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Effect of *Lactobacillus* supernatant on swarming-related gene expression in *Proteus mirabilis* isolated from urinary tract infections

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Abstract. *Proteus mirabilis isolates have been intensively researched for their capacity to cause urinary tract infections (UTIs) and their swarming motility, although little is known about this phenomenon. Probiotic Lactobacillus species, which are beneficial bacteria, are being studied worldwide as therapeutic and preventative agents against bacterial infections. This study investigated Lactobacillus supernatants as a potential new treatment against Proteus mirabilis. In addition to testing their antimicrobial and anti-swarming activities, the research also aimed to understand the genetic mechanisms behind the observed phenotypic changes.*

Methods. A total of 150 urine specimens were collected from UTI patients at various hospitals in Baghdad. Direct culture was performed by streaking the specimens on differential media. RNA was extracted and purified from the bacterial isolates, and then reverse transcription and quantitative PCR were used to evaluate swarming-related gene expression. Gene expression was assessed relative to a reference gene to reveal how probiotics regulate swarming behavior at the genetic level. Gene expression patterns varied, indicating complex genomic responses to Lactobacillus exposure.

Results. UTIs affected 50 males (33.33%) and 100 females (66.66%) of various ages. Proteus mirabilis was identified in 30 (20%) of the 150 samples. Resistance was observed in 25 (83.33%) isolates for azithromycin and amoxicillin/clavulanic acid, and in 22 (73.33%) isolates for meropenem. Real-time PCR showed significant alterations in the expression of four swarming-related genes (rsbA, umoD, ZapA, and FliL). The rsbA gene showed a notable increase in expression, while another sample displayed a decrease. The umoD gene exhibited the largest change, with expression doubling in some cases. ZapA showed the greatest increase, nearly tripling in expression in one sample. FliL expression also rose in multiple isolates. Swarming activity was positively correlated with gene expression levels for rsbA ($r = 0.8$, $p = 0.009$), umoD ($r = 0.635$, $p = 0.045$), ZapA ($r = 0.942$, $p = 0.001$), and FliL ($r = 0.894$, $p = 0.001$).

Conclusions. The study reveals a complex gene network regulating the swarming motility of Proteus mirabilis. It suggests that Lactobacillus acidophilus supernatants can modify gene expression and bacterial motility, potentially aiding in the treatment of UTIs.

Keywords: *Lactobacillus, swarming, Proteus mirabilis, urinary tract infections.*

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Вплив супернатанту *Lactobacillus* на експресію генів, асоційованих з роїнням *Proteus mirabilis*, ізольованого від пацієнтів з інфекціями сечової системи

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Резюме. *Ізолати Proteus mirabilis інтенсивно досліджуються через їхню здатність викликати інфекції сечової системи (ІСС) та властивість роїння, хоча про це явище мало що відомо. Пробіотичні види Lactobacillus вивчаються в усьому світі як терапевтичні та профілактичні засоби проти бактеріальних інфекцій. У цьому дослідженні було вивчено супернатанти Lactobacillus як потенційний новий засіб лікування ІСС, викликаних Proteus mirabilis. Крім тестування антимікробної та анти-роїльної активності, дослідження також мало на меті визначити генетичні механізми, які лежать в основі спостережуваних фенотипічних змін.*

Методи. Загалом було зібрано 150 зразків сечі від пацієнтів з ІСС. Пряме культивування проводили шляхом нанесення зразків на диференціальні середовища. РНК була екстрагована та очищена з бактеріальних ізолятів, після чого за допомогою зворотної транскрипції та кількісної ПЛР було оцінено експресію генів, асоційованих із роїнням. Експресію генів оцінювали відносно референсного гена для того, щоб з ясувати, як пробіотики регулюють роїльну поведінку на генетичному рівні.

Результати. ІСС діагностовано у 50 чоловіків (33,33%) і 100 жінок (66,66%) різного віку. Proteus mirabilis був визначений у 30 (20%) із 150 зразків. Резистентність була виявлена в 25 (83,33%) ізолятів до азитроміцину та амоксицциліну/клавуланової кислоти, і в 22 (73,33%) ізолятів до меропенему. Кількісна ПЛР показала значні зміни в експресії чотирьох генів, асоційованих із роїнням (rsbA, umoD, ZapA та FliL). Ген rsbA продемонстрував підвищення рівня експресії, тоді як в іншій пробі спостерігалося зниження. Ген umoD продемонстрував найбільші зміни, при цьому в деяких випадках експресія подвоїлася. Експресія гена ZapA збільшилася найбільше, майже потроївшись в одній з проб. Експресія FliL також зросла в декількох ізолятах. Активність роєння позитивно корелювала з рівнями експресії генів rsbA (r = 0,8, p = 0,009), umoD (r = 0,635, p = 0,045), ZapA (r = 0,942, p = 0,001) та FliL (r = 0,894, p = 0,001).

Висновки. Представлене дослідження демонструє складну генетичну мережу, яка регулює роїльну мотильність Proteus mirabilis та припускає, що супернатанти Lactobacillus acidophilus можуть модифікувати експресію генів і бактеріальну рухливість, що потенційно може сприяти лікуванню ІСС.

Ключові слова: *Proteus mirabilis, Lactobacillus, роїння, інфекції сечової системи.*

Introduction. The bacterial genus *Proteus* consists of various species, including *P. vulgaris, P. mirabilis, P. myxofaciens, P. penneri, and P. hauseri* [1]. These species can either exist as saprophytes in the gastrointestinal tracts of animals and humans or act as opportunistic pathogens in urinary tract infections (UTIs). In UTIs involving structural abnormalities or indwelling catheters, *Proteus mirabilis* is typically present [2]. The rise in drug resistance among *Proteus* species highlights the need for regular antimicrobial susceptibility monitoring to ensure effective therapeutic treatment [3]. A significant virulence factor of Proteus species, particularly *P. mirabilis*, is swarming motility, which is defined as "a rapid and coordinated movement of bacteria across surfaces, allowing them to spread quickly and colonize new areas." This trait enables the bacteria to rapidly spread across surfaces in a bull's-eye pattern, increasing their resistance to antibiotics and predation while providing competitive advantages through the secretion of surfactants [4].

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Non-motile, rod-shaped, Gram-positive *Lactobacillus* species may offer the potential to treat antibiotic resistance. Microorganisms in nutrient-rich habitats contribute to health benefits. According to Salvetti and O'Toole [5], *Lactobacillus* supernatants can impact *P. mirabilis* gene expression linked to swarming, suggesting a novel avenue for antibacterial treatment. Research shows that fermentative *Lactobacillus* species compete with harmful bacteria. These bacteria, part of the human gut microbiota, can adjust gene regulation to inhibit pathogenic quorum sensing. The antimicrobial effect of *Lactobacillus* is attributed to the production of bacteriocins, hydrogen peroxide, and organic acids. *Lactobacillus* supernatants may prevent *P. mirabilis* swarming and colonization of the urinary tract by eliminating other harmful bacteria [6–8].

Swarming genes such as *rsbA*, *umoD*, *ZapA*, and *FliL* in *P. mirabilis* control their movement. The *rsbA* gene encodes a sensor kinase in a two-component regu-

latory system that regulates the initiation of swarming [1, 9]. Mutations in the *rsbA* gene result in early swarming, demonstrating its role in timing. *UmoD*, a key up-regulator of the umo operon, enhances flagellar gene expression and swarming motility during the transition from swimmer to swarmer cells. Swarming also increases the expression of the Zn-dependent metalloprotease encoded by the *ZapA* gene, which may degrade extracellular proteins and enhance movement. The *FliL* gene, a flagellar basal body gene, is essential for proper flagellar function and directly impacts swarming dynamics [10, 11]. Comparing gene expression during and after swarming can provide insights into the complex genomic networks controlling swarming in *P. mirabilis*. This will further elucidate the biology and molecular mechanisms of swarming [12].

The swarming motility of *P. mirabilis* accelerates colonization and involves cell differentiation and collective movement [13]. Swarming enhances antibiotic resistance; the increased permeability of swarmer cells and the expression of resistance genes may help these bacteria survive antimicrobial treatment. *Lactobacillus* supernatants may influence *P. mirabilis* gene expression, swarming behavior, and antibiotic sensitivity [14, 15]. While the antibacterial capabilities of *Lactobacillus* supernatants have been extensively studied, their effects on *P. mirabilis* swarming motility, a critical virulence factor in urinary tract infections, remain largely unexplored. Previous investigations into the antibacterial and probiotic activities of *Lactobacillus* have overlooked its potential to regulate gene expression related to swarming. This gap in the literature calls for further research into how sub-inhibitory concentrations of *Lactobacillus* supernatants affect critical gene expression.

The present study aimed to examine the antibacterial and anti-swarming properties of sub-inhibitory doses of *Lactobacillus* supernatant and to evaluate its influence on the gene expression of swarming-related genes in *P. mirabilis*.

Materials and methods. This study was conducted in accordance with ethical guidelines, and approval was obtained from the institutional review board of [Institution Name] (approval no. [xxx]). Written informed consent was obtained from all participants, and all procedures adhered to the principles of the Declaration of Helsinki.

Isolation and identification of Proteus spp. Specimen collection. One hundred urine specimens were obtained from UTI patients at various hospitals in Baghdad. The specimens were streaked on blood agar and MacConkey agar, then incubated aerobically at 37°C for 24 hours.

Identification of isolates. Morphological identification was performed based on Bergey's Manual [16], and biochemical tests were conducted according to references [11-13]. The identification of *P. mirabilis* and *Lactobacillus* acidophilus was confirmed using the VITEK 2 compact system. The antibiotic susceptibility test was applied on Mueller-Hinton agar medium, and the disc diffusion method was employed [17]. The inhibition zones formed around the discs were measured in millimeters (mm) [18]. The antibiotics and their concentrations (µg/disc) used were: Gentamicin 10 (India), Azithromycin 15 (Germany), Meropenem 10 (United Kingdom), Ceftazidime 30 (Canada), Imipenem 10 (Germany), Levofloxacin 5 (U.S.), Ciprofloxacin 5 (Germany), Amoxicillin/clavulanic acid 30 (India), Trimethoprim 10 (U.S.), and Vancomycin 5 (Denmark).

Swarming motility assay (central spot inoculation method). Blood agar plates were inoculated with an overnight broth culture (0.01 ml), and the plates were incubated at 37°C overnight [19].

Preparation of Lactobacillus cell-free culture supernatants. Agar plates and MRS broth were used to cultivate *Lactobacillus*. The optical density of the standard cell suspension was adjusted using McFarland standard no. 0.5 turbidity. The supernatant was prepared by incubating 0.1 ml of the standard cell suspension in MRS broth at 37°C for 24 hours. *Lactobacillus* cells were removed through centrifugation and filtration. The supernatant stock solution was used to examine its inhibitory activity [20].

Antimicrobial activity of Lactobacillus isolates. Various concentrations of *Lactobacillus* supernatant were tested for antibacterial activity using the agar dilution method [21]. Briefly, distilled water (D.W.) was used to dilute the supernatant by 1/2, 1/4, 1/8, 1/16, and 1/32, to reach a final volume of 10 ml. Proteus mirabilis was inoculated in nutrient broth for 24 hours. Double-concentration Muller-Hinton agar was autoclaved, then mixed with the appropriate supernatant concentrations in sterile Petri dishes. *P. mirabilis* isolates were spotted in microliters onto each plate and incubated at 37°C for 24 hours. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of supernatant that inhibited bacterial growth. The swarming potential of the isolates was re-evaluated using sub-inhibitory concentrations of *Lactobacillus* supernatant.

Gene expression analysis. RNA extraction and purification. RNA extraction was performed using GeneAid reagents (South Korea), and RNA concentration and purity were measured with a NanoDrop 1000 spectrophotometer. After overnight ulture, bacteria were centrifuged, lysed, and mixed with ethanol. The supernatant was transferred to an RNA binding column, followed by washing with Pre-Wash Buffer and Wash Buffer. The column was dried, and RNase-free water was added to elute the pure RNA. This process ensured precise and efficient RNA extraction.

cDNA synthesis, RT-PCR, and quantitative RT-PCR. cDNA synthesis, reverse transcription-PCR (RT-PCR), and quantitative RT-PCR were performed on RNA samples, excluding those set aside for RNA sequencing (RNA-Seq). Dissociation curve analysis confirmed product specificity. Gene expression levels were quantified using the relative method with rpoA as the reference gene. The following primer sequences were used:

- rpoAF: 5'-GCGTGTTATAGCCCAGTTGA-3', rpoAR: 5'-AGGCTGACGAACATCACGTA-3'
- *rsbA*F: 5'-CTATACCTACCGCACCATGT-3', *rsbA*R: 5'-GAAGTCCCATCCGTTGATAC-3'
- *FliL*F: 5'-GGTGATCGCCATTATTGCAG-3', *FliL*R: 5'-AGCGTAACGTGATCCCTATG-3'
- *umoD*F: 5'-CAAGAGTGCCGTGTTTTCTATA-3', *umoD*R: 5'-CGATGATATCGCCCGGTTTAA-3'
- *ZapA*F: 5'-GGCCAAGCATGGTTTAGTGA-3', *ZapA*R: 5'-GGCGACTATCTTCCGCATAA-3'

Results. *Isolation proportions of Proteus mirabilis.* The study involved the collection of 150 urine samples from patients with UTIs admitted to various hospitals. The patient population consisted of 33.33% male and 66.66% female participants. These samples were cultivated on blood and MacConkey agar to identify *Proteus mirabilis*. *P. mirabilis* was isolated in 20% of the samples (30 samples), with a higher incidence in female patients (12.66%) compared to male patients (7.33%). The remaining 80% (120 samples) were negative for bacteria, comprising 54% female and 26% male samples. This data highlights a gender-based variation in the incidence of *P. mirabilis* in the tested population.

Patterns of antibiotic susceptibility in Proteus mirabilis. The disc diffusion assay revealed the antibiotic resistance patterns of *P. mirabilis* isolates. Susceptibility is indicated by the presence of inhibition zones around antibiotic-impregnated discs, while resistance is signified by smaller or absent zones of inhibition. The isolates demonstrated resistance to Amoxicillin/clavulanic acid, Azithromycin, and Meropenem, with resistance rates of 25 (83.33%), 25 (83.33%), and 22 (73.33%), respectively. Resistance rates for Ceftazidime, Gentamicin, and Vancomycin were 21 (70%), 20 (66.66%), and 16 (53.33%), respectively. Conversely, sensitivity rates were 26 (86.66%) for Imipenem, Levofloxacin, and Ciprofloxacin, and 18 (60%) for Trimethoprim.

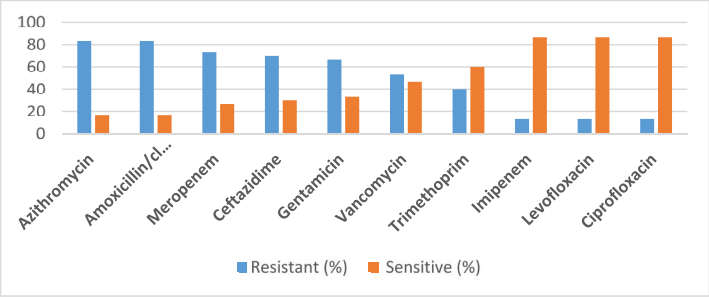

Fig. 1. Test of antibiotic sensitivity for isolates of Proteus mirabilis.

Determination of minimum inhibitory concentration (MIC). Sixteen antibiotics were tested using VITEK 2 Compact and an AST card. The antibiotics showed resistance to Piperacillin (≥128 µg/ml), Clavulanic acid (tazobactam/16–64 µg/ml), and Ticarcillin, with MIC values of 25 (83.33%). Additionally, 66.66% of isolates demonstrated resistance to Ceftazidime with MICs of at least 64 µg/ml. Sixteen isolates (53.33%) showed resistance to Imipenem (≤0.25 µg/ml), Aztreonam (≥64 µg/ml), Meropenem (1 µg/ml), and Cefepime. Approximately 50% of samples showed resistance to Amikacin (≤2 µg/ml) and Gentamicin (2 µg/ml). Resistance rates for Sulfamethoxazole (≥320 µg/ml), Trimethoprim, and Minocycline (8 µg/ml) were around 40% (12 isolates). Fewer isolates (13.33%) showed resistance to Tobramycin (≤1 µg/ml), Ciprofloxacin (≤0.25 µg/ml), and Piperacillin/Tazobactam (≤4 µg/ml).

Swarming behavior of Proteus mirabilis on blood agar. The bacterium exhibits a unique swarming growth pattern, characterized by tendril-like extensions from the central inoculation point, demonstrating its rapid colonization and spreading ability across solid surfaces. On blood agar, Proteus mirabilis forms these tendrils, indicating quick diffusion. Initial swarming diameters were 8.3 cm, 6.7 cm, 7.7 cm, and 6.6 cm. After treatment with *Lactobacillus* supernatant, these diameters decreased to 3.1 cm, 2.4 cm, 3.2 cm, and 3.0 cm, indicating reduced swarming. This significant reduction suggests that chemicals in *Lactobacillus* supernatants inhibit *P. mirabilis* motility on blood agar. The observed interaction highlights the antagonistic relationship between *Lactobacillus* acidophilus and *P. mirabilis*, suggesting that *Lactobacillus* products could be used to treat infections caused by swarming pathogens.

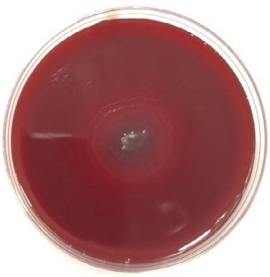

Fig. 2. Swarming activity of Proteus mirabilis on blood agar in the presence of *Lactobacillus* acidophilus.

Estimation of gene expression. Real-time PCR revealed changes in the expression levels of four genes—*rsbA*, *umoD*, *ZapA*, and *FliL*—in isolates treated with *Lactobacillus* acidophilus supernatants compared to a reference or control. After treatment, the expres-

sion of the *rsbA* gene in isolate 77 increased by 1.62-fold, while there was no significant change in isolate 55 (0.96). Isolates 80 and 90 exhibited reductions in expression, with 0.54 and 0.83-fold decreases, respectively. *UmoD* expression showed a significant increase of 2-fold in isolate 77. Isolate 55 was upregulated by 1.25, while isolates 80 and 90 were downregulated by 0.59 and 0.68, respectively. The *ZapA* gene was upregulated in isolate 77 with a 2.93-fold increase, while isolate 55 showed a fold change of 1 (0.85), and isolates 80 and 90 were downregulated (0.61 and 1.11, respectively). The *FliL* gene showed a 1.25-fold upregulation in isolate 77, with isolate 55 showing a slight increase (1.05) and isolate 80 downregulated by 0.66. Isolate 90 exhibited a 1.29-fold increase. These changes suggest that *Lactobacillus* acidophilus supernatants may influence the gene expression of swarming-related genes in *P. mirabilis*.

Table 1

Gene expression levels of *rsbA, umoD, ZapA*, and *FliL* before and after treatment with *Lactobacillus* acidophilus supernatants

Isolate code	pre			post			ΔΔCt	Fold change
	hkg	*rsbA*	ΔCt	hkg	*rsbA*	ΔCt		
80	8.45	13.23	4.78	8.5	14.16	5.66	0.88	0.5433674
77	8.18	15.36	7.18	8.56	15.04	6.48	-0.7	1.6245048
55	13.32	14.74	1.42	10.83	12.31	1.48	0.06	0.9592641
90	8.49	12.68	4.19	10.44	14.89	4.45	0.26	0.8350879
Isolate code	**pre**			**post**			**ΔΔCt**	**Fold change**
	hkg	*umo*	ΔCt	hkg	*umo*	ΔCt		
80	8.45	10.13	1.68	8.5	10.92	2.42	0.74	0.5987394
77	8.18	11.41	3.23	8.56	10.79	2.23	-1	2
55	13.32	15.17	1.85	10.83	12.35	1.52	-0.33	1.2570134
90	8.49	9.55	1.06	10.44	12.04	1.6	0.54	0.6877709
Isolate code	**pre**			**post**			**ΔΔCt**	**Fold change**
	hkg	*ZapA*	ΔCt	hkg	*ZapA*	ΔCt		
80	8.45	10.21	1.76	8.5	10.97	2.47	0.71	0.6113201
77	8.18	12.12	3.94	8.56	10.95	2.39	-1.55	2.9281714
55	13.32	13.92	0.6	10.83	11.67	0.84	0.24	0.8467453
90	8.49	10.24	1.75	10.44	12.04	1.6	-0.15	1.1095695
Isolate code	**pre**			**post**			**ΔΔCt**	**Fold change**
	hkg	*fli*	ΔCt	hkg	*fli*	ΔCt		
80	8.45	9.98	1.53	8.5	10.62	2.12	0.59	0.6643429
77	8.18	10.52	2.34	8.56	10.57	2.01	-0.33	1.2570134
55	13.32	14.54	1.22	10.83	11.98	1.15	-0.07	1.0497167
90	8.49	9.87	1.38	10.44	11.45	1.01	-0.37	1.2923528

Gene expression and swarming correlation. Table 2 reveals a strong positive correlation between gene expression and Proteus mirabilis swarming. Statistically significant correlations between the expression of *rsbA*, *UmoD*, *ZapA*, and *FliL* suggest that these genes regulate swarming behavior. Although gene activity increased after *Lactobacillus* supernatant treatment, swarming rates decreased, suggesting a complex regulatory mechanism involving multiple genes. Investigating these networks is essential for understanding microorganism motility patterns.

Table 2

Correlation between gene expression levels and swarming activity

Gene	Pearson's r	*p*-value
rsbA	0.8	0.009
UmoD	0.635	0.045
ZapA	0.942	0.001
FliL	0.894	0.001

Discussion. In 2023, Al-Ezzy et al. reported that 63.33% of Proteus mirabilis cases in Iraq occurred in females, while 36.67% were in males. The 17–23 age group had a 33.33% infection rate, while the 24–30 age group had a rate of 16.67% [22]. In 2022, Al-Obaidi and Al-Hashimy found 22 *P. mirabilis* isolates among UTI patients in Baghdad hospitals, with an isolation rate of 21.34% [23]. Similarly, in 2022, Al-Jubouri and Shami studied UTIs in Iraq and identified microbial growth in 62.3% of 180 urine samples, with no growth in 37.7%. *P. mirabilis* was isolated in 2.7% of patients, though the actual number of cases was not provided [24]. These studies show that the frequency of *P. mirabilis* varies by gender and age. It is most common in individuals aged 17 to 37, but recent research suggests it is more frequent in boys under 14 [25].

Under the oil immersion lens of a compound light microscope, the bacterial isolates appeared as reddish-pink rods, a characteristic typically associated with Proteus bacteria. This observation aligns with previous findings [26]. In the present study, *Lactobacillus* species were identified using Gram staining, appearing as Gram-positive bacilli and cocco-bacilli, either solitary or paired. Several studies have reported similar results [27]. This investigation supports both domestic and international research findings.

Mohammad et al. found that *P. mirabilis* isolates from patients with kidney failure and UTIs in Baghdad exhibited resistance to ciprofloxacin [28]. Al-Nabhani and Shami reported *P. mirabilis* resistance to ceftazidime (86.1%) and gentamicin (76.9%) [29]. Ciprofloxacin resistance was moderate at 40%, and resistance to amoxicillin-clavulanic acid was 56.9%. Levofloxacin (33.8%), imipenem (15.3%), and meropenem (1.5%) had lower resistance rates [30]. Antibiotic susceptibility of *P. mirabilis* isolates has varied across studies. For instance, isolates from Baqubah Hospital were 80% sensitive to ciprofloxacin, 60% sensitive to gentamicin, and 82.6% sensitive to imipenem. Newer carbapenems, such as imipenem, are more effective against Gram-negative bacteria due to their lower resistance rates [31]. The increasing resistance to gentamicin in local hospitals underscores the importance of aminoglycosides in treating UTIs. The complex structure of Proteus spp.'s outer cytoplasmic membrane likely contributes to its resistance mechanisms. Additionally, improper antimicrobial use has been linked to antibiotic resistance in Proteus spp. and other microorganisms [29, 32–34].

Minocycline, a bacteriostatic tetracycline that inhibits protein synthesis by binding to the 30S ribosomal subunit, showed resistance in 71.4% of isolates in recent studies [35]. Meropenem had the lowest resistance rate at 2.85%, and gentamicin and imipenem were highly effective against *P. mirabilis*, with a 97.2% success rate [36]. However, Proteus spp.' swarming motility, a virulence factor, poses challenges for microbiological studies and diagnosis due to its association with high virulence enzymes. *Lactobacillus* acidophilus has demonstrated the ability to displace harmful bacteria on mucosal surfaces and combat various infections [37]. This study found that L. acidophilus can inhibit *P. mirabilis*, with implications for antibiotic resistance profiles. These results underscore the urgent need for novel therapeutic approaches and precise diagnostic methods in the face of increasing antibiotic resistance. Probiotics, particularly *Lactobacillus* species, may influence the virulence of *P. mirabilis*, potentially leading to new methods for controlling harmful behaviors [38, 39].

Swarming motility, a coordinated and rapid surface movement exhibited by certain bacterial species, is closely associated with virulence, colonization, and biofilm formation [40]. In the case of *P. mirabilis*, swarming plays a crucial role in urinary tract infections, especially in catheterized patients, where it facilitates bacterial colonization [41]. The two samples with the highest swarming activity on blood agar support phenotypic observations that suggest a link between swarming and the expression of the *rsbA* and Zap genes [42]. The *rsbA* gene has been associated with bacterial stress responses, which are believed to play a role in host colonization [43]. Its high expression in the most active swarming samples suggests it may be involved in limiting swarming under harsh or resource-limited conditions, such as the urinary tract [44]. While the exact role of the Zap gene in swarming remains unclear, its increased expression in the most active samples suggests it plays a significant role in this behavior [45].

This study demonstrates that swarming in *P. mirabilis* is influenced by multiple genes, rather than a single one. Genes like Zap and *FliL* are involved in flagellar assembly and extracellular matrix production, which are essential for swarming. However, the moderate associations of genes like *rsbA* and *UmoD* suggest that regulatory or context-dependent factors may mediate their roles. This highlights the complex genetic regulation of bacterial swarming behavior, indicating that other factors or pathways are likely involved. Swarming regulation is known to be influenced by various stimuli and interconnected regulatory networks [46]. The moderate association of *UmoD* may reflect the complexity of its role, which could be context-specific or require interaction with other regulatory elements. Overall, multiple genes contribute to swarming behavior, which is governed by a complex genetic network [47]. Genes involved in flagellar function, such as Zap, show a strong correlation with swarming, corroborating previous studies indicating that these genes are highly

activated during swarming [48]. *FliL* genes, linked to extracellular components, support swarming motility, aligning with research that suggests swarming is aided by structures like lipopolysaccharides and other membrane components [49]. The moderate correlations observed for *rsbA* and *UmoD* suggest a more intricate regulatory network. Studies have shown that regulatory proteins like *RsbA* control the timing of swarming, supporting findings that gene overexpression or mutations can result in premature swarming [49, 50].

Some studies suggest that *P. mirabilis* coordinates virulence factor expression with swarming differentiation, including the regulation of toxins, adhesins, and other virulence genes [51]. The complex genetic regulation observed in this study is consistent with transcriptomic research showing widespread changes in gene expression during swarming, including both upregulation and downregulation of various gene sets [48].

When evaluating the effect of *Lactobacillus* spp. supernatant on swarming-related genes, the PCR approach is generally recommended. This molecular technique is widely applied across various fields of microbiology. It has been used to diagnose pathogenic bacteria, including Proteus mirabilis [35, 52], Staphylococcus aureus [53], and Pseudomonas aeruginosa [54], as well as to assess the severity of Coronavirus Disease 19 [55]. Additionally, PCR has been employed to explore the roles of bacterial neuraminidase and hyaluronidase in cancer cell interactions in vivo [56, 57], investigate mutations in gastric cancer [58], and measure gene expression levels of biomarkers in a range of diseases [59, 60]. Furthermore, the PCR technique has been used to examine small nuclear RNA host gene 3 as a potential therapeutic target in breast cancer through metabolic reprogramming [61], evaluate the significance of mitochondrial DNA quantification for blastocyst transfer potential [62], and used miRNA-126 as a Biomarker for Cancer Stem Cells [63].

Several limitations were encountered in this study. First, the sample size was relatively small, which may affect the generalizability of the results. A larger, more diverse sample size would provide a more comprehensive understanding of *P. mirabilis* swarming and gene expression. Second, this study focused on isolates from a specific geographical region (Baghdad), limiting its applicability to other regions. Future research should include isolates from different regions to provide a more global perspective. Third, while gene expression was analyzed using real-time PCR, additional molecular techniques, such as transcriptomics or proteomics, could offer more detailed insights into the regulatory pathways involved in swarming. Finally, the study did not explore the long-term effects of *Lactobacillus* supernatant on swarming behavior, and further research is needed to determine the sustainability of its inhibitory effects over time.

Conclusions. The study reveals that swarming motility in Proteus mirabilis is regulated by multiple genes, including *rsbA*, *UmoD*, *ZapA*, and *FliL*. Additionally, *Lactobacillus* acidophilus supernatants can influence gene expression and modulate bacterial motility, suggesting potential therapeutic applications for managing UTIs.

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The authors' contributions. Both authors contributed equally to the development of the research plan, the statistical analysis of the results, and the writing of the manuscript.

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