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Detection of the antibacterial activity of Lactobacillus CFS against Proteus bacteria

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Abstract

Urinary tract infections are among the most common bacterial infections affecting humans, with Proteus species frequently identified as a primary cause. The increasing resistance of these bacteria to antibiotics has prompted the search for safe biological alternatives, such as probiotics. In this study, the antibacterial action of the cell-free supernatant obtained from Lactobacillus isolates was evaluated against Proteus spp. adjusted to 0.50 McFarland turbidity using the well diffusion technique. Out of 150 urine samples collected from patients showing UTI symptoms, 20 samples (13.3%) were confirmed positive for Proteus. The infection rate was highest among patients aged between 21 and 40 years, Pearson Chi-square p-value = 0.000, which is less than 0.05, (statistically significant), relationship between age groups and the isolation of *Proteus* isolates, whereas there was no statistically significant relationship (p = 1.000) between gender (male and female) and *Proteus* isolation Meanwhile, 200 specimens taken from both clinical sources (healthy urine and vaginal swabs) and non-clinical sources (fermented foods) yielded 20 *Lactobacillus* isolates (10%). The supernatant of *Lactobacillus* exhibited a clear inhibitory effect on all Proteus isolates, forming inhibition zones ranging from 9 to 18 mm. Overall, these findings suggest the potential use of Lactobacillus supernatant as a natural suppressive agent against resistant Proteus strains in urinary tract infections.

Keywords: Proteus, Urinary Tract Infection, Lactobacillus, probiotic, CFS

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التحري عن الفعالية التثبيطية للراشح الخلوي CFS لبكتريا *Lactobacillus* ضد بكتريا ال *Proteus*

خالد جمال صالح [1]، هبه تقي رشيد [2]

المستخلص

تَعدُّ عدوى المسالك البولية من أكثر العدوى البكتيرية شيوعًا التي تُصِيب الإنسان، حيث تُعَدُّ أنواع *Proteus* سَبَبًا رئيسيًّا في كثيرٍ من الأحيان، وقد حَفَّزَت الزِّيادةُ في مقاومة هذه البكتيريا للمُضادات الحيوية البحثَ عن بدائل بيولوجية آمنة مثل البروبيوتك. في هذه الدِّراسة، فُحِصَ التأثير المضاد للرَّاشِح الخالي من الخلايا Cell-Free Supernatant المتحصل من عزلات *Lactobacillus* ضد أنواع *Proteus* المُعَدَّلة لعكارة بقدر ٠,٥٠ McFarland باستخدام تقنية الانتشار في الآبار Well Diffusion Technique، ومِن بين ١٥٠ عَيْنَة بَوْل جُمِعَت مِن مرضى تَظهَر عليهم أعراض عدوى المسالك البولية UTI، تَأَكَّدَت إيجابية ٢٠ عَيْنَة (١٣,٣٪) لوجود *Proteus*، حيث كان مُعَدَّل الإصابة أعلى في الفئة العُمُرية بين ٢١ و ٤٠ سنة، بقيمة p-value = 0.000 حَسَب اختبار Pearson Chi-square، وهي أقل من ٠,٠٥ (ذات دَلالة إحصائية) مُشِيرَةً إلى عِلاقة بين الفئات العمرية وعَزْل بكتيريا *Proteus*، في حين لم تكُن هناك علاقة ذات دَلالة إحصائية p = 1.000 بين الجنس (ذكور وإناث) وعملية العزل. في المُقابِل، أدَّى فحص ٢٠٠ عَيْنَة مأخوذة من مصادر سريرية (بول أصحاء ومَسْحات مهبلية) وأخرى غير سريرية (أطعمة مُخَمَّرة) إلى الحصول على ٢٠ عزلة من *Lactobacillus* (١٠٪)، وأَظْهَر راشِح *Lactobacillus* تأثيرًا تَثْبيطيًّا واضحًا على جميع عزلات *Proteus* حيث شَكَّل هالات تثبيط تراوح قُطْرُها بين ٩ و١٨ ملم، وبشكل عام تُشير هذه النتائج إلى الإمكانية الواعدة لاستخدام راشِح *Lactobacillus* كعامل تثبيط طبيعي ضد السلالات المقاومة للمضادات الحيوية من *Proteus* في حالات عدوى المسالك البولية.

الكلمات المفتاحية : Proteus، التهاب المجاري البولية، Lactobacillus، البروبايوتك، الراشح الخلوي

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معلومات البحث
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Introduction

Urinary tract infections (UTIs) are among the most common bacterial diseases affecting humans, and are among the leading causes of community- and hospital-acquired infections. They are particularly concerning because of their recurrent nature, association with antibiotic resistance, and potential to develop into severe complications such as pyelonephritis or bacteremia if not diagnosed effectively [1]. Among the uropathogens implicated in UTIs, Proteus species are widely distributed. These Gram-negative bacilli are highly motile, urease-producing organisms that not only colonize the urinary tract but are also causative agent to stone formation, recurrent UTIs infections, and treatment failures. The ability of *Proteus* isolates to thrive in diverse environments, coupled with their capacity for biofilm formation and virulence factor expression, highlights their importance as opportunistic pathogens [2]. In recent years, the global medical community has been challenged by the rising antibiotic resistance of *Proteus* isolates to conventional antibiotics, including β-lactams, aminoglycosides, and fluoroquinolones. These developments reduce therapeutic options and underscore the urgent need for alternative or complementary strategies in infection control [3]. *Probiotics*, particularly lactic acid bacteria, have emerged as promising candidates. Among them, *Lactobacillus* species are of special interest due to their ability to colonize mucosal surfaces, produce antimicrobial metabolites, and compete with pathogenic bacteria for adhesion sites. Their production of organic acids, hydrogen peroxide, and bacteriocins contributes to a clear inhibitory effect against pathogens, including uropathogenic *Proteus* spp. [4]. While numerous studies have investigated the antimicrobial properties of *Lactobacillus* in gastrointestinal and vaginal ecosystems, fewer studies have been directed toward understanding their interactions with urinary tract pathogens such as *Proteus*. This knowledge gap is particularly evident in local contexts, where data on the prevalence of probiotic strains in clinical and non-clinical sources, as well as their inhibitory action, remain limited. Addressing this gap is crucial not only for expanding scientific understanding but also for exploring cost-effective, naturally derived strategies in infection prevention and management [5]. The present research focuses on assessing the antagonistic activity of *Lactobacillus* cell-free supernatants (CFS) against *Proteus* isolates using in vitro assays well diffusion assay. Furthermore, to strengthen the accuracy of microbial identification and deepen insight into the nature of bioactive metabolites, advanced molecular and analytical tools will be employed in subsequent stages. These include 16S rRNA sequencing for precise taxonomic resolution and high-performance liquid chromatography (HPLC) for profiling inhibitory compounds of *Lactobacillus* CFS. Such approaches are expected to provide a comprehensive perspective on the mechanisms underlying probiotic action, bridging classical microbiology with modern molecular and chemical analyses [6]. In summary, this introduction positions the research within the global challenge of antibiotic resistance, emphasizes the clinical significance of *Proteus* in UTIs, highlights the probiotic potential of *Lactobacillus*, and outlines the study's contribution toward novel biotherapeutic approaches [7].

Materials and methods

Equipment and instruments

The equipment and instruments used in this study are listed in Table (1).

Table (1): Equipment and instruments

Equipment and instruments	Manufacturer/	Origin
Anaerobic jar	Oxoid	England
Digital –sensitive balance	Laptech	Korea
Autoclave	Hirayama	Japan
Distillator	Laptech	Korea
Deep freezer	Revco	USA
Incubator	Froilabo	France
Micropipette	IsoLab	France
pH-Meter	Infinite	China
Centrifuge	Hettich	Germany
Cold centrifuge	Eppendorf	Germany
light microscope	Olympus	Japan
Oven	Elekata	Japan
Refrigerator	Concord	Lebanon
UV Trans illuminator	UVP	USA
Water bath,	Memmert	Germany
Micro centrifuge	Heraius	Germany
Laminar air flow hood	Cryste	Korea
Turbidity meter (McFarland standard)	BioMérieux	France
Hot plate with magnetic stirrer	Heidolph	Germany
Millipore filters (0.45mm)	scharlau	Spain
Vortex mixer	Griffin	England

Culture Media

The culture media used in this study are included in Table (2).

Table (2) Culture Media

Medium name	Use	Company	Country of origin
Blood agar base	enriched medium used for the cultivation and preliminary identification of Proteus spp.	Liofilchem	Italy
Brain Heart Infusion (BHI) broth	Rich medium used with glycerol for long-term preservation of Proteus spp.	Liofilchem	Italy
MacConkey agar	Selective medium used for	Liofilchem	Italy

	the isolation and preliminary identification of Proteus spp.		
Modified de Man, Regoza, and Sharpe (MRS) broth	Enriched medium used for the primary cultivation of Lactobacillus spp.	Himedia	India
Modified de Man, Regoza, and Sharpe (MRS) agar	Enriched solid medium used for the isolation of Lactobacillus spp. by subculturing from MRS broth.	Himedia	India
Mueller-Hinton agar	Simple medium used for testing the antibacterial effect of Lactobacillus cell-free supernatant (CFS) against Proteus spp.	Liofilchem	Italy
Simon citrate media	Media used for biochemical tests	Himedia	India
Triple Sugar Iron (TSI)agar		Himedia	India
Urea-based agar		Liofilchem	Italy
MR-VP broth(Buffered glucose broth)		Liofilchem	Italy
Buffered peptone water (indole)		Condalab	Spain

Reagents

Reagents used in this study are included in Table (3).

Table (3): Reagents

Reagents	Company	Origin
Oxidase	Himedia	India
Kovac's	Himedia	India
Catalase	Himedia	India
Methyl red	Himedia	India

Samples Collection and Sources

A total of 150 clinical urine samples were collected from patients diagnosed with urinary tract infections at Al-Kut Pediatric and Gynecology Hospital for the isolation of Proteus isolates. In parallel, 200 samples were collected for the isolation of Lactobacillus isolates, including 100 clinical samples (60 urine samples and 40 vaginal swabs) and 100 non-clinical samples (40 pickled products, 30 cheese samples, and 30 yogurt samples) obtained from local markets. All samples were collected during the period from 1st September 2024 to 1st February 2025 and were immediately transported to the microbiology laboratory under appropriate conditions for further processing.

***Proteus* identification**

1- Morphological Identification For the preliminary isolation, urine samples suspected of urinary tract infections were streaked on MacConkey agar and Blood agar using the plate streaking technique. The plates were incubated aerobically at 37 °C for 24 hours to allow the development of distinct colonies. Observation of colony growth on these selective and differential media was applied as the first step in the morphological characterization of *Proteus* isolates before microscopic and biochemical confirmation [8].

2- Microscopic Identification Pure colonies obtained from the primary culture were subjected to Gram staining according to standard bacteriological protocols. Smears were prepared on clean glass slides, heat-fixed, and sequentially treated with crystal violet, Gram's iodine, decolorizing agent, and safranin as the counterstain. The stained cells were then examined under a compound light microscope using an oil-immersion objective lens to determine cellular morphology and Gram reaction [9].

3- Biochemical Identification

A-Catalase test, Slide (drop) method

A small portion of the bacterial colony was placed on a clean glass slide, and a drop of 3% hydrogen peroxide was added. The immediate formation of bubbles indicated a positive result, while the absence of bubbles indicated a negative result [10].

B- Oxidase Test

A piece of filter paper was moistened with oxidase reagent (tetramethyl-p-phenylenediamine), and a small amount of the bacterial colony was rubbed on the paper. The appearance of a dark purple color within a few seconds was considered a positive result, while no color change indicated a negative result [10].

C- Indole Test

The bacterial isolate was inoculated into peptone broth and incubated at 37 °C for 24 hours. After incubation, a few drops of Kovac's reagent were added, and the appearance of a red ring on the surface indicated a positive result, while the absence of a color change indicated a negative result [11].

D- Methyl Red Test

The bacterial isolate was inoculated into MR-VP broth and incubated at 37 °C for 24 hours. After incubation, five drops of methyl red indicator were added; the development of a stable red color indicated a positive result, while yellow or no color change indicated a negative result [12].

E-Triple Sugar Iron (TSI) Test

The bacterial isolate was streaked on the slant and stabbed into the butt of a TSI agar tube, followed by incubation at 37 °C for 24 hours. Changes in slant and butt color were recorded to indicate sugar fermentation, while gas production was observed by cracks or bubbles in the medium, and blackening indicated hydrogen sulfide (H_2S) production [13].

F- Urease Test

Each slant was streaked aseptically with the bacterial isolate using a sterile loop and incubated at 37°C for 24 hours under standard laboratory conditions; a change in the medium color to pink indicated a positive result, while no color change indicated a negative result [14].

G- Citrate Utilization Test

The bacterial isolate was streaked onto Simmons citrate agar slant and incubated at 37 °C for 24–48 hours; a color change of the medium from green to blue indicated a positive result, while no color change indicated a negative result [15].

4- Molecular Identification

During the well diffusion assay, six Proteus isolates that exhibited the strongest response to the Lactobacillus cell-free supernatant were selected for molecular confirmation. The isolate that demonstrated the highest inhibition level among this group was subjected to 16S rRNA sequencing, and these advanced molecular procedures will be fully presented and discussed in the Scopus-indexed research focusing on the molecular component of the study.

Lactobacillus Identification

1- Morphological Identification

The bacterial isolates were cultured on de Man, Rogosa, and Sharpe (MRS) agar plates and incubated at 37 °C for 48 hours. After incubation, the colony characteristics were examined macroscopically to record morphological features [16].

2- Microscopic Identification

A bacterial smear was prepared on a clean glass slide, heat-fixed, and subjected to the Gram staining procedure. The stained slides were then examined under a light microscope using the oil immersion lens to study the microscopic characteristics of the isolates [17].

3- Biochemical Identification

A-Catalase test, Slide (drop) method

B-oxidase test

C-Indole test

D-methyl red test

E-Triple sugar iron agar (TSI) test

F-Urease test

G-Citrate utilization test

The biochemical tests applied for the identification of *Lactobacillus* isolates were conducted using the same standard procedures described previously in the biochemical characterization of *Proteus*; the only modification was that all culture were incubated carried out under anaerobic conditions to suit the growth requirements of *Lactobacillus* isolates [18].

4- Molecular Identification

Six Lactobacillus isolates (KJ1, KJ135, KJ136, KJ140, KJ162, and KJ163) were subjected to PCR amplification targeting the 16S rRNA gene, as these isolates had previously exhibited the highest

mean inhibition zones against Proteus isolates (PS11, PS63, PS78, PS132, PS142, and PS147) in the well diffusion assay. Among this group, isolate KJ162—which demonstrated the strongest inhibitory effect—was further selected for species-level identification using Sanger sequencing of the 16S rRNA gene.

Lactobacillus Cell Free Supernatant preparation (CFS)

The cell-free supernatant (CFS) was prepared by cultivating a *Lactobacillus* isolate in MRS broth under anaerobic conditions at 37 °C for 24 hours. After incubation the cultures were centrifuged at 10,000 rpm for 15 minutes using a refrigerated centrifuge. The resulting supernatant was then carefully filtered through a sterile 0.45 µm membrane filter to ensure complete removal of residual cells. The clear filtrate was collected for subsequent antibacterial testing, while the pellet was discarded [19].

Detection of the antibacterial Activity of _Lactobacillus_ cell-free supernatant _(CFS)_ against _Proteus_ isolates

The antibacterial activity was assessed using the agar well diffusion method. Sterile Mueller-Hinton agar plates were uniformly swabbed with standardized *Proteus* suspensions at dilution level (0.50) to allow comparative analysis. Wells of 6 mm diameter were created in the solidified agar using a sterile cork borer, and each well was filled with 100 µL of freshly prepared *Lactobacillus* CFS under aseptic conditions. The plates were incubated at 37 °C for 24 hours under standard laboratory conditions, after which the inhibition zones around the wells were observed and measured to evaluate the antibacterial effect of the CFS against the tested *Proteus* isolates [20].

Results

Cultural results of _Proteus_ isolates

Out of the 150 urine samples obtained from patients with symptoms of urinary tract infection, 20 isolates were confirmed as *Proteus*- positive isolates. The distribution of positive cases according to age revealed that the highest frequency occurred in the 21–40 years' group (n = 9, 45.0%), followed by the 41–60 years group (n = 6, 30.0%), and the 1–20 years group (n = 5, 15.0%). Statistical analysis using the Pearson Chi-square test demonstrated a significant association between age and the isolation of *Proteus* isolates (p.value = 0.000), which is less than 0.05 indicating that age is an important determinant in infection distribution [21]. Regarding gender, females accounted for the majority of cases (n=13, 65.0%) compared to males (n=7, 35.0%), However, statistical analysis, Pearson Chi-Square, showed no significant relationship between gender and *Proteus* isolation (p.value = 1.000), greater than 0.05, suggesting that the observed difference was not statistically meaningful [22].

Identification results of _Proteus_ Isolates

A- Morphologically

The isolates demonstrated consistent and distinguishable cultural features. On MacConkey agar see figure (1), they produced pale colonies that were non-lactose fermenting, a characteristic feature of enteric pathogens such as *Proteus* [8].

Figure (1): Morphological appearance of *Proteus* colonies on MacConkey agar after 18-24 hours at 37°C under aerobic conditions

On Blood agar as seen in figure (2), all 20 positive *Proteus* isolates exhibited a typical swarming motility pattern with concentric rings, reflecting their active surface migration [23].

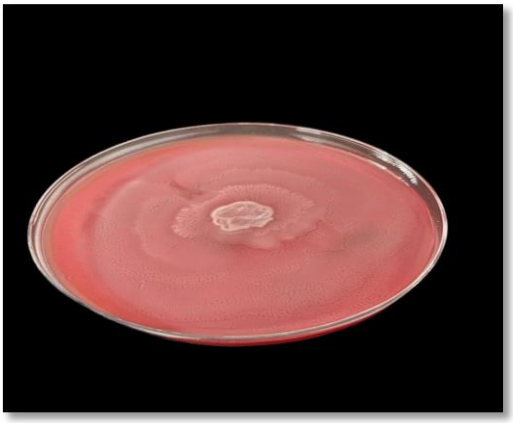

Figure (2): Swarming motility pattern of Proteus isolates on Blood Agar, showing concentric spreading rings characteristic of active surface migration

B- Microscopically

The *Proteus* isolates appeared as Gram-negative, pink-colored rods, consistent with their known morphological characteristics see Figure (3), and were observed arranged singly or in short chains under oil immersion [9].

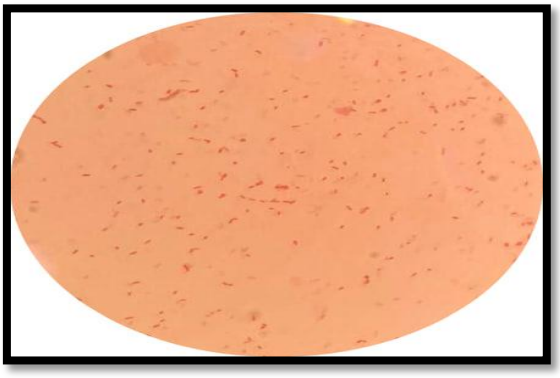

Figure (3): Microscopic appearance of Gram-negative *Proteus* under 100x oil immersion

C- Biochemical test results for *Proteus* identification

Further validating the phenotypic identification of the bacterial isolates, a series of conventional biochemical tests was performed on all 20 positive as seen in Table **(4)**, and Figure (4)

Table (4): Results of Biochemical Tests Used for Identification of *Proteus* Isolates

Sr. No	Tests	Results	Interpretation
1	Catalase Test	Positive	Bubbling
2	Oxidase Test	Negative	No color appears
3	Citrate Test	Negative	Absence of utilization of citrate as the sole carbon source
4	Urease Test	Positive	Ammonia production
5	Indole Test	Negative	No indole production
6	TSI Test - Slant	Acidic	lactose and sucrose fermentation (yellow slant)
	TSI Test - Butt	Acidic	Glucose fermentation(yellow butt)
	Gas Production	Positive	Gas formation
	H2S Production	Positive	Hydrogen sulfide production(black precipitation)
7	Methyl red test	Positive	Glucose fermentation

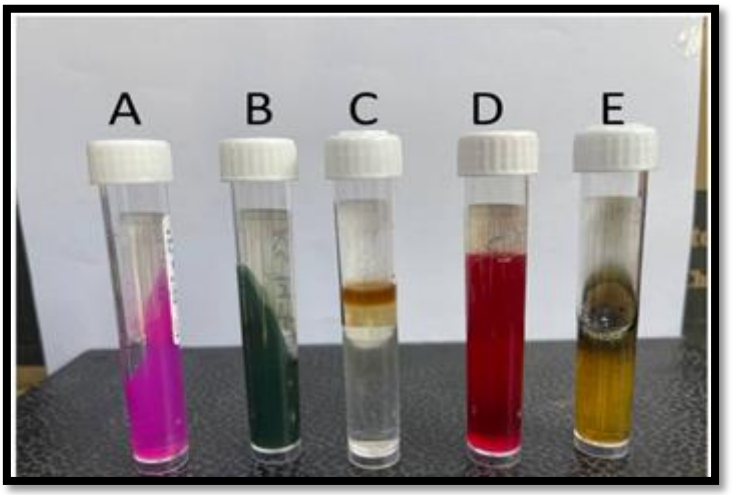

Figure (4): Biochemical tests for *Proteus* isolates: A Urease test, B: Citrate utilization test, C: Indole test, D: Methyl red test, E: TSI test

Identification results of *Lactobacillus isolates*

A- Morphologically

On MRS agar, all positive isolates developed as small, round, creamy-white colonies with smooth surfaces and entire margins, which are consistent with the typical phenotypic profile of *Lactobacillus* isolates see figure (5). Such uniformity in colonial appearance provides a reliable preliminary indication of their classification as lactic acid bacteria [16].

Figure (5) Morphological appearance of *Lactobacillus* colonies on MRS agar after 48 hours at 37°C under anaerobic conditions

.

B-Microscopically

All positive isolates were observed as Gram-positive, non-motile, non-spore forming bacilli, appearing either singly or arranged in short chains when examined under oil immersion, see figure (6). This cellular morphology is a well-recognized trait of *Lactobacillus* species and further supports their classification within lactic acid bacteria [17].

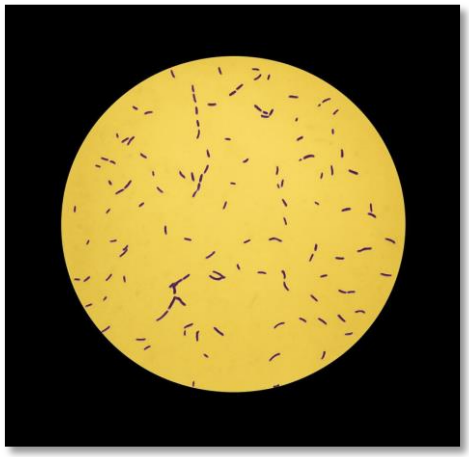

Figure (6): Microscopic appearance of Gram-positive Lactobacillus spp under 100x oil immersion.

B-Microscopically

C- Biochemical test results for *Lactobacillus* identification

All results of biochemical tests that were used to identify the *Lactobacillus* isolates are presented in Table (5), and Figure (7).

Table (5): Results of Biochemical Tests for Identification of Lactobacillus Isolates

Sr. No	Tests	Results	Interpretation
1	Catalase Test	Negative	No Bubbling

2	Oxidase Test	Negative	No color change with the reagent used
3	Citrate Test	Negative	Unable to utilize citrate
4	Urease Test	Negative	No Ammonia production
5	Indole Test	Negative	No indole production
6	TSI Test - Slant	Acidic	lactose and sucrose fermentation
	TSI Test - Butt	Acidic	Glucose fermentation
	TSI Test -Gas Production	Negative	No Gas formation
	TSI Test -H2S Production	Negative	No Hydrogen sulfide production
7	Methyl red test	Positive	Glucose fermentation

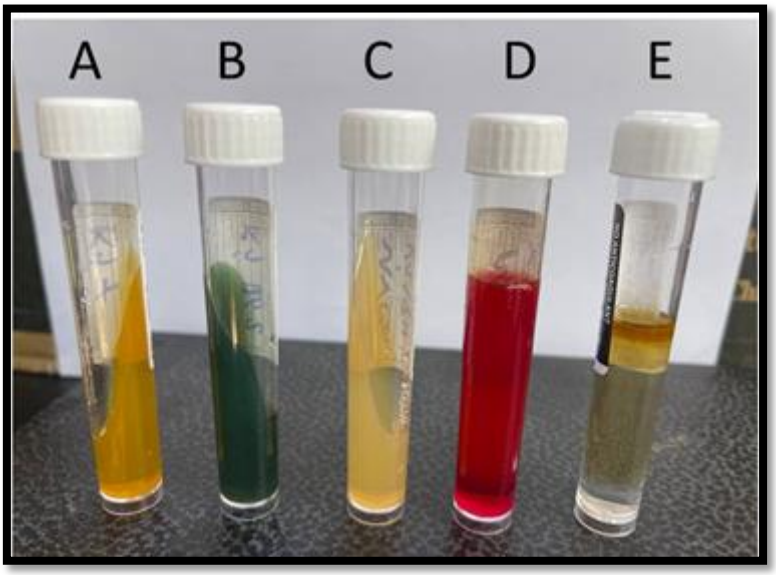

Figure (7): Biochemical tests for *Lactobacillus* isolates: A TSI test, B: Citrate utilization test, C: Urease test, D: Methyl red test, E: Indole test

Inhibition Assay Analysis (Well diffusion test)

All twenty positive isolates of *Proteus* exhibited clear inhibition zones in response to each of the twenty Lactobacillus cell-free supernatants (CFS) applied via the well diffusion method, see figure (8) and table **(**6**).** Mueller-Hinton agar plates covered with *Proteus* suspensions at turbidity levels of 0.50 revealed consistent inhibition across all treatments, with diameters ranging from 9 mm to 20 mm. Notably, 0.50 McFarland *Proteus* was used to assess the inhibitory effect of *Lactobacillus* CFS, in alignment with previous, on the level of *Proteus* spp, suspensions were adjusted to 0.50 McFarland to evaluate the inhibitory efficacy of *Lactobacillus* cell-free supernatant (CFS), in alignment with previous research. Locally, a study conducted at the University of Mosul College of Veterinary, Iraq [24]**.**

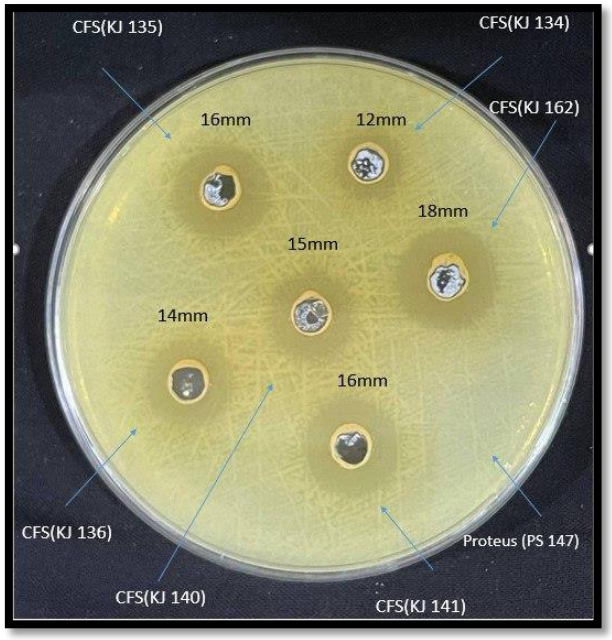

Figure (8) Antibacterial activity of *Lactobacillus* isolates (KJ134, KJ135, KJ136, KJ140, KJ141, and KJ162) against *Proteus* isolate (PS147) using the agar well diffusion method.

Table (6): Inhibition zone diameters (mm) of Proteus isolates at 0.50 dilution level treated with Lactobacillus CFS

Sample code	PS 11	PS 23	PS 34	PS 47	PS 50	PS 63	PS 71	PS 78	PS 84	PS 89	PS 95	PS 100	PS 106	PS 111	PS 124	PS 127	PS 132	PS 139	PS 142	PS 147	inhibition zones mean(mm)
KJ 1	17	16	14	16	16	14	14	15	17	14	14	15	14	15	16	14	16	14	14	16	15.5
KJ 7	11	10	9	12	12	11	10	12	11	10	10	9	9	11	12	9	12	12	10	11	10.65
KJ 17	9	12	11	10	9	12	10	12	11	12	12	9	10	10	10	9	11	9	11	11	10.5
KJ 19	12	11	10	12	10	10	12	10	9	10	12	9	10	9	11	10	10	9	11	11	10.5
KJ 96	12	10	9	12	11	11	9	10	12	12	10	12	12	10	12	10	9	11	11	9	10.7
KJ 120	12	9	12	9	12	12	12	11	10	11	10	12	11	10	11	11	9	9	9	9	10.55
KJ 131	10	11	11	10	12	11	9	12	11	11	11	11	9	11	12	10	11	9	10	10	10.6
KJ 134	12	9	10	10	12	12	9	9	12	9	12	10	12	10	10	11	9	11	11	12	10.6
KJ 135	18	18	18	18	18	18	18	17	18	17	17	17	18	17	17	18	17	17	18	16	17.6
KJ 136	17	14	15	16	15	15	14	17	14	15	16	16	16	15	17	14	15	14	15	14	15.35

KJ 138	**10**	**12**	**10**	**11**	**10**	**10**	**12**	**12**	**10**	**12**	**10**	**11**	**12**	**10**	**11**	**10**	**9**	**9**	**10**	**12**	**10.65**
KJ 140	**14**	**15**	**16**	**14**	**16**	**15**	**17**	**16**	**16**	**14**	**15**	**17**	**14**	**17**	**15**	**16**	**18**	**15**	**15**	**14**	**15.45**
KJ 141	**12**	**11**	**13**	**14**	**14**	**11**	**11**	**11**	**11**	**13**	**13**	**12**	**12**	**14**	**12**	**14**	**14**	**12**	**11**	**16**	**12.55**
KJ 143	**12**	**9**	**10**	**12**	**12**	**11**	**10**	**10**	**11**	**11**	**10**	**9**	**9**	**12**	**10**	**10**	**10**	**12**	**9**	**9**	**12.25**
KJ 147	**10**	**10**	**11**	**10**	**10**	**12**	**9**	**11**	**11**	**10**	**12**	**10**	**12**	**10**	**11**	**11**	**12**	**10**	**10**	**9**	**10.55**
KJ 162	**18**	**18**	**17**	**18**	**18**	**17**	**18**	**18**	**18**	**17**	**18**	**17**	**17**	**18**	**17**	**18**	**18**	**18**	**18**	**18**	**17.7**
KJ 163	**12**	**14**	**13**	**12**	**12**	**14**	**11**	**13**	**11**	**11**	**13**	**14**	**13**	**14**	**13**	**14**	**13**	**11**	**15**	**14**	**12.85**
KJ 176	**11**	**10**	**11**	**11**	**12**	**10**	**12**	**12**	**9**	**9**	**11**	**9**	**10**	**10**	**12**	**12**	**9**	**12**	**9**	**11**	**10.6**
KJ 179	**12**	**12**	**9**	**12**	**9**	**11**	**10**	**10**	**9**	**12**	**10**	**11**	**10**	**10**	**12**	**9**	**11**	**11**	**10**	**10**	**10.5**
KJ 183	**9**	**12**	**12**	**9**	**9**	**9**	**9**	**12**	**12**	**9**	**11**	**9**	**10**	**11**	**11**	**12**	**9**	**9**	**10**	**10**	**10.2**

Conclusion

The present study revealed that the cell-free supernatants (CFS) obtained from *Lactobacillus* isolates produced a stable inhibitory effect against uropathogenic *Proteus* spp., with inhibition zones ranging from 9 to 18 mm, This uniform trend suggests that the inhibitory activity resulted from the combined influence of multiple bioactive factors commonly secreted by *Lactobacillus*, including organic acids, antimicrobial peptides, and hydrogen peroxide, the consistent pattern of inhibition across isolates reflects a balanced metabolic performance and underlines the reliable antagonistic potential of *Lactobacillus* isolates as promising supportive bio therapeutic alternative to chemical drugs against antibiotic-resistant *Proteus* pathogens associated with urinary tract infections.

Recommendations

1. Future studies should concentrate on the extraction, purification, and chemical characterization of the bioactive inhibitory substances produced by *Lactobacillus* cell-free supernatants.
2. Further investigations are needed to evaluate the impact of these inhibitory compounds on additional virulence factors of *Proteus* isolates, particularly urease production and swarming motility.
3. Analyzing the main antimicrobial molecules secreted by *Lactobacillus* that inhibited *Proteus isolate*s

Suggestions

Advanced analytical approaches, such as high-performance liquid chromatography (HPLC) and molecular assays, are suggested to characterize the metabolites in *Lactobacillus* CFS and their mechanisms of action.

References

[1] Adugna B, Sharew B, Jemal M. Bacterial Profile, Antimicrobial Susceptibility Pattern, and Associated Factors of Community-and Hospital-Acquired Urinary Tract Infection at Dessie Referral Hospital, Dessie, Northeast Ethiopia. International journal of microbiology. 2021;2021(1):5553356.

[2] Chakkour M, Hammoud Z, Farhat S, El Roz A, Ezzeddine Z, Ghssein G. Overview of Proteus mirabilis pathogenicity and virulence. Insights into the role of metals. Frontiers in Microbiology. 2024;15:1383618.

[3] Oliveira M, Antunes W, Mota S, Madureira-Carvalho Á, Dinis-Oliveira RJ, Dias da Silva D. An overview of the recent advances in antimicrobial resistance. Microorganisms. 2024;12(9):1920.

[4] Fallah MS, Nikoo HR, Jamali A, Mohebbi A, Ghaemi EA. A cross-sectional study on the prevalence of multidrug-resistant clinical isolates of Proteus species in Northern Iran. The Journal of Infection in Developing Countries. 2024;18(12):1855-60.

[5] Scillato M, Spitale A, Mongelli G, Privitera GF, Mangano K, Cianci A, et al. Antimicrobial properties of Lactobacillus cell-free supernatants against multidrug-resistant urogenital pathogens. Microbiologyopen. 2021;10(2):e1173.

[6] Mahmood Mahdy D, Nazar Mahmood N, Shawkat Ali W. Antagonistic Activity of Bacteriocin-Producing Lactobacillus Local Isolates Against Select Pathogenic Bacterial Strains. Iranian Journal of Medical Microbiology. 2023;17(4):432-40.

[7] Bada P, Bharathi B, Thanagala N, Indracanti M, Dokka MK. Therapeutic Potential Of Probiotic Lactobacillus Strains: Antimicrobial And Antibiofilm Activities Against Multidrug-Resistant Pathogens. International Journal of Environmental Sciences. 2025;11(9s):908-16.

[8] Rodrigues PM, Ribeiro P, Tavaria FK. Distinction of different colony types by a smart data-driven tool. Bioengineering. 2022;10(1):26.

[9] Ekhuemelo C, Iordye M. MICROBIAL ASSESSMENT OF AFRICAN MESQUITE (PROSOPIS AFRICANA GUILL. AND PERR.) CONDIMENT SOLD IN MAKURDI METROPOLIS, BENUE STATE, NIGERIA. Nigerian Journal of Mycology Vol. 2022;14:111.

[10]Augustine EI. Identification of Proteus Vulgaris using a Series of Biochemical Tests and Staining Technique: Ecology, Pathogenicity (Enteric Pathogen), Description and Prevention of Its Diseases. International Journal of Contemporary Microbiology. 2024;10(1).

[11] Hovhannisyan HG, Danielyan LV, Baghdasaryan LG, Melkumyan IE, Barseghyan AH. The influence of the alcoholic extract of lemon balm on the growth of Lactobacillus helveticus MDC 9602 and the technological, physicochemical, organoleptic, and antioxidant properties of fermented milk. Journal of Food Science and Technology. 2024:1-10.

[12] Ezeagu GG, Bukar M, Fadayomi M. Determination of bacterial species associated with contamination of poultry feeds produced by three different companies in Abuja, Nigeria. UMYU Journal of Microbiology Research. 2024;9(1):102-8.

[13] Farooqui MA, Shaker M, Farooqui MA, Jaweria R, Khan AM, Shaikh J. Isolation and

characterization of bacterial isolates from PAH contaminated soil. IJSAT-International Journal on Science and Technology. 2025;16(1).

[14] Terrones-Fernandez I, Rodero-De-Lamo L, López A, Peiró S, Asensio D, Castilla R, et al. Microwave oven application for the preparation and sterilization of microbiological culture media: a feasible method with an adapted water bath and perforable cap. Applied Sciences. 2024;14(6):2340.

[15] Hamed RA, Wahhab DN, Qasim D, Obead AR. Biochemical test for Proteus mirabilis isolated from urine and wound samples. Journal of Advanced Multidisciplinary Research. 2024;3(1):58-63.

[16] Liping D. Screening and molecular identification of lactic acid bacteria in environmental samples.2024 ;9(3):124 .

[17] Singh S. Microbial assay techniques. Medical Microbiology. 2022;177.

[18] Szczerbiec D, Bednarska-Szczepaniak K, Torzewska A. Antibacterial properties and urease suppression ability of Lactobacillus inhibit the development of infectious urinary stones caused by Proteus mirabilis. Scientific Reports. 2024;14(1):943.

[19] Das S, Konwar BK. Prophylactic application of vaginal lactic acid bacteria against urogenital pathogens and its prospective use in sanitary suppositories. International Microbiology. 2024;27(1):179-202.

[20] Wang G, Zeng H. Antibacterial effect of cell-free supernatant from Lactobacillus pentosus L-36 against Staphylococcus aureus from bovine mastitis. Molecules. 2022;27(21):7627.

[21] Fares R, Debabza M, Mechai A. Detection and prevalence of extended-spectrum β-lactamases production among Enterobacteriaceae isolated from urinary tract infections. Biosystems Diversity. 2023;31(2):163-9.

[22] Khalid Ibrahim S. Phenotypic and genotypic analysis of antibiotic resistance in Proteus vulgaris isolated from ICU patients in Baghdad hospitals. Iranian Journal of Medical Microbiology. 2023;17(5):585-95.

[23] Veisi M, Hosseini-Nave H, Tadjrobehkar O. Biofilm formation ability and swarming motility are associated with some virulence genes in Proteus mirabilis. BMC Microbiology. 2025;25(1):388.

[24] Alchalaby, A. Y., & Al-Abedi, S. F. (2023). Inhibitory effect of Probiotics on some Gram-positive and negative Bacteria. *BASRAH JOURNAL OF VETERINARY RESEARCH*, *22*(1), 69-82.