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The Correlation of Bacteriocin Production with Lactobacillus spp. Habitat and the presence of plnI , plnD , orf38 , and orf12 Genes

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Abstract

The relation of bacteriocin production with the habitat of *Lactobacillus* spp. and the presence of target genes was investigated by isolating *Lactobacillus* spp. from stool samples of 163 diarrheal and 90 healthy children. Ninety-seven of 111 *Lactobacillus* spp. isolates exhibited antibacterial activity (against *Salmonella typhi*, *Shigella* spp., and *Aeromonas hydrophilia* pathogens) in primary screening, whereas only 18 of them demonstrated bacteriocin production effectiveness in secondary screening (showed an inhibition zone against the three indicators), with a higher frequency in healthy (21.6%) than patient isolates (16.3%). Molecular identification using the *16S rRNA* gene revealed that only 15 of the 18 active isolates belonged to *Lactobacillus* spp. Their sequencing showed that two isolates showed 100% similarity with reference *Lactiplantibacillus plantarum,* four with reference *Limosilactobacillus fermentum*, and two with reference *Lacticaseibacillus rhamnosus*, all submitted to GenBank. The incidence of the *plnD*, *plnI*, *orf12*, and *orf38* genes in eight isolates revealed a higher significant variation (P=0.0084**). *Lactiplantibacillus plantarum* (SMHA16) obtained from the patient habitat revealed the incidence of *plnD, plnI*, and *orf38* genes and showed a higher average of bacteriocin production activity.

Keywords: *Lactiplantibacillus plantarum*, bacteriocin genes, *Salmonella typhi*, *Shigella* spp., *Aeromonas hydrophilia,* phylogenetic tree.

علاقة إنتاج البكتريوسين مع موطن بكتيريا *Lactobacillus* spp. ووجود جينات *plnI* و *plnD* و *orf38* و*orf12*

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الخلاصة

تم التحري عن علاقة إنتاج البكتريوسين مع موطن *Lactobacillus* spp. ووجود الجينات المستهدفة ، وذلك من خلال عزل *Lactobacillus* spp. من عينات براز 163 طفلًا يعانون من الإسهال و90 طفلًا سليماً. أظهرت 97 من أصل 111 عزلة *Lactobacillus* spp. نشاطًا مضادًا للممراضات *Salmonella typhi*, *Shigella* spp., *Aeromonas hydrophilia* في الغربلة الأولية، في حين 18 منها فقط أظهرت فعالية إنتاج للبكتريوسين في الغربلة الثانوية (اظهرت منطقة تثبيط ضد المؤشرات الثلاثة) مع ملاحظة ارتفاع معدل العزلات عند الاصحاء (21.6%) مقارنة بالمرضى (16.3%). بينت نتائج التشخيص الجزيئي للعزلات ال 18(باستعمال الجين *16SrRNA*) أن 15 منها تنتمي إلى *Lactobacillus* spp. وأعطت تحليلات

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التسلسل لها وجود تطابقاً لعزلتين بنسبة 100% مع المرجع *Lactiplantibacillus plantarum*، ولأربع عزلات مع *Limosilactobacillus fermentum*، ولعزلتين مع *Lacticaseibacillus rhamnosus*، وتم تسجيل العزلات في قاعدة بيانات GenBank. ان وجود الجينات *plnD, plnI, orf12, orf38* في هذه العزلات الثمانية أظهر تبايناً معنوياً عالياً(P=0.0084) . وأن العزلة *Lactiplantibacillus plantarum* (SMHA16) التي تم الحصول عليها من المرضى أظهرت متوسطًا أعلى لنشاط إنتاج البكتريوسين ووجود للجينات *plnD, plnI, orf38*.

1. Introduction

The human gastrointestinal (GI) microbiota represents a complex ecosystem of hundreds of bacterial species, including lactic acid bacteria (*Lactobacillus* spp.) and bifidobacteria, which play a pivotal role in maintaining intestinal microbiota balance. These beneficial microorganisms support immune function and protect against infections [1]. *Lactobacillus* spp. contributes to various biological processes such as pathogen clearance, intestinal barrier strengthening, immune response regulation, and metabolic activity enhancement; thus, they are recognized as functional probiotics [2]. One of the key mechanisms through which *Lactobacillus* spp. exert their beneficial effects is the production of specific bioactive compounds, including organic acids, hydrogen peroxide, inhibitory enzymes, and bacteriocins, which are multifunctional, ribosomally synthesized proteinaceous substances with antibacterial activity at specific doses [3]. Bacteriocins are categorized into four primary classes based on their chemical characteristics, genetic structure, mode of action, and molecular weight. These include Class I (lantibiotics), Class II (small heat-stable proteins), Class III (large heat-labile proteins), and Class IV (complex bacteriocins containing lipid or carbohydrate moieties) [4]. Bacteriocins produced by *Lactobacillus* spp. have appeared as promising therapeutic agents. These peptides not only show a broad spectrum of activity against Gram-positive bacteria but also show effectiveness against certain Gram-negative bacteria [5]. The production of bacteriocins is affected by the genes associated with bacteriocin synthesis, such as *plnD*, *plnI*, *plnG*, *PlnA*, *PlnW*, *PlnE*, *GaT*, *orf38*, *orf12*, *orf30,* and *PlnB*, and the strains of *Lactobacillus*, i.e., *L. plantarum* strains, were documented for their capacity to produce bacteriocins with potent antimicrobial properties [6][7]. *Lactobacillus* spp. is effective in treating acute infectious diarrhea and preventing antibiotic-induced diarrhea [8]. The imbalance in gut microbiota, known as dysbiosis, significantly increases susceptibility to pathogens, leading to various diseases, including diarrhea [9]. Acute diarrhea, often caused by contaminated food and water, is primarily attributed to pathogens such as *Salmonella typhi*, *Campylobacter* spp., *Shigella* spp., and *Escherichia coli*-producing Shiga toxin [10]. Diarrhea remains one of the most prevalent health challenges, defined as the passage of loose or watery stools [11]. The habitat in which *Lactobacillus* spp. live affects their ability to produce bacteriocin, also the presence or absence of some effective genes [12,6]. This study aimed to investigate the correlation between bacteriocin production, habitat, and the presence of the four target genes. To achieve this aim, *Lactobacillus* spp. were isolated from the stool samples of patients and healthy children, and the assessment of their ability to inhibit the growth of intestinal pathogens (produce bacteriocin) was performed. Then, the incidence of some genes that are responsible for bacteriocin production was detected.

2. Materials and Methods

Collection of Samples

Clinical samples were collected from Childe Hospital in the city of medicine, Ibn Al Baladi, and Fatima Alzahraa hospitals in Baghdad between October 1, 2023, and April 30, 2024. Two hundred fifty-three stool samples were collected from children (90 healthy and 163 patients) with aged between 1 day and 11 years. The samples were collected in a plastic

container and transferred to the laboratory to detect *Lactobacillus* spp. and pathogenic bacteria. Research Ethics Committee in the College of Sciences, University of Baghdad, Iraq, approved the study protocol (Ref. No. CSEC/0724/0050).

Isolation and Identification of Lactobacillus spp.

The samples were transferred into test tubes containing 5mL of normal saline to form a10^{-1} dilution. Serial dilutions were prepared, 10^{-2} to 10^{-5}, by passing 1 ml from each test tube to the next. Loopfuls of the 10^{-3}, 10^{-4}, and 10^{-5} dilutions were cultured in test tubes with 9 ml of De Man–Rogosa–Sharpe (MRS) broth and incubated for 48 hrs. at 37°C. Loopful MRS from broth culture was streaked onto MRS agar and incubated for 48 hrs. at 37°C. The suspected colonies were purified by re-culturing twice on MRS agar containing 1% $CaCO_3$ and incubated anaerobically at 37 ºC for 48 hrs. Bacterial isolates were identified by colony morphology, cellular microscopic properties (100X magnification with oil immersion lenses), and biochemical tests, including catalase, oxidase, blood hemolysis and indole tests [13, 14], and then the *16S rRNA* was detected by molecular method. The isolates of *Lactobacillus* spp. were designated as 'Lac' followed by a numerical identifier (e.g., Lac1) for identification."

Isolation and Identification of the Pathogenic Bacteria

Patients' stool samples were cultured on MacConkey agar, Xylose Lysine Deoxycholate agar, Hekaton enteric agar, and Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) agar, followed by overnight incubation at 37°C. The growing colonies were re-cultured on the same media, and their morphological characters were recorded. Then biochemical tests were conducted, including catalase, oxidase, indole, citrate utilization, motility, Triple Sugar Iron (TSI), and urease tests. Finally, identification of the pathogenic isolates was confirmed by the Vitek 2 compact system.

Antibiotic Sensitivity Determination

The Kirby-Bauer method was used to conduct antibiotic sensitivity tests for *Salmonella typhi*, *Shigella* spp., and *Aeromonas hydrophila* to 12 distinct antibiotic discs: Amikacin (AK), Ampicillin (Amp), Aztreonam (AZM), Cefepime (CPM), Cefotaxime (CFM), Ceftazidime (CAZ), Ceftriaxone (CRO), Chloramphenicol (C), Imipenem (IMI), Piperacillin tazobactam (PIT), and Trimethoprim-sulfamethoxazole (COT). The inhibition zone sizes of pathogenic isolates were compared with those of CLSI [15].

Biofilm Formation

Biofilm formation assay for *Salmonella typhi*, *Shigella* spp., and *Aeromonas hydrophila* was conducted using the microtiter plate's method [16].

Assessment of Bacteriocin Production

Lactobacillus spp. isolates were subjected to primary screening using the agar plug diffusion method by cultivating the isolates on MRS agar plates and incubating anaerobically at 37ºC for 48 hrs. Plugs from each isolate with 7 mm diameter were formed using a sterile cork borer. These plugs were placed upside down on Muller-Hinton agar (MH) that was streaked with 100 µl of indicator culture (1×10^8 cells/ml). The plates were incubated overnight at 37 ºC, and then inhibition zones around the bacterial plugs were measured to determine the antibacterial activity of each isolate [17].

The active isolates were subjected to secondary screening using the agar well diffusion method in order to recognize the most bacteriocin producers by culturing in MRS broth under anaerobic conditions at 37ºC for 48 hrs. The cultures were centrifuged at 6000 rpm for 15 minutes at 4ºC, and then the cell-free supernatant (CFS) was collected. The activated indicator was cultured on MH agar by spreading 0.1 ml of 10^8 cell/ml. Wells (7 mm diameter) were

formed using a sterile cork borer and then filled with 100 µl of CFS. The plates were kept at room temperature for 2 hrs. and then incubated at 37°C for 18-24 hrs. The inhibition zones formed around the wells were compared with the control group, which contained MRS broth only [18]. Three indicators were used, *Salmonella typhi*, *Shigella* spp., and *Aeromonas hydrophilia*, in the primary and secondary screening.

DNA Extraction

The genomic DNA of eighteen *Lactobacillus* spp. isolates was extracted and purified using the ABIOpure TM kit and following its protocol. DNA concentration was measured by the Quantus Fluorometer to assess sample quality for further applications. Diluted Quant fluor dye (200 µl) was added to 1 µl of DNA, and DNA concentration values were recorded after five minutes of incubation at room temperature.

PCR Amplification

The PCR method was performed to confirm *Lactobacillus* spp. identification using the *16SrRNA* gene and detected bacteriocin production association genes with specific primers (Table 1). These primers amplified the regions 345bp, 415bp, 450bp, 99bp, and 99bp of *16SrRNA*, *PlnD, PlnI, Orf12,* and *Orf38* genes, respectively. The reaction solution (25µl) contained 12.5µl of Master Mix, 1.5µl of each primer (forward and reverse), 4.5µl of nuclease-free water, and 5µl of the purified DNA template. The program used is shown in Table 2. The products of PCR were analysed by 1.5% w/v agarose gel electrophoresis, formed from 1X TAE buffer with 1µl of ethidium bromide, then powered at 100 v/m Amp for 60 minutes. The stained bands were displayed under a UV transilluminator [19].

Table 1: The primers used in this study (were from Macrogen® /Korea).

The gene	Primer sequence 5′→3′	Product size (bp)	Reference
16S rRNA	F-GCAGTAGGGAATCTTCCA R-ATTYCACCGCTACACA	345	[20]
PlnD	F-TGAGGACAAACAGACTGGAC R-GCATCGGAAAAATTGCGGATAC	415	[6]
PlnI	F-CTCGACGGTGAAATTAGGTGTAAG R-CGTTTATCCTATCCTCTAAGCATTGG	450	
Orf12	F-AGCTTGGAAAAGATTGCTGGT R-CAGACTACCAGTAAGCACGC	99	[7]
Orf38	F-CGGCAGGTATTGGATCAGGA R-ACCCAACCGCTCCTAAGTTT		

Table 2: PCR program for amplification of *16SrRNA*, *plnD*, *plnI*, *orf12*, and *orf38* genes.

Steps	Temperature	Time (m:s)	Number of cycles
Initial Denaturation	95°C	05:00	1
Denaturation	95°C	00:30	30
Annealing *16SrRNA*	55°C	00:30	
Annealing *plnD*	57°C	00:30	
Annealing *plnI*	59°C	00:30	
Annealing *orf12*	60°C	00:30	
Annealing *orf38*	60°C	00:30	
Extension	72°C	00:30	
Final extension	72°C	07:00	1
Hold	10°C	10:00	

Gene Sequencing

The PCR products of *16SrRNA*, and *plnD,* were sent for Sanger sequencing using the ABI 3730XL automated DNA sequencer by Macrogen Corporation, Korea. Sequencing the *16SrRNA* gene provides accurate information on genetic composition and advances our knowledge of evolutionary links across species. While *plnD* genes encode a key enzyme involved in the biosynthesis of bacteriocin, this provides insights into the antimicrobial capabilities of the investigated strain. The phylogenetic trees were constructed for eight local *Lactobacillus* spp. strains based on *16SrRNA* gene sequences and also for two locals *Lactiplantibacacillus plantarum* isolates based on *plnD* gene sequences utilizing the neighbor joining (NJ) method.

Statistical Analysis

The Statistical Packages of Social Sciences-SPSS (2019) program was used to detect the effect of different factors on the studied parameters. Least significant difference (LSD) and the T-test was used to significantly compare the means. The Chi-square test was used to significantly compare percentages (0.05 and 0.01 probability) in this study.

3. Results and Discussion

Isolation and identification of Lactobacillus spp.

One hundred and eleven *Lactobacillus* spp. isolates were determined by morphological characteristics and biochemical tests, 49 from patients and 62 from healthy individuals, (Table 3). *Lactobacillus* spp. isolates were able to grow on MRS agar after anaerobic incubation at 37 ºC for 48 hrs. their colonies appeared single, small, circular, with a rough surface, white to creamy with regular edges, slightly mucoid, round form, convex, and strong odor. Microscopically, the cells appear as Gram-positive bacilli or coccobacilli in single, paired, or chain arrangements. *Lactobacillus* spp. isolates were non-hemolytic on blood agar and reacted negatively for catalase, oxidase, and indole tests [13, 14]. In Iraq, it was found that out of 120, 90 (75%) healthy infant's stool samples were positive for *Lactobacillus* spp. [21]. In Iran, 105 *Lactobacillus* spp. isolates were isolated from 120 stool samples from Iranian infants under the age of 12 months and suggested that *Lactobacillus* spp. is a prevalent member of the infant gut microbiota and plays an important role in early life development [22].

The prevalence of *Lactobacillus* spp. isolates was higher in healthy individuals (68.89%) compared with patients (30.01%) (Table 3). The decrease of *Lactobacillus* spp. in patients may be related to an imbalance in the gut microbiota, which may be caused by diarrhea and other gastrointestinal problems. This imbalance shows the importance of keeping the gut microbiota stable and varied because any changes may support the colonization of dangerous pathogens [23]. Based on bacterial therapy, the gastrointestinal tract constitutes a healthy microbiota throughout life, which plays an important role of preventing and treating diarrhea [24]. *Lactobacillus* spp. are regarded as a key component of a healthy gut microbiota, particularly in early life [21]. It is a normal gastrointestinal tract flora and acts to regulate luminal pH, enhance barrier function by increasing mucus production, and secrete antimicrobial peptides [25].

Table 3: Prevalence of *Lactobacillus* spp. in stool samples.

Groups	Sample size	*Lactobacillus* spp. positive	Prevalence (%)
Patients	163	49	30.01%
Healthy	90	62	68.89%
Total	253	111	43.9%

Distribution of Lactobacillus spp. isolates in age groups

The distribution of *Lactobacillus* spp. in patients and healthy individuals gut microbiota was investigated across various age groups: 1day to 1 year, 1-4 years, 4-8 years, and 8-11 years (Table 4). The analysis revealed a generally higher prevalence of *Lactobacillus* spp. in newborns and infants (41.4%) (group 1) than in the older individuals, groups 2, 3, and 4 (29.7%, 17.1%, and 12.9%, respectively). The prevalence of *Lactobacillus* spp. isolates in both patients and healthy individuals decreased with an increase in age groups. Generally, *Lactobacillus* spp. isolates revealed more prevalence in healthy individuals (62) than in patients (49). Table 4 shows a notable difference in the distribution within different age groups, which was higher in all healthy groups than in patient groups, except group 1. This may relate to the fact that the gastrointestinal tract of a fetus is sterile, but after birth, the normal flora of the infant begins to develop during the first year of life. A study mentioned that the predominant bacteria during early colonization include lactic acid bacteria and Bifidobacteria [26].

Table 4: Distribution of *Lactobacillus* spp. isolates in age groups of patients and healthy individuals.

Age Groups		***Lactobacillus* spp. in patient No. = 49 (44.1%)**	***Lactobacillus* spp. in Healthy No. = 62 (55.9%)**	**The total *Lactobacillus* spp. No. = 111**
Group 1	1day-1 year	26 (53.1%)	20 (32.3%)	46 (41.4%)
Group 2	1-4 years	13 (26.5%)	19 (30.6%)	32 (28.8%)
Group 3	4-8 years	6 (12.2%)	15 (24.2%)	21 (18.9%)
Group 4	8-11years	4 (8.2%)	8 (12.9%)	12 (10.8%)

Isolation and identification of pathogenic bacteria

Morphological, cultural, and biochemical tests indicated the incidence of various bacterial colonies, such as *Escherichia coli*, *Proteus* spp., *Klebsiella* spp., *Staphylococcus* spp., *Acinetobacter* spp., *Salmonella* spp., *Shigella* spp., *Aeromonas* spp., and others. Various strains of these bacteria were regarded as gut normal flora, thus, *Salmonella* spp., *Shigella* spp., and *Aeromonas* spp. were used as indicators in the bacteriocin production test. These bacteria are the most common intestinal pathogenic bacteria that cause diarrhea [27]. Table 5 illustrates the cultural and biochemical properties of the picked pathogens. The employment of the Vitek 2 compact system showed that the probabilities of those isolated organisms being *Salmonella typhi*, *Shigella* spp., and *Aeromonas hydrophila* were 99%, 98%, and 97%, respectively.

Table 5: The biochemical test for *Salmonella* spp., *Shigella* spp., and *Aeromonas* spp.

Biochemical test		***Salmonella* spp.**	***Shigella* spp.**	***Aeromonas* spp.**
MacConkey agar		Pale colonies	Transparent or colorless colonies	Pale colonies
XLD agar		Red colonies with a black center	Pure pink colonies without a black center	----------
HE Agar		Transparent green or blue-green colonies with or without a black center	Transparent green or blue-green colonies without a black center	----------
TCBS agar		----------	----------	Yellow colonies
Catalase test		+	+	+
Oxidase test		-	-	+
Urease test		-	-	-
Simmon citrate		-	-	+
Indole test		-	-	+
Motility test		+	-	+
(TSI)	**Slant/butt**	K/A	K/K	K/A
	H_2S	+	-	-
	Gas	-	-	+

+: Positive. -: Negative. K: Alkaline. A: Acidic

Antibiotic sensitivity test

The result of the antibiotic susceptibility test showed that *Salmonella typhi* and *Shigella* spp. were resistant to ampicillin, cefotaxime, and ceftriaxone antibiotics. *Aeromonas hydrophila* was resistant to cefotaxime, ciprofloxacin, cefepime, ceftazidime, and piperacillin-tazobactam. On the other hand, *Salmonella typhi* and *Shigella* spp. were sensitive to aztreonam, ciprofloxacin, trimethoprim sulfamethoxazole-chloramphenicol, and meropenem, while *Shigella* spp. was also sensitive to imipenem. However, *Aeromonas hydrophila* was sensitive to only 3 antibiotics, including imipenem, meropenem, and amikacin. Statistical analysis revealed highly significant P-values for *Salmonella typhi*, *Shigella* spp., and *Aeromonas hydrophilia* isolates; P-values were 0.0098, 0.0082, and 0.0089, respectively (Table 6). It's obvious that the three tested pathogens have high rates of antibiotic resistance, with multidrug resistance observed against some critical antibiotics, such as cefotaxime, ceftriaxone, and ciprofloxacin, indicating caution with antibiotic treatments. Meanwhile, *Aeromonas hydrophila* exhibited more resistance than *Shigella* spp. and *Salmonella typhi*. Many studies mentioned that *Salmonella* spp., *Shigella* spp., and *Aeromonas hydrophila* isolates have high rates of antibiotic resistance. The extensive use of antibiotics by humans is more relevant for antibacterial resistance development among enteropathogenic bacteria, including *Salmonella* spp. and *Shigella* spp. self-medication and using antibiotic drugs without a prescription are frequently observed in the developing world [28, 29].

Table 6: Antibiotic susceptibility test of the pathogenic indicators.

Antibiotic	*Salmonella typhi*	*Shigella* spp.	*Aeromonas hydrophilia*
Ampicillin (AMP)	R	R	----------
Aztreonam (AZM)	S	S	----------
Cefotaxime (CTX)	R	R	R
Ceftriaxone (CRO)	R	R	
Ciprofloxacin (CIP)	S	S	R
Trimethoprim- sulfamethoxazole (COP)	S	S	----------
chloramphenicol (C)	S	S	----------
Imipenem (IMI)	I	S	S
Meropenem (MRP)	S	S	S
Cefepime (CPM)	----------	----------	R
Ceftazidime (CAZ)	----------	----------	R
Piperacillin tazobactam (PIT)	----------	----------	R
Amikacin (AK)	----------	----------	S
P-value	0.0098 **	0.0082 **	0.0089 **
	** (P<0.01)		

R: resistant, S: sensitive, I: intermediate.

Biofilm formation

The results indicated that the isolates *Salmonella typhi*, *Shigella* spp., and *Aeromonas hydrophilia* have a moderate capacity for biofilm formation (Table7). A study by Drprabhurajeshwar and Kelmani tested 31 *Salmonella* spp. isolates for biofilm production ability, they found that all the isolates can form biofilm, and 11 (35.48%) isolates have strong biofilm production ability. Another study investigated the biofilm formation ability of 32 MDR *Shigella* spp. isolates [30]. The clinical isolates produced biofilm in varying degrees; strong biofilm production was observed in *S. dysenteriae* (93.8%), *S. flexneri* (77.8%), and *S.*

sonnei (100%); moderate biofilm formation was less common and observed in *S. dysenteriae* (6.3%), while weak biofilm production was rare, primarily seen in *S. sonnei*. These findings emphasize the significant role of biofilm formation in antimicrobial resistance [31]. Vestby *et al*., estimated the differences in biofilm formation degrees by *Aeromonas hydrophila* at three different temperatures. They found that 96% of them were biofilm producers at 35ºC, while 84% were able to produce biofilm at 25°C, and 52% demonstrated biofilm formation at 4°C. They concluded that biofilm production by *Aeromonas hydrophila* facilitates adhesion to the host intestinal epithelium, which aids in the establishment of disease [32]. Biofilms production in bacteria employs several survival strategies to avoid host defenses. These bacteria can avoid the immune system by staying dormant, causing damage to tissue, which can lead to severe infections [33].

Table 7: Biofilm intensity measured with an ELISA device at a wavelength of 630.

The isolates	Biofilm capacity	Mean of OD for treatment	Mean of OD for control
Salmonella typhi	Moderate	0.261	0.062
Shigella spp.	Moderate	0.346	0.055
Aeromonas hydrophila	Moderate	0.321	0.058

Antibacterial activity of Lactobacillus spp. isolates

The antibacterial activity *Lactobacillus* spp. (111 isolates) against *Salmonella typhi*, *Shigella* spp., and *Aeromonas hydrophila* was tested using the agar-plug diffusion method. 97 isolates (87.4%) involved,46 patients and 51 healthy individuals, inhibited the pathogens by forming zones ranging from 9 to 22 mm. *Lactobacillus* spp. isolates obtained from patients (93.9%) exhibited greater antibacterial activity compared to those obtained from healthy individuals (82.3%), against all bacterial indicators (Table 8). The high antimicrobial activity rate of *Lactobacillus* spp. may be related to their production of antimicrobial agents, including hydrogen peroxide (H_2O_2), organic acid, diacetyl, inhibitory enzymes, bacteriocin, and carbon monoxide (CO), as well as other substances such as bio-emulsifiers [6].

In general, *Salmonella typhi* revealed a greater sensitivity against *Lactobacillus* spp. isolates (96.4%), followed by *Shigella* spp. (91.0%), then *Aeromonas hydrophila* (88.3%), with a highly significant difference between them (P-value = 0.0001**). [33] found that of 30 *Lactobacillus* spp., 8 isolates inhibited the growth of *Staphylococcus aureus*, *E. coli*, and *Streptococcus aureus*. Another study demonstrated that *Lactobacillus* spp. isolates, isolated from the feces of healthy individuals, inhibited the growth of enteropathogenic *E. coli* (EPEC), *enteroaggregative E. coli* (EAEC), *Salmonella typhi*, and *Shigella dysenteriae* using the agar-plug diffusion method [1]. [17] showed that 80% of their *Lactobacillus* spp. isolates (isolated from 120 vaginal samples) exhibited antibacterial activity against *Pseudomonas aeruginosa*, *Escherichia coli, Klebsiella pneumoniae*, and *Proteus* spp.

Table 8: Antibacterial activity of *Lactobacillus* spp. against pathogenic indicators, represented in inhibition zone (mm).

Indicators bacteria	Pantient No. = 49	Healthy No. = 62	Total No.=111	L.S.D. (P-value)
Salmonella typhi	49 (100%)	58 (93.6%)	107 (96.4%)	6.027 ** (0.0001)
Shigella spp.	47 (95.9%)	54 (87.1%)	101 (91.0%)	5.941 ** (0.0001)
Aeromonas hydrophila	46 (93.9%)	52 (83.9%)	98 (88.3%)	5.602 ** (0.0001)
The three indicators	46 (93.9%)	51 (82.3%)	97 (87.4%)	* (P<0.05) ** (P<0.01)

The effectiveness of Lactobacillus spp. bacteriocin

Ninety-seven *Lactobacillus* spp. isolates showing inhibition activity against three indicators via the agar-plug diffusion method were subjected to secondary screening using the more accurate agar-well diffusion method. This method assessed bacteriocin effectiveness against *Salmonella typhi*, *Shigella* spp., and *Aeromonas hydrophila*. Only 18 (18.6%) isolates, including 16.3% of patients and 21.6% of healthy individuals, exhibited the ability to inhibit all bacterial indicators with inhibition zones ranging from 11 to 16 mm (Table 9). The percentage of *Lactobacillus* spp. isolates that exhibited effectiveness against the bacterial indicators varied between patients and healthy individuals with the different indicators. The percentages for patients' isolates were 23.3%, 51.2%, and 39.5%, while for healthy isolates they were 49.0%, 41.2%, and 37.3%, against *Salmonella typhi*, *Shigella* spp., and *Aeromonas hydrophila*, respectively. In general, the bacteriocin produced by *Lactobacillus* spp. isolates showed higher activity against *Shigella* spp. (44.3%), followed by *Aeromonas hydrophila* (37.1%), then *Salmonella typhi* (36.1%), with a highly significant difference between them (P-value = 0.0001**). A study by Bibalan *et al*., obtained 72 *Lactobacillus* spp. strains of 434 LAB isolates, isolated from 43 fecal samples. Their antimicrobial activity test showed that 40% of *Lactobacillus* spp. isolates were active against one or more used indicators: Enteropathogenic *E. coli* (EPEC), Enteroaggregative *E. coli* (EAEC), *Salmonella typhi*, and *Shigella dysenteria*e, while only 17.4% of these strains were active against all the indicators [1]. Another study mentioned that LAB's cell-free supernatants could inhibit gram-positive and gram-negative bacteria, such as *Salmonella* spp., *E. coli*, *S. aureus*, and *P. aeruginosa* [34]. As indicated by [35] the bacteriocin-like raw material extracted from *Lactobacillus acidophilus* showed antibacterial activity against foodborne pathogens such as *S. typhi* and *S. aureus*, supporting its potential use as an alternative to conventional antibiotics.

Table 9: The effectiveness of *Lactobacillus* spp. bacteriocin against pathogenic indicators.

The isolates number The indicators	Pantient No. = 43	Healthy No. = 51	Total number No. = 97	P-value
Salmonella typhi	10 (23.3%)	25 (49.0%)	35 (36.1%)	0.0001 **
Shigella spp.	22 (51.2%)	21 (41.2%)	43 (44.3%)	0.0001 **
Aeromonas hydrophila.	17 (39.5%)	19 (37.3%)	36 (37.1%)	0.0001 **
The three indicaters	7 (16.3%)	11 (21.6%)	18 (18.6%)	* (P<0.05) ** (P<0.01)

Molecular study

PCR technique using the 16S rRNA gene for Lactobacillus spp.

Eighteen bacterial isolates were subjected to DNA extraction, and then the gene*16S rRNA* was amplified by specific primers (Table 2), in order to diagnose *Lactobacillus* spp. The gel electrophoresis result of the amplified PCR products revealed that fifteen isolates had bands at 345 bp; thus, they belonged to *Lactobacillus* spp.

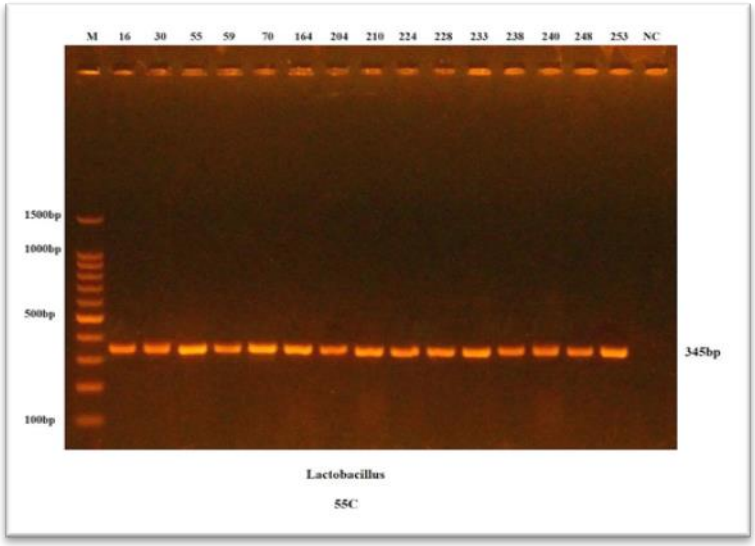


Figure 1: Amplification product of *16S rRNA* gene in *Lactobacillus* spp. isolates after electrophoresis on 1.5% agarose gel in the presence of DNA Ladder (1500 bp).

Sequencing of Lactobacillus spp. 16S rRNA gene

The PCR products of fifteen *Lactobacillus* spp. isolates were subjected to sequencing by Macrogene Company in Korea. Sequencing analysis confirmed the genus and species identification, and the quality of the sequences was evaluated. Consensus sequences were generated utilizing Geneious version 2023.0.7. Subsequently, BLASTN analysis was employed to compare the *16SrRNA* gene sequences of the 15 local isolates with a reference strain in the GenBank database. Sequencing analysis revealed that 8 out of 15 local isolates were identified as *Lactobacillus* species. Two isolates were found to be *Lactiplantibacillus plantarum* (identical to the Nigerian isolate ID: PP978768.1), four isolates were *Limosilactobacillus fermentum* (identical to the Malaysian isolate ID: MT645492.1), and two isolates were matched *Lacticaseibacillus rhamnosus* (identical to the Chinese isolate ID: MT645513. The eight *Lactobacillus* spp. isolates were recorded at the gene bank, in the National Center for Biotechnology Information (NCBI). The identification and accession numbers were presented in Table 10. It was found that diabetic patients predominantly had *L. acidophilus, L. fermentum, L. reuteri, L. ingluviei, and L. salivarius,* while non-diabetic individuals showed higher prevalence of *L. plantarum, L. gasseri, L. rhamnosus, L. paracasei*, and *L. delbrueckii*, indicating distinct microbial profiles between diabetic and healthy individuals, with implications for metabolic and gut health [4].

Table 10: The sequence analysis and accession numbers of *Lactobacillus* spp. isolates according to the gene bank, National Center Biotechnology Information (NCBI).

The isolate No.	Sequence analysis result (isolate name)	Accession number according to NCBI
Lac16	*Lactiplantibacillus plantarum* (SMHA16)	PQ 309568
Lac30	*Limosilactobacillus fermentum* (SMHA30)	PQ 309569
Lac 208	*Lactiplantibacillus plantarum* (SMHA208)	PQ 309570
Lac 210	*Limosilactobacillus fermentum* (SMHA210)	PQ452299.1
Lac 224	*Limosilactobacillus fermentum* (SMHA224)	PQ45200.1
Lac 228	*Lacticaseibacillus rhamnosus* (SMHA228)	PQ 309571
Lac 240	*Limosilactobacillus fermentum* (SMHA240)	PQ452301.1
Lac 253	*Lacticaseibacillus rhamnosus* (SMHA253)	PQ452302.1

Phylogenetic analysis

Eight local *Lactobacillus* spp. intestinal stool isolates were sequenced for *16S rRNA*, displayed in the phylogenetic tree constructed by the neighbor-joining method. The optimal tree with the sum of branch length = 0.18922414 is shown in Figure 2. The tree is drawn to scale, with branch lengths (above the branches) in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the maximum composite likelihood method and are in the units of the number of base substitutions per site. This analysis involved 23 nucleotide sequences. There was a total of 290 positions in the final dataset. Among Iraqi isolates from Baghdad province, three groups were identified: *Lactiplantibacillus plantarum* (2 isolates), *Limosilactobacillus fermentum* (4 isolates), and *Lacticaseibacillus rhamnosus* (2 isolates). The local isolated analysis by phylogenetic indicated that the first group, *Lactiplantibacillus plantarum*, closely matched isolates from Nigeria, China, Iraq, Iran, and Turkey, with the highest similarity to Nigeria (ID: PP978768.1), followed by China (ID: OR879325.1), Iraq (ID: OQ933119.1), Iran (ID: MW36248.1), and Turkey (ID: MW853617.1). The second group, *Limosilactobacillus fermentum*, closely matched isolates from Malaysia, Iraq, Turkey, Iran, and India, with the highest similarity to Malaysia (ID: MT645492.1), followed by Iraq (ID: OQ933120.1), Turkey (ID: MN372309.1), Iran (ID: ON384533.1), and India (ID: PP864358.1). The third group, *Lacticaseibacillus rhamnosus*, closely matched isolates from China, Japan, Iran, the USA, and Iraq, with the highest similarity to China (ID: MT645513.1), followed by Japan (ID: LC463254.1), Iran (ID: OQ74244.1), the USA (ID: PP864358.1), and Iraq (ID: MW897741.

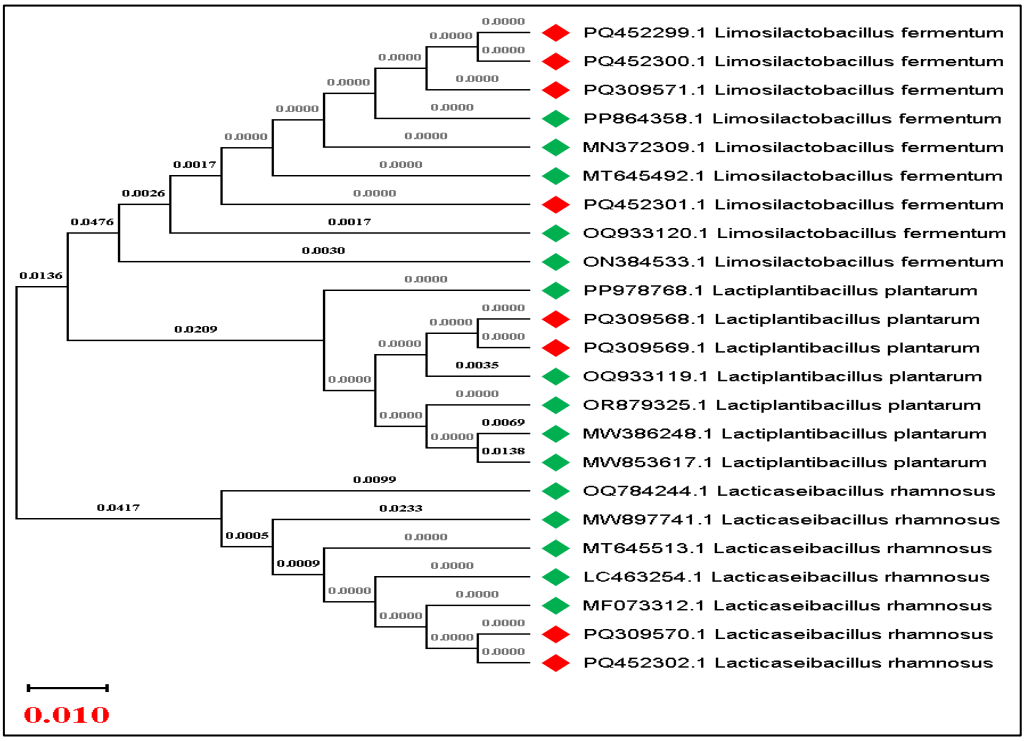


Figure 2: Phylogenetic tree including eight local isolates sequences of the *16S rRNA* (indicated by red) and fifteen isolates from Nigeria, Malaysia, China, Iraq, Japan, Iran, and the USA.

Investigation of some bacteriocin production genes

The eight *Lactobacillus* isolates were subjected to PCR to detect the *plnD, plnI, orf12*, and *orf38* genes involved with bacteriocin production. Gel electrophoresis showed that three isolates have some of these genes (Table 11 and Figure 3). The *plnD, plnI,* and *orf38* genes were present in 25% of the isolates with significant variation (P = 0.043 *). The gene *orf12*

was found in 12.5% of the isolates, with a higher significant variation (P = 0.0084 **). A previous study found that *plnI* genes in 28 isolates (56%) and the *plnD* gene in 36 isolates (72%) in 50 *Lactobacillus* spp. isolates, isolated from 60 fecal samples [6].

Table 11: The presence of bacteriocin production responsible genes in *Lactobacillus* spp. isolates.

***Lactobacillus* strains**	**The gene bacteriocin**			
	Pln I	***PlnD***	***orf38***	***orf12***
Lactiplantibacillus plantarum (SMHA 16)	+	+	+	-
Limosilactobacillus fermentum (SMHA30)	-	-	-	-
Lactiplantibacillus plantarum (SMHA208)	+	+	-	-
Limosilactobacillus fermentum (SMHA210)	-	-	-	-
Limosilactobacillus fermentum (SMHA224)	-	-	-	-
Lacticaseibacillus rhamnosus (SMHA228)	-	-	-	-
Limosilactobacilus fermentum (SMHA240)	-	-	+	+
Lacticaseibacillus rhamnosus(SMHA253)	-	-	-	-
P-value	0.043 *	0.043 *	0.043 *	0.0084 **

* (P<0.05), ** (P<0.01)

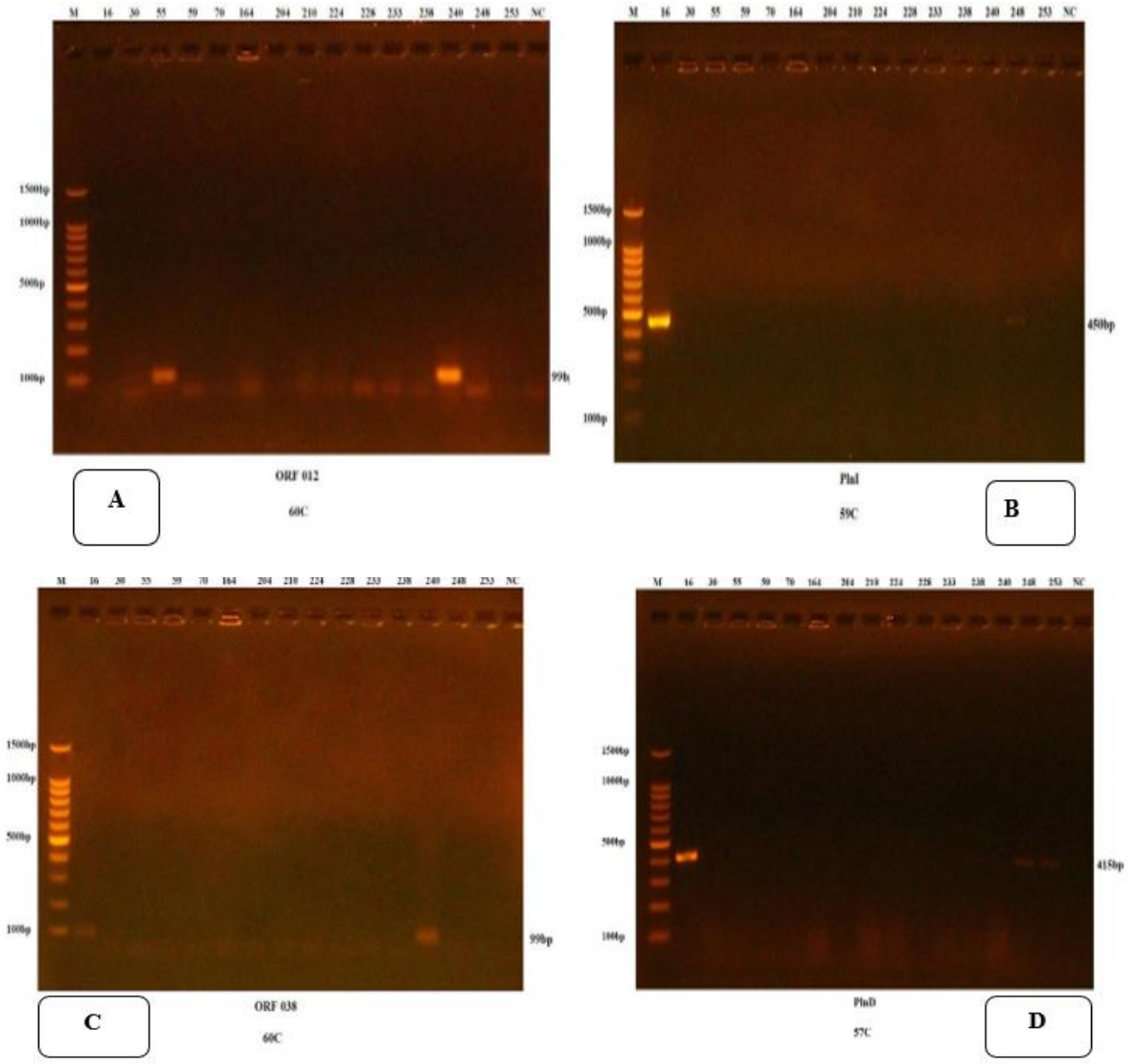
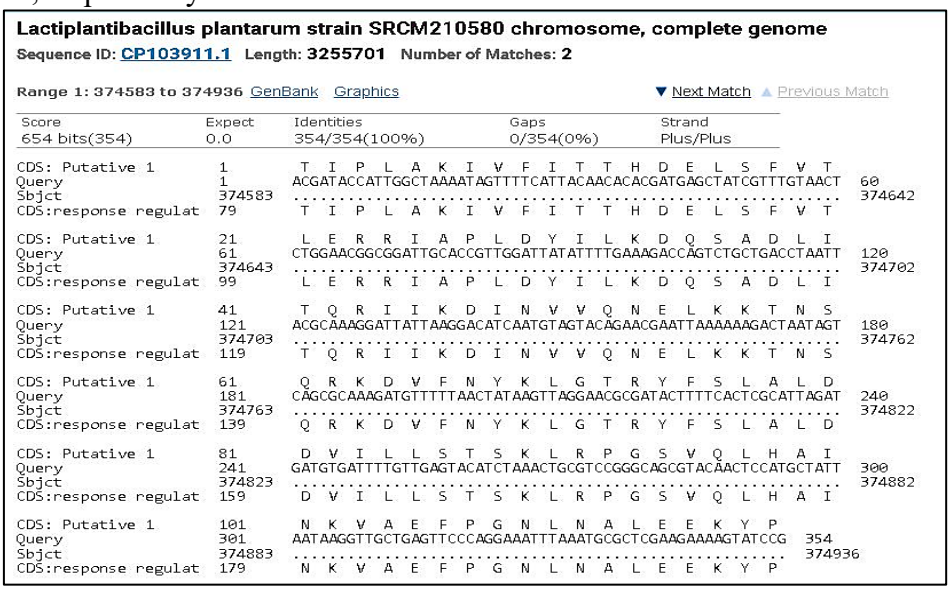


Figure 3: Detection of bacteriocin genes in *Lactobacillus* spp. (A) *orf12,* (B) *plnI,* (C) *orf38,* and *(D) PlnD.*

Sequencing of PlnD gene

The PCR products related to *plnD* gene in *Lactiplantibacillus plantarum* (SMHA16) and *Lactiplantibacillus plantarum* (SMHA208) were sent to Koria (Macrogene Company) for sequencing. The *plnD* gene was analyzed and compared with a GenBank reference strain (NCBI). BLASTN analysis at NCBI showed 100% compatibility for *Lactiplantibacillus plantarum* isolates. The nucleotide sequences were compared with those from South Korea (accession ID: CP103911.1) and showed lower identity with isolates from Taiwan (CP070847.1), China (CP073266.1), Argentina (CP031140.1), Sweden (CP059168.1, GU584090.1), and Mexico (CP129567.1) (Figures 4 and 5). The sequenced *plnD* gene for the *Lactiplantibacillus plantarum* SMHA16 and *Lactiplantibacillus plantarum* SMHA208 were recorded at the gene bank of NCBI, under the accession numbers PQ365542.1 and PQ365543.1, respectively.



Lactiplantibacillus plantarum strain SRCM210580 chromosome, complete genome

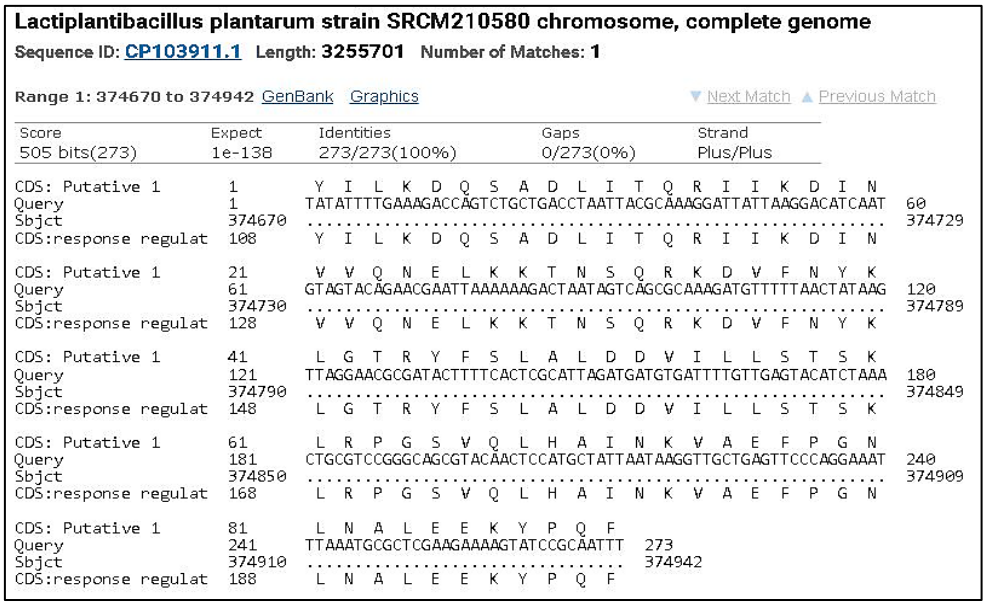
Sequence ID: CP103911.1 Length: 3255701 Number of Matches: 2

Range 1: 374583 to 374936 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
654 bits(354)	0.0	354/354(100%)	0/354(0%)	Plus/Plus

```
CDS: Putative 1       1       T  I  P  L  A  K  I  V  F  I  T  T  H  D  E  L  S  F  V  T
Query                 1       ACGATACCATTGGCTAAAATAGTTTTCATTACAACACACGATGAGCTATCGTTTGTAACT  60
Sbjct                 374583  ............................................................  374642
CDS:response regulat  79      T  I  P  L  A  K  I  V  F  I  T  T  H  D  E  L  S  F  V  T

CDS: Putative 1       21      L  E  R  R  I  A  P  L  D  Y  I  L  K  D  Q  S  A  D  L  I
Query                 61      CTGGAACGGCGGATTGCACCGTTGGATTATATTTTGAAAGACCAGTCTGCTGACCTAATT  120
Sbjct                 374643  ............................................................  374702
CDS:response regulat  99      L  E  R  R  I  A  P  L  D  Y  I  L  K  D  Q  S  A  D  L  I

CDS: Putative 1       41      T  Q  R  I  I  K  D  I  N  V  V  Q  N  E  L  K  K  T  N  S
Query                 121     ACGCAAAGGATTATTAAGGACATCAATGTAGTACAGAACGAATTAAAAAAGACTAATAGT  180
Sbjct                 374703  ............................................................  374762
CDS:response regulat  119     T  Q  R  I  I  K  D  I  N  V  V  Q  N  E  L  K  K  T  N  S

CDS: Putative 1       61      Q  R  K  D  V  F  N  Y  K  L  G  T  R  Y  F  S  L  A  L  D
Query                 181     CAGCGCAAAGATGTTTTTAACTATAAGTTAGGAACGCGATACTTTTCACTCGCATTAGAT  240
Sbjct                 374763  ............................................................  374822
CDS:response regulat  139     Q  R  K  D  V  F  N  Y  K  L  G  T  R  Y  F  S  L  A  L  D

CDS: Putative 1       81      D  V  I  L  L  S  T  S  K  L  R  P  G  S  V  Q  L  H  A  I
Query                 241     GATGTGATTTTGTTGAGTACATCTAAACTGCGTCCGGGCAGCGTACAACTCCATGCTATT  300
Sbjct                 374823  ............................................................  374882
CDS:response regulat  159     D  V  I  L  L  S  T  S  K  L  R  P  G  S  V  Q  L  H  A  I

CDS: Putative 1       101     N  K  V  A  E  F  P  G  N  L  N  A  L  E  E  K  Y  P
Query                 301     AATAAGGTTGCTGAGTTCCCAGGAAATTTAAATGCGCTCGAAGAAAAGTATCCG  354
Sbjct                 374883  ....................................................  374936
CDS:response regulat  179     N  K  V  A  E  F  P  G  N  L  N  A  L  E  E  K  Y  P
```

Figure 4: Sequences of *Lactiplantibacillus plantarum* (SMHA16) *plnD* gene matched with *Lactiplantibacillus plantarum* strain SRCM210580. No differences were found in the nucleotides of this study query and the subject.



Lactiplantibacillus plantarum strain SRCM210580 chromosome, complete genome

Sequence ID: CP103911.1 Length: 3255701 Number of Matches: 1

Range 1: 374670 to 374942 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
505 bits(273)	1e-138	273/273(100%)	0/273(0%)	Plus/Plus

```
CDS: Putative 1       1       Y  I  L  K  D  Q  S  A  D  L  I  T  Q  R  I  I  K  D  I  N
Query                 1       TATATTTTGAAAGACCAGTCTGCTGACCTAATTACGCAAAGGATTATTAAGGACATCAAT  60
Sbjct                 374670  ............................................................  374729
CDS:response regulat  108     Y  I  L  K  D  Q  S  A  D  L  I  T  Q  R  I  I  K  D  I  N

CDS: Putative 1       21      V  V  Q  N  E  L  K  K  T  N  S  Q  R  K  D  V  F  N  Y  K
Query                 61      GTAGTACAGAACGAATTAAAAAAGACTAATAGTCAGCGCAAAGATGTTTTTAACTATAAG  120
Sbjct                 374730  ............................................................  374789
CDS:response regulat  128     V  V  Q  N  E  L  K  K  T  N  S  Q  R  K  D  V  F  N  Y  K

CDS: Putative 1       41      L  G  T  R  Y  F  S  L  A  L  D  D  V  I  L  L  S  T  S  K
Query                 121     TTAGGAACGCGATACTTTTCACTCGCATTAGATGATGTGATTTTGTTGAGTACATCTAAA  180
Sbjct                 374790  ............................................................  374849
CDS:response regulat  148     L  G  T  R  Y  F  S  L  A  L  D  D  V  I  L  L  S  T  S  K

CDS: Putative 1       61      L  R  P  G  S  V  Q  L  H  A  I  N  K  V  A  E  F  P  G  N
Query                 181     CTGCGTCCGGGCAGCGTACAACTCCATGCTATTAATAAGGTTGCTGAGTTCCCAGGAAAT  240
Sbjct                 374850  ............................................................  374909
CDS:response regulat  168     L  R  P  G  S  V  Q  L  H  A  I  N  K  V  A  E  F  P  G  N

CDS: Putative 1       81      L  N  A  L  E  E  K  Y  P  Q  F
Query                 241     TTAAATGCGCTCGAAGAAAAGTATCCGCAATTT  273
Sbjct                 374910  .................................  374942
CDS:response regulat  188     L  N  A  L  E  E  K  Y  P  Q  F
```

Figure 5: Sequences of *Lactiplantibacillus plantarum* (SMHA208) *plnD* gene matched with *Lactiplantibacillus plantarum* strain SRCM210580. No differences were found in the nucleotides of this study query and the subject.

Phylogenetic analysis of plnD gene

The sequenced *plnD* gene for the two local isolates, *Lactiplantibacillus plantarum* SMHA16 and *Lactiplantibacillus plantarum* SMHA208, was displayed in the phylogenetic tree constructed by the neighbour-joining method. The optimal tree with the sum of branch length = 0.45381526 is shown in Figure 6. The tree is drawn to scale, with branch lengths (above the branches) in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the maximum composite likelihood method and are in the units of the number of base substitutions per site. This analysis involved 9 nucleotide sequences. There was a total of 249 positions in the final dataset. Phylogenetic analysis reveals that this Iraqi *Lactiplantibacillus plantarum* isolates from Baghdad province form distinct clusters and exhibit close relatedness to strains from various countries, with the highest similarity to a south Korean strain (CP103911.1) and lower similarities to strains from Taiwan (CP070847.1), China (CP073266.1), Argentina (CP031140.1), Sweden (CP059168.1), Germany (GU584090.1), and Mexico (CP129567.1).

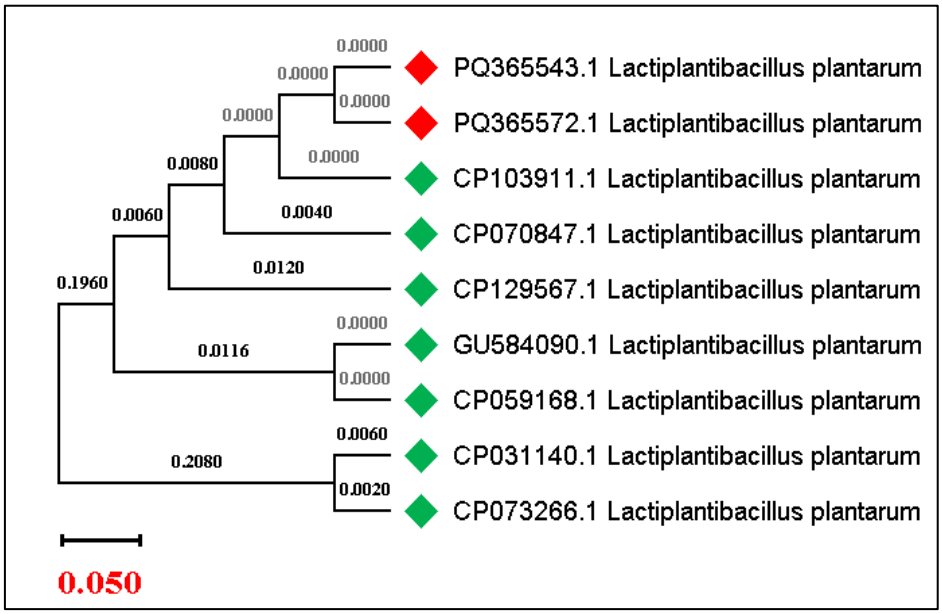


Figure 6: Phylogenetic tree including two local isolates sequences in the *plnD* (indicated by red) and 7 isolates from South Korea, Taiwan, China, Argentina, Sweden, Germany, and Mexico clustering based on the neighbor-joining maximum composite likelihood method by using the MEGA X program version 11.0.13.

The relation between the isolate habitat and the gene appearance

The investigation of some genes involved in the production of bacteriocin (*plnI, plnD, orf12*, and *orf38*) by *Lactobacillus* spp. isolates indicated the incidence of *plnI, plnD*, and *orf38* in one isolate of the two patient source isolates. However, only two of the six healthy source isolates involved the incidence of some detected genes; *plnI* and *plnD* were found in the Lac 208 (*Lactiplantibacillus plantarum* (SMHA208)) isolate, while *orf12*, and *orf38* were found in the Lac 240 *(Limosilactobacilus fermentum* (SMHA240)) isolate (Table 12). The incidence of the detected 4 genes involved in the production of bacteriocin in the eight tested isolates revealed a highly significant difference (P-value = 0.0078 **). It was found that 60% of *Lactobacillus* spp. that were isolated from stool contain bacteriocin-encoding genes [12]. Meanwhile, there was a higher percentage of positive bacteriocin genes in healthy and IBD-recovered patients than in IBD patients. The presence of these genes is related to gut health,

and their higher prevalence in healthy and recovered individuals indicates their potential role in maintaining gut health and aiding recovery from inflammatory conditions like IBD

Table 12: The relationship between the gene presence in *Lactobacillus* spp. isolates and their habitat (patient and healthy).

The genes	The isolates source							
	Patient (No.2)		Healthy (No.6)					
	Lac16	Lac30	Lac208	Lac210	Lac224	Lac228	Lac240	Lac253
plnI	+	-	+	-	-	-	-	-
PlnD	+	-	+	-	-	-	-	-
orf12	-	-	-	-	-	-	+	-
orf38	+	-	-	-	-	-	+	-
P-value	0.0078 **							
** (P<0.01)								

+: sign denotes the presence gene, - : denotes the absence gene.

The relation between bacteriocin production and the appearance of gene

The relation between the presence of *plnI*, *plnD*, *orf12*, and *orf38* genes with bacteriocin production activity, the diameter of the inhibitory zone, by *Lactobacillus* spp. against *Salmonella typhi*, *Shigella* spp., and *Aeromonas hydrophila* was determined. The isolates *Lactiplantibacillus plantarum* (SMHA16), *Lactiplantibacillus plantarum* (SMHA208), and *Limosilactobacilus fermentum* (SMHA240) revealed the incidence of the detected genes and showed a higher average of bacteriocin production activity. On the other hand, the isolates *Limosilactobacillus fermentum* (SMHA30), *Lacticaseibacillus rhamnosus* (SMHA228), *Limosilactobacillus fermentum* (SMHA210), and *Limosilactobacillus fermentum* (SMHA224) which revealed the absence of the detected genes, showed a lower average of bacteriocin production activity (Table 13). Meanwhile, the isolate Lac 253 revealed the absence of the detected genes and a higher average of bacteriocin production activity, and this may be due to the presence of other undetected genes.

A study investigated the relationship between genes responsible for bacteriocin production through gene expression analysis using real-time PCR, by interacting with pathogenic bacteria. Their study revealed that *L. plantarum* No.14 was positive for 11 genes and demonstrated the highest bacteriocin production [6]. Therefore, they suggested that this strain could be used as an antimicrobial agent against enteric bacteria.

The strain ZBK1-5 of *Lactiplantibacillus plantarum* derived from fermented ginger revealed the presence of *plnEF*, *plnA*, and *plnKJ* genes, bacteriocin-related genes, suggesting the impact of habitat on the presence of specific bacteriocin gene clusters [36].

Table13: The relation between bacteriocin production and gene presence.

***Lactobacillus* spp.**	**Diameter of the inhibition zone**	**The genes for bacteriocin**			
		PlnD	***plnI***	***orf38***	***orf12***
Lac16 (*Lactiplantibacillus plantarum* (SMHA 16))	16	+	+	+	-
Lac30 (*Limosilactobacillus fermentum* (SMHA30))	13	-	-	-	-
Lac208 (*Lactiplantibacillus plantarum* (SMHA208))	15.8	+	+	-	-
Lac210 (*Limosilactobacillus fermentum* (SMHA210))	11.2	-	-	-	-
Lac224 (*Limosilactobacillus fermentum* (SMHA224))	11.2	-	-	-	-
Lac228 (*Lacticaseibacillus rhamnosus* (SMHA228))	13	-	-	-	-
Lac240 (*Limosilactobacillus fermentum* (SMHA240))	14.2	-	-	+	+
Lac253 (*Lacticaseibacillus rhamnosus* (SMHA253))	15.1	-	-	-	-

4. Conclusion

Lactobacillus spp. isolates were more prevalent in healthy individuals than in patients with diarrhea, with the highest frequency observed among infants and young children, decreasing with increased age. The diarrhea-associated pathogens bacteria, *Salmonella typhi*, *Shigella* spp., and *Aeromonas hydrophila*, emerged as the most prevalent. Notably, *Limosilactobacillus fermentum* exhibited a higher prevalence in stool samples. *Lactiplantibacillus plantarum* exhibited more bacteriocin production against the pathogenic bacteria. *Lactiplantibacillus plantarum* (SMHA16) harboring *plnD*, *plnI*, and *orf30* genes demonstrated higher bacteriocin production efficacy, which indicates the relation between the incidence of these genes and bacteriocin production. On the other hand, *Lacticaseibacillus rhamnosus* (SMHA253)that demonstrated high bacteriocin production despite the absence of the detected genes, suggesting the involvement of other genes.

Bacteriocin production is associated with the habitat of isolates and the presence of specific genes, highlighting its role as an antibacterial agent and its contribution to gut health. This positive correlation of the genes incident and healthy habitat in enhancing bacteriocin production suggests that the bacteriocin production of *Lactobacillus* spp. are key factors that contribute to human intestinal health and combat pathogenic bacteria.

Acknowledgment

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Disclosure and conflict of interest

The authors declare that they have no conflict of interest.

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