofilm-forming microorganisms are responsible for around 65–80% of illnesses, according to research published by the Centers for Disease Control and the National Institutes of Health (El-Deeb *et al.*, 2018). Probiotics, particularly *Lactobacillus* species, have been shown in numerous medical trials to suppress the production of biofilms, hence interfering with the uropathogenic bacteria responsible for severe illnesses such as CAUTI (Mekky *et al.*, 2022).

Thus, the control of biofilm formation by uropathogenic bacteria using LAB was studied. Mekky *et al.* (2022) showed that *L. plantarum*, when applied to catheters, can suppress biofilm formation on the catheters. This inhibitory activity may be attributed to the removal of the bacterial uropathogenic bacteria's adhesion surface as well as the synthesis of antimicrobial compounds. The ability of an *L. plantarum* isolate to produce robust biofilms and its longer-lasting inhibitory activity against many spoilage and pathogenic bacteria was assessed by Jalilsood *et al.* (2015) The *L. plantarum* biofilms took seven days to form, and up to six days, they dramatically decreased the amount of pathogens when exposed to bacteria. It was discovered that *L. pentosus* and *L. plantarum* metabolites extracted from fermented dairy products had antibiofilm properties against *P. aeruginosa* and *Bacillus cereus* biofilms (Jalilsood *et al.*, 2015). According to (Shokri *et al.* 2018), two strains of *L. fermentum* can either prevent *P. aeruginosa* strains from forming biofilm or eliminate any that has already been produced. Many researchers have concentrated on using *Lactobacillus spp.* as alternative agents in the treatment of biofilm-associated infections since they were shown to have anti-biofilm effects against antibiotic-resistant strains of *S. aureus* and *P. aeruginosa*. Because *L. acidophilus* inhibits the production of biofilms by pathogenic isolates, it may be a viable alternative to battle the pathogenicity of *P. aeruginosa* (Díaz *et al.*, 2020), Comparing CFS to pathogenic strains of *P. aeruginosa* alone, the adhesion capacity was decreased, which in turn



decreased the production of biofilms.

Probiotics have been shown by (Barzegari *et al.*2020) to be beneficial in suppressing quorum-sensing signals, which are essential for the formation of biofilms and the survival of pathogens. This interference with biofilm integrity leads to the eradication of biofilms. Furthermore, *Lactobacilli* isolates showed robust biofilm formation and strong anti-biofilm activity against MDR uropathogenic *E. coli*.

Determination of Total Phenolic and Total Flavonoid Contents

The antimicrobial efficacy of the *Lactobacillus* extract is attributed to the bioactive chemical constituents within this medicinal substance, such as phenols and flavonoids. These compounds can precipitate bacterial cell proteins and deactivate enzymes crucial for amino acid synthesis necessary for cellular proliferation (Al-Obaidi, 2013). Additionally, *Lactobacillus* can create a protective biofilm as a barrier to hinder the adhesion of pathogenic bacteria. The presence of hydroxyl groups (-OH) in phenols enables the formation of hydrogen bonds between these compounds' hydroxyl groups and water molecules within the bacterial cell, constituting 99% of its mass. This interaction disrupts essential cellular functions in the bacterial organism.

Limitations: the study was based on hospitals in Baghdad (Medical City Teaching Hospitals, Al-Karkh General Hospital, Al-Kindy Teaching Hospital, and Al-Nu'man Teaching Hospital) hence, the findings don't represent the whole population.

CONCLUSIONS

The results showed that *L. plantarum* might be used as a potent probiotic to treat pathogenic bacteria like *S. aureus* and *P. aeruginosa* that produce biofilms, hence curing conditions like nosocomial infections. Moreover, *Lactobacillus* species can be effective anti-virulence agents, particularly those that target biofilms. Furthermore, the outcomes demonstrated that naturally occurring *lactobacilli* possess potent probiotic qualities against bacteria resistant to multiple drugs. The antimicrobial effectiveness of the *Lactobacillus* extract is ascribed to the bioactive chemical components present in this therapeutic agent, including flavonoids and phenols.

Authors' Contributions

Nuha Khaldoun and Huda S. A. Al-Hayanni made a direct, intellectual, and substantial contribution to the work and approved it for publication.

Authors' Declaration

Conflicts of Interest: There are no conflicts of interest.

We confirm that all the Tables and Figures in the manuscript are ours.

Funding: none



REFERENCES

1. Abdelraheem, W. M., Abdelkader, A. E., Mohamed, E. S., and Mohammed, M. S. (2020). Detection of biofilm formation and assessment of biofilm genes expression in different *Pseudomonas aeruginosa* clinical isolates. 23, 100646.
2. Al-Hayanni, H. S. (2022). The Antibacterial and anti-biofilm effects of Sumac (*Rhus coriaria* L) fruits extracts against some multidrug-resistant pathogenic bacteria. *Journal of the Faculty of Medicine Baghdad*, 64(3), 183-188.
3. Al-Hayanni, H. S., & El-Shora, H. (2021). Various extracts of some medicinal plants as inhibitors for beta-lactamase activity. *Baghdad Science Journal*, 18(1), 0047-0047.
4. Al-Lami, R. A. H., Al-Hayanni, H.A.S., Shehab, Z.H. (2022). Molecular Investigation of Some Beta-lactamase Genes by PCR and DNA Sequencing Techniques in Clinical *Escherichia coli*. *Iraqi Journal of Science*, 63(10), 4205–4212.
5. Al-Obaidi, O. 2013. Water and alcohol extraction from the plant Sumac and study its effectiveness against bacteria and tumors and to study the possibility of removal of heavy metals from industrial water pollution and natural reagent. *The First Scientific Conference for women – Baghdad*, 2013.
6. Bahaddad, S. A., Almalki, M. H. K., Alghamdi, O. A., Sohrab, S. S., Yasir, M., Azhar, E. I., and Chouayekh, H. (2023). *Bacillus* Species as direct-fed microbial antibiotic alternatives for monogastric production. *Probiotics and Antimicrobial Proteins*, 15(1), 1–16.
7. Barzegari A., Kheyrolahzadeh K., Hosseiniyan Khatibi S.M., Sharifi S., Memar M.Y., Zununi Vahed S. (2020). The battle of probiotics and their derivatives against biofilms. *Infect. Drug Resist.* 13:659–672.
8. Breijyeh, Z., Jubeh, B., & Karaman, R. (2020). Resistance of gram-negative bacteria to current antibacterial agents and approaches to resolve it, 25(6), 1340.
9. Carvalho, F. M., Teixeira-Santos, R., Mergulhao, F. J., & Gomes, L. C. (2020). The use of probiotics to fight biofilms in medical devices: *A systematic review and meta-analysis*, 9(1), 27.
10. Cheung, G. Y., Bae, J. S., & Otto, M. (2021). Pathogenicity and virulence of *Staphylococcus aureus*. *Virulence*, 12(1), 547-569.
11. El-Deeb, B., Al-Talhi, A., Mostafa, N., & Abou-assy, R. (2018). Biological synthesis and structural characterization of selenium nanoparticles and assessment of their antimicrobial properties. *Am. Sci. Res. J. Eng. Technol. Sci*, 45(1), 135-170.
12. El-Mokhtar, M. A., Hassanein, K. M., Ahmed, A. S., Gad, G. F. M., Amin, M. M., & Hassanein, O. F. E. (2020). Antagonistic activities of cell-free supernatants of lactobacilli against extended-spectrum β lactamase producing *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. *Infection and Drug Resistance*, 543–552.



13. Elshikh, M., Ahmed, S., Funston, S., Dunlop, P., McGaw, M., Marchant, R., & Banat, I. M. (2016). Resazurin-based 96-well plate microdilution method for the determination of minimum inhibitory concentration of biosurfactants. *Biotechnology letters*, 38, 1015-1019.
14. Jeong, G. J., Khan, F., Khan, S., Tabassum, N., Mehta, S., & Kim, Y. M. (2023). *Pseudomonas aeruginosa* virulence attenuation by inhibiting siderophore functions. *Applied Microbiology and Biotechnology*, 107(4), 1019-1038.
15. Holt, J.G., Krieg, N.R., Sneath, P.H.A., Stanley, J.T. and William, S.T. .1994. Bergey's Manual of Determinative Bacteriology. Williams and Wilkins, Baltimore.
16. Hu CH, Ren LQ, Zhou Y, Ye BC. (2019) Characterization of antimicrobial activity of three *Lactobacillus plantarum* strains isolated from Chinese traditional dairy food. *Food Sci Nutr*. Apr 29;7(6)
17. J. Yayan, B. Ghebremedhin, and K. Rasche (2015) "Antibiotic resistance of *Pseudomonas aeruginosa* in pneumonia at a single university hospital center in Germany over a 10-year period," *PLoS One*, vol. 10, no. 10, p. e0139836.
18. Jalilsood, T., Baradaran, A., Song, A.AL. et al. (2015) Inhibition of pathogenic and spoilage bacteria by a novel biofilm-forming *Lactobacillus* isolate: a potential host for the expression of heterologous proteins. *Microb Cell Fact* 14, 96 .
19. Kaur S., Sharma P., Kalia N., Singh J., Kaur S.(2018) Anti-biofilm properties of the fecal probiotic lactobacilli against *Vibrio* spp. *Front. Cell. Infect*.
20. Khalil W. L., Al-Hayanni H. S. (2024) Molecular Detection of the *mecA* and some Virulence Determinants in Methicillin-Resistant *Staphylococcus aureus*. *Journal Fac Medical Baghdad*; 66(2):247-53.
21. Layus, B. I., Gerez, C. L., and Rodriguez, A. V. (2020). Antibacterial activity of *Lactobacillus plantarum* CRL 759 against methicillin resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *Arabian Journal for Science and Engineering*, 45, 4503–4510.
22. Lei, S., Zhao, R., Sun, J., Ran, J., Ruan, X., & Zhu, Y. (2020). Partial purification and characterization of a broad-spectrum bacteriocin produced by a *Lactobacillus plantarum* zrx03 isolated from infant's feces. *Food Science and Nutrition*, 8(5), 2214–2222.
23. Mekky AF, Hassanein WA, Reda FM,& Elsayed HM. (2022) Anti-biofilm potential of *Lactobacillus plantarum* Y3 culture and its cell-free supernatant against multidrug-resistant uropathogen *Escherichia coli* U12. *Saudi Journal Biological Science*. Apr;29(4):2989-2997..
24. Muhammad Z, Ramzan R, Abdelazez A, Amjad A, Afzaal M, Zhang S,& Pan S.(2019) Assessment of the Antimicrobial Potentiality and Functionality of *Lactobacillus plantarum* Strains Isolated from the Conventional Inner Mongolian Fermented Cheese Against Foodborne Pathogens. *Pathogens*. May 21;8(2):71



25. Nouraldin, A. A. M., Baddour, M. M., Harfoush, R. A. H., & Essa, S. A. M. (2016). Bacteriophage-antibiotic synergism to control planktonic and biofilm producing clinical isolates of *Pseudomonas aeruginosa*. *Alexandria Journal of Medicine*, 52(2), 99–105.
26. Onbas, T., Osmanagaoglu, O., & Kiran, F. (2019). Potential properties of *Lactobacillus plantarum* F-10 as a bio-control strategy for wound infections. *Probiotics and Antimicrobial Proteins*, 11, 1110–1123.
27. Rasigade, J. P., Dumitrescu, O., & Lina, G. (2014). New epidemiology of *Staphylococcus aureus* infections. *Clinical Microbiology and Infection*, 20(7), 587-588.
28. Shinde, S; Lee, L. H; and Chu, T. (2021). Inhibition of biofilm formation by the synergistic action of EGCG-S and antibiotics. *Antibiotics*, 10(2), 1–21.
29. Shokri, D., Khorasgani, M. R., Mohkam, M., Fatemi, S. M., Ghasemi, Y., & Taheri-Kafrani, A. (2018). The inhibition effect of lactobacilli against growth and biofilm formation of *Pseudomonas aeruginosa*. *Probiotics and antimicrobial proteins*, 10(1), 34-42.
30. Verderosa, A. D., Totsika, M., & Fairfull-Smith, K. E. (2019). Bacterial biofilm eradication agents: a current review. *Frontiers in Chemistry*, 7, 824.