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# A New Record of Lactic Acid Bacteria Strains from the Contents of Adult Chicken Intestines

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**Abstract**: The study was conducted in the Microbiology Laboratory of the Department of Food Science at Basrah University's, Faculty of Agriculture from 15/11/2020 to 26/12/2020, to study the flora of lactic acid bacteria in digestive parts (Jejunum, Ileum, and Ceca) for healthy adult chickens and identifying the target part that contains the largest number of lactic acid bacteria for different types of poultry. As a result, as well as isolating bacterial colonies after three times, and being genetically Identified with PCR, seven new species of lactic acid bacteria were acquired and registered at the National Center for Biotechnology Information (NCBI) as new local strains, both in Iraq and around the world, as follows: *Lactobacillus gasseri* strain Al-Salhi-1, *Lactobacillus helveticus* strain Al-Salhi-2, *Lactiplantibacillus plantarum* strain Al-Salhi-3, *Limosilactobacillus reuteri* strain AhQuSa-1, *Limosilactobacillus* sp. strain AhQuSa-2, *Ligilactobacillus salivarius* strain AhQuSa-3, *Lactobacillus Johnsonii* strain AhQuSa-4. The results also showed a significant superiority ($P \leq 0.05$) in the logarithmic numbers of lactic acid bacteria in the jejunum for each of : the group of birds bred in local environments, as well as in the group of birds bred in commercial fields in comparison with the Ileum and Ceca, as the bacterial content was in the group of local birds : 6.52, 5.21, 4.15 (Cfu/g) for each of the jejunum, ileum and Ceca, respectively, while in the group of commercial birds it reached: 6.35, 5.02, 3.92 (Cfu/g) for each of the jejunum, ileum and Ceca, respectively.

**Keywords:** Intestinal microflora, *Lactobacillus*, Microbial Count, Poultry, Strains.

## Introduction

The intestinal flora in the gut of domestic birds consists of a symbiotic microbial community. Markowiak & Śliżewska (2018) mentioned that about 90% of the intestinal flora consists of lactic acid-producing bacteria such as *Lactobacillus* and *Enterococcus*. The remaining percentage, about 10%, includes *Escherichia coli*, *Clostridium, Staphylococcus, Pseudomonas*, and others. The pH of the poultry's digestive tract has been divided into five areas, each ideal for a certain type of bacteria. These bacteria can be found throughout the digestive tract. Lactic acid bacteria are less acid resistant than others. They cannot grow spontaneously and in significant numbers in this location, as they cannot in other sections of the digestive system (glandular stomach, gizzard, and small intestine). Other bacterial species cannot grow in it because the pH is low, ranging between 1-

2, save for bacteria resistant to high acidity and creating huge quantities of lactic acid, such as Lactobacilli and Enterococci. In contrast, in the large intestine and the cecum, Clostridia and Bacilli predominate (Shang *et al.,* 2018). The intestinal flora present in the gut of domestic birds into two groups, according to the degree of their endemicity, namely:

Bacteria that are freely present in the space of the alimentary canal, such as *Enterococcus faecum*, cannot adhere to or settle in the epithelial layer lining the alimentary canal for the intestines.

The bacteria that are endemic in the alimentary canal and can stick to the epithelial layer lining the alimentary canal, such as *Lactobacillus* bacteria, and their adhesion helps to supply the alimentary canal with continuous movements, especially in areas where the worm's movement increases and here lies the importance of adhesion (Akalu *et al.,* 2017; Mohamed *et al*., 2019).

Lactic acid bacteria have multiple benefits. Their biological properties improve the value of nutritional compounds (Ghazal *et al*., 2021). They contribute to decreasing cholesterol (Nasser *et al.,* 2021) and are included in the manufacture of probiotics, which are considered functional foods.

The current study investigates the microbial content of parts of the alimentary canal and different types of adult poultry bred in commercial fields to determine the largest microbial community of beneficial bacteria and name their location in the gut parts. Moreover, the study aims to confirm the quality of the dominant microorganisms in the gut of poultry by polymerase chain reaction PCR technique and determine the type of lactic acid bacteria present in the small intestine.

## Materials & Methods

This study was conducted in the Microbiology Laboratory of the Department of Food Science at the College of Agriculture at Basrah University from 15/11/2020 to 26/12/2020; To isolate and purify microorganisms from the intestines of adult chickens and study their types and numbers. Fig. (1) shows the design scheme of the experiment.

### Culture Media

The culture media used in the study, Nutrient Agar, MRS Agar, and MRS Broth, were prepared according to the manufacturer's instructions, then were sterilized by Autoclave at 121°C, and at a pressure of 15 pound / inch$^2$, for 15 minutes, Skim Milk was used as a carrier to activate bacterial cultures, and 0.1% Peptone was used to prepare decadal dilutions (Da Silva *et al*., 2019).

### Total bacterial counts in poultry alimentary parts

Bacterial counts of total and lactic acid bacteria, were carried out using a pour plate method (Da Silva *et al.,* 2019).

### Isolation of lactic acid bacteria

After the birds were slaughtered and the intestine parts (Jejunum, Ileum, and Ceca) were removed, samples were taken from those parts by cotton swabs and sterile conditions. Anaerobic at 37°C for 48 hours, purification and screening were carried out by loop and streaking method on MRS Agar medium.

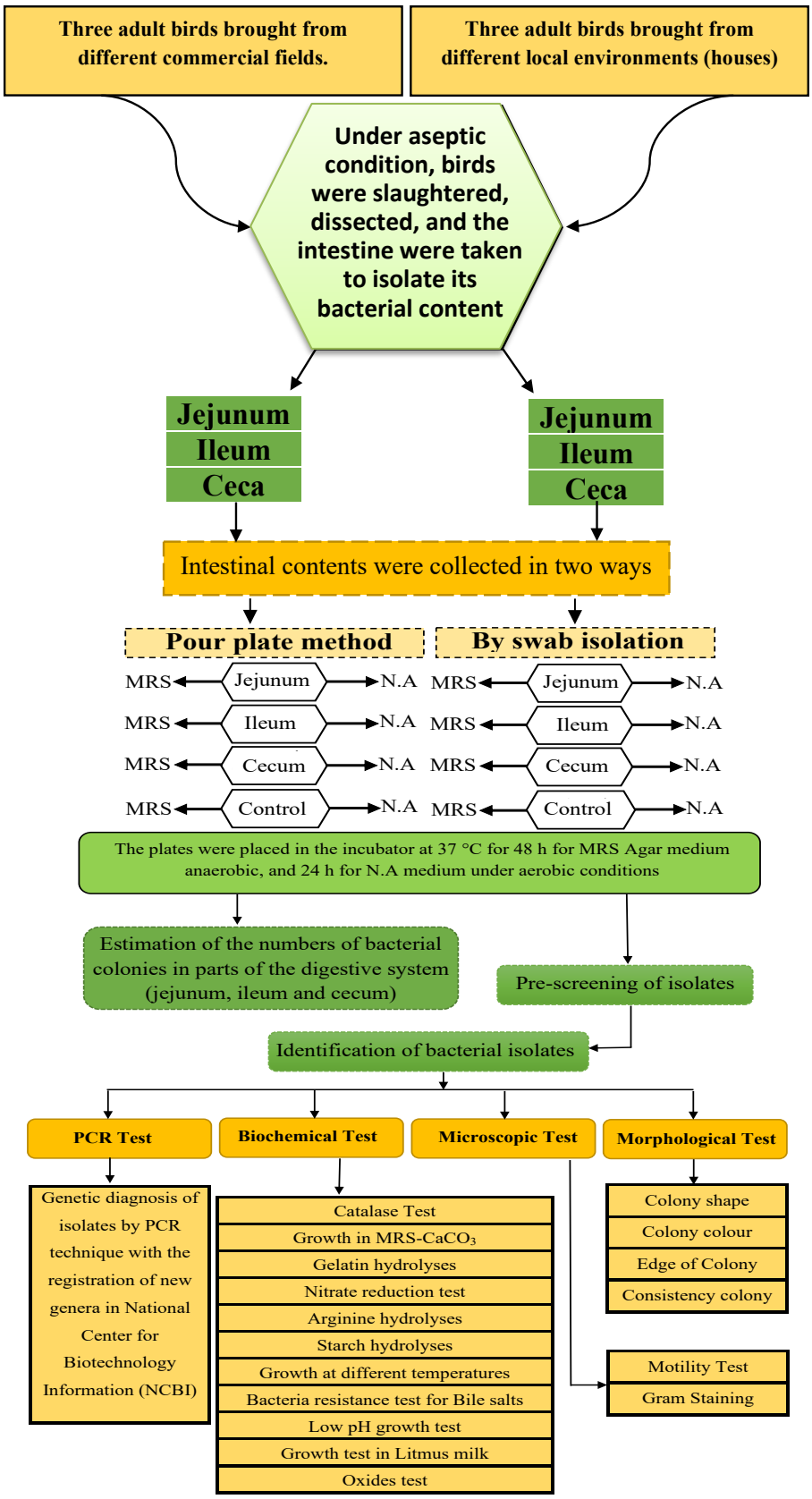



**Fig. (1): Experiment design scheme.**

**Purification of bacterial isolates**

After preparing the culture media, the medium used MRS agar was inoculated with the loop carrier from the media containing the activated primary isolates. Then the plates were incubated at 37°C for 48 hours under anaerobic conditions. The process was repeated three times in succession to obtain pure and single colonies (where the convex and spindle-shaped and creamy white colonies were selected) and conduct diagnostic tests for these isolates.

**Methods of preserving bacterial isolates**

About the daily work cultures, the use and preservation of isolates, they are inoculum in the MRS broth medium and incubated for 48 hours under anaerobic conditions. After incubation, they are kept in the refrigerator until use with the renewal of the cultures every month. As for long-term preservation cultures, sterile glycerol is added by 20% to MRS Broth sterile culture medium and inoculated with bacteria growing on it at 48 hours after incubation. They were then stored at -20°C (Jain *et al*., 2020).

**Activation of bacterial isolates**

Before each test, the process of activating the isolates was performed by taking a part of the developing colony, mediated by the loop carrier (Loop) and spreading it on the MRS Agar solid medium; then, they were transferred to the incubator at a temperature of 37°C for 48 hours.

**Identification of bacterial isolates**

The selected isolates were identified and obtained after purification three successive times, as these bacterial isolates were diagnosed based on phenotypic, microscopic, and biochemical tests and were confirmed by genetic examination using PCR technique.

**Morphological examinations**

After growing the selected bacterial isolates on MRS Agar culture media, the morphological characteristics of the bacterial colonies were studied in terms of color, size, elevation, colony edge, external appearance, and the emission of the characteristic lactic acid smell after opening the dishes.

**Microscopic tests**

The sterile MRS Agar medium plates were inoculated with the selected colonies and incubated at 37°C for 48 hours under anaerobic conditions. After which, the Gram staining and all bacterial cultures in preparation for microscopic examination to identify the shape and motility of bacterial cells (Johnson & Case, 2019)

**Biochemical Tests**

The biochemical tests for bacterial colonies included a wide range of confirmatory tests, which are:

Catalase test, Growth of MRS-$CaCO_3$ (Procop *et al*., 2017), Gelatin hydrolysis test, Nitrate reduction test, Arginine hydrolysis test, Starch hydrolysis test, Growth in different temperature, Bile salt resistance test, Oxidase test, Growth test in Litmus Milk, Low pH resistance test pH=4 (Cappuccino & Welsh, 2019).

**Stages of the molecular assay (PCR) for selected isolates**

**DNA extraction**

The ready-made kits supplied by the Korean company Intron biotechnology/Korea were used as the 17045 i-genomic BYF DNA Extraction Mini Kit to extract deoxygenated DNA from the selected bacterial samples. After the purification process that was conducted three times in a row, where the process of DNA extraction and amplification of PCR products was carried out in the

laboratory of Wahej Al-Dana Company (for training, rehabilitation, and services) in Baghdad, according to the protocol attached with the ready-made kit.

**Detection of deoxyribonucleic acid**

Electrophoresis was carried out for the detection of DNA fragments after the extraction process, and based on Sambrook *et al.* (2001) according to the following steps:

**Preparation of agarose gel**

Agarose gel was prepared at a concentration of 1.5% by dissolving 1.5 g of agarose gel in 100 ml of TBE transfer solution, which is included with the kits.

**Sample preparation**

Macerate 3 µl of the Processor Loading Buffer prepared by the Korean company (Intron/ Korea) was mixed with 5 µl of DNA samples. After the mixing process, the pits of the gel were filled, and the electric power. The supply was turned on at a voltage of 60 volts for 1 hour. Then the gel was lifted and immersed in a basin containing a solution consisting of 30 µl of Red safe dye with 500 ml of distilled water. Then, the gel was placed in a UV generator with a wavelength of 336 nm to observe the fluorescence of the DNA bundles that were extracted from lactic acid bacteria.

**Gene amplification**

The primer prepared by the Korean company Bioneer for *Lactobacillus* bacteria was used to amplify the 16sRNA gene. According to what was mentioned by Walter *et al.* (2000) and table (1) shows, the sequences and properties of the primer used to amplify the 16sRNA gene.

**Table (1): Sequences and properties of the primer used to amplify the 16sRNA gene.**

| primer | First sequences | Amplification temperature | GC (%) |
|---|---|---|---|
| Forward | 5'-TCGCTAGTAATCGCGGATCAGC - 3' | 61.6 | 54.6 |
| Reverse | 5'-GCATATCGGTGTTAGTCCCGTCC - 3’ | 62.0 | 56.5 |

**Table (2): A phase that has been programmed in a PCR device to amplify the gene.**

| No. | phase | Temperature ͦc | Time | Number of cycles |
|---|---|---|---|---|
| **1** | Initial Denaturation | 95 | 3 min | 1 cycle |
| **2** | Denaturation -2 | 95 | 45 Sec | 35 cycle |
| **3** | Annealing | 48 | 45 Sec | |
| **4** | Extension-1 | 72 | 1min | |
| **5** | Extension -2 | 72 | 7 min | 1 cycle |

The amplification was performed using a PCR device. The device was adjusted by entering the data for the 16sRNA amplification program and table (2) explaining the steps programmed to provide the optimal conditions for amplifying the 16SrRNA gene.

**Electrophoreses of DNA amplification products**

PCR products using Agarose gel, with a concentration of 1.5% and a 5volt and 1% TBE buffer, were left at a 1.5 hour, using a DNA ladder ranging from (100-1500) bp.

**DNA extraction from agarose gel**

DNA was extracted from agarose gel (Vogelstein & Gillespie, 1979)

**Genetic sequence and gene analysis**

The genetic sequence was performed at the Biotechnology Laboratory at the National Instrumentation Center for Environmental Management (NICEM) in South Korea using the DNA sequencer 3730XL application Biosystem. After relaying PCR products to the 2% agarose gel concentration and exposing it to UV rays, with a 302 nm wavelength. It was stained with red dye. The results were analyzed to detect the type of genetically diagnosed isolation using Basic Local Alignment, available at the National Center for Biotechnology Information.

**Statistical analysis**

The complete random design (CRD) was used to study the effect of different treatments on the qualities studied and compared the moral differences between averages by Duncan test multi-border below the moral level of 0.05, and the program SPSS (2018) was used in statistical analysis.

## Results & Discussion

**Bacterial counting results for the contents of digestive parts**

It is clear from figs. (2 and 3) the presence of total bacteria and lactic acid bacteria in parts of the digestive system (Jejunum, Ileum, and Ceca) of the group of birds raised in local environments and the group of birds raised in commercial fields. The statistical analysis results of the logistic numbers of lactic acid bacteria show high moral differences ($P \leq 0.05$) in the Jejunum area compared to the Ileum. Moreover, Ceca regions, where the microbial content of lactic acid bacteria in the group of birds raised in local environments in form (2): 6.52, 5.21, and 4.15 ($cfu.g^{-1}$) for both Jejunum, Ileum, and Ceca, respectively. The results do not differ much in the group of birds raised in the Commercial fields in terms of lactic acid bacteria in parts of the digestive system (Jejunum, Ileum, and Ceca), amounted to: 6.35, 5.02, and 3.92 ($cfu.g^{-1}$) per part respectively.

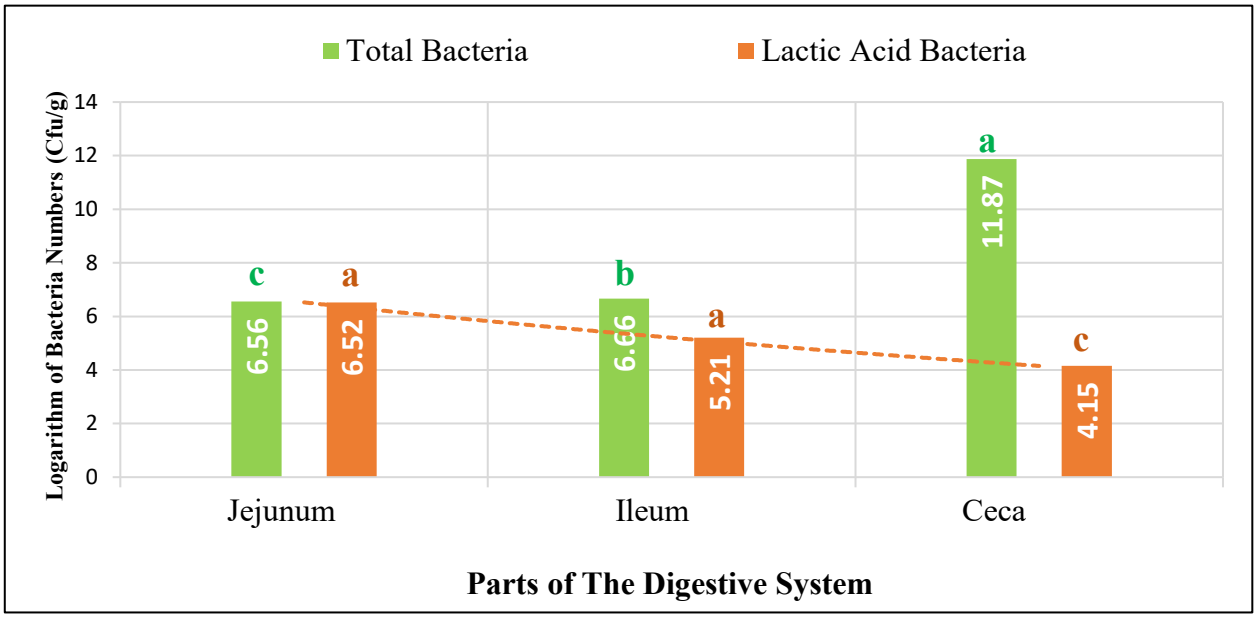
This indicates that total bacteria approach the results of lactic acid bacteria, and the total bacteria in the Jejunum area mostly of lactic acid bacteria (beneficial), unlike other parts (Ileum and Ceca) in which they are concentrated less, because of the presence of other types of different bacteria, specifically in the Ceca. According to the indicators of the total bacteria for both groups, and through the results we reached, the jejunum area in the small intestine was determined because it occupies a large number of lactic acid bacteria and is mostly pure. This result agreed with Sjofjan & Adli (2020) that the numbers of lactic acid bacteria are concentrated in the jejunum part in poultry.

**Isolated lactic acid bacteria from the Jejunum**

The isolation of lactic acid bacteria colonies from the Jejunum area of the small intestine of different types of domestic birds showed 27 bacterial isolates. After being isolated, 20 bacterial isolates were selected (understudy), excluding seven bacterial isolations, was excluded; Because it's duplicate, according to the biochemical tests of the Bergey's manual, and table (3) shows the results of the initial isolation of bacterial colonies.

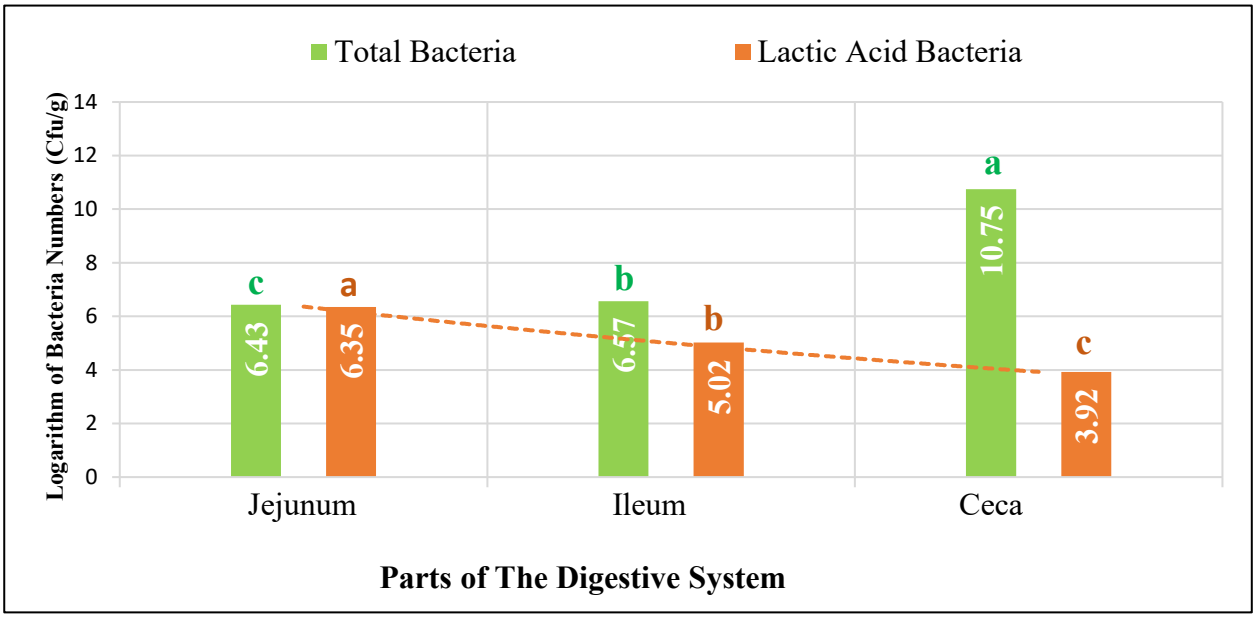
**Table (3): Results of the initial isolation of bacterial colonies.**

| No. | Total number of isolates | Number of selected isolates | Number of isolates excluded |
|---|---|---|---|
| 1 | 27 | 20 | 7 |




Characters in different columns within the same color indicate moral differences in bacterial content, between groups of parts of the digestive system (Jejunum, Ileum, and Ceca) at (P≤0.05).

**Fig. (2): The numbers of total bacteria and lactic acid bacteria present in parts of the digestive system (Jejunum, Ileum, and Ceca) of the group of domestic birds raised in local environments.**




Characters in different columns within the same color indicate moral differences in bacterial content, between groups of parts of the digestive system (Jejunum, Ileum, and Ceca) at (P≤0.05).

**Fig. (3): The numbers of total bacteria and lactic acid bacteria present in parts of the digestive system (Jejunum, Ileum, and Ceca), for the group of poultry raised in commercial fields.**

**Table (4): Results of tests for morphology and microscopy of bacteria isolated**

| Morphological tests | | Colony colour | Colony shape | Gram staining | Motility | Spores formation |
|---|---|---|---|---|---|---|
| Bacterial colonies isolated from the Jejunum area | 1 | cream | stick | + | - | - |
| | 2 | cream | stick | + | - | - |
| | 3 | cream | stick | + | - | - |
| | 4 | cream | stick | + | - | - |
| | 5 | cream | stick | + | - | - |
| | 6 | cream | stick | + | - | - |
| | 7 | cream | stick | + | - | - |
| | 8 | cream | stick | + | - | - |
| | 9 | cream | stick | + | - | - |
| | 10 | cream | stick | + | - | - |
| | 11 | cream | stick | + | - | - |
| | 12 | cream | stick | + | - | - |
| | 13 | cream | stick | + | - | - |
| | 14 | cream | stick | + | - | - |
| | 15 | cream | stick | + | - | - |
| | 16 | cream | stick | + | - | - |
| | 17 | cream | stick | + | - | - |
| | 18 | cream | stick | + | - | - |
| | 19 | cream | stick | + | - | - |
| | 20 | cream | stick | + | - | - |

- + : Means positive for the Gram stain.
- - : Means immovable and non-formed for the spores .

## Morphological and microscopic tests of bacterial isolated

Table (4) shows the results of morphological (appearance) and microscopic examinations of bacterial colonies isolated from the Jejunum area in the small intestine of local and commercial domestic birds. It turns out that all bacterial colonies isolated from the Jejunum area of the small intestine of birds gave the same results in terms of color, shape, and general casting as well as the results of the examination of motility and formation of spores, where the colors of all colonies appeared in creamy color and bacilli shape and gave results positive for gram stain. It also gave negative results in its motility and formation of spores. These results agreed with the results of microscopic examinations mentioned in Bergey's Manual (Vos *et al.,* 2009).

## Results of biochemical tests of bacteria

It is clear from table (5) that the results of chemical tests of bacterial colonies isolated from the Jejunum area in the small intestine of local and commercial domestic birds, like some results of chemical tests of lactic acid bacteria colonies, showed a positive result. This means it can grow in the media of MRS-$CaCO_3$, grow in different temperatures (35 and 45) $^{\circ}$C, carry it for bile salts 0.3%, low pH (pH=4) in addition to its ability to grow in Litmus milk, it also gave a negative result of oxidase test.

Other tests of lactic acid bacteria colonies showed a negative result in the catalase test, gelatin hydrolysis, reduced nitrates, and ammonia production from arginine. These results agree with the results of biochemical tests mentioned in Bergey's Manual (Holt, 2004).

**Table (5): Results of biochemical tests of isolates bacteria from the jejunum.**

| Diagnostic tests | Bacterial isolates | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
| Catalase | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| MRS - $CaCO_3$ | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| hydrolysis of Gelatin | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Nitrate reduction | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Ammonia formation of arginine | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Growth at 35 °C | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Growth at 45 °C | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Bile Salts 0.3% | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Growth in pH=4 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Growth in Litmus milk | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Starch hydrolysis | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Oxidase test | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

- + : Means growth of bacterial colonies.
- - : Means no growth of bacterial colonies.

**Results of PCR identification of lactic acid bacteria**

The results of the identification of lactic acid bacteria with PCR, bacterial gene bands, 1250 bp genes compared to the standard marker as shown in figs. (4) and (5). The presence of some lactic acid bacteria extensively in the Jejunum area through their frequent appearance despite the different sequence of some nitrogen bases.

**Registration of lactic acid bacteria isolates from the jejunum**

After boycotting the results of the isolations mentioned in table (6), the screening of isolates was carried out by excluding repeated isolates, where seven pure bacterial isolations were obtained from lactic acid bacteria strains and registered at the National Center for Biotechnology Information (NCBI). A new record, some at the level of Iraq and others worldwide, with the frequency ratio for each bacterial isolation recorded and by table (6).

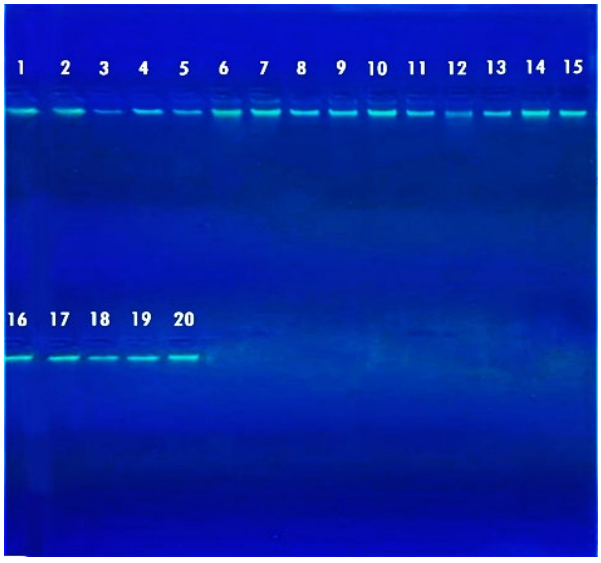



**Fig. (4): Electrophoresis using Agarose gel 1% for genome lactic acid bacteria isolates, 5 volt /cm$^2$, for 1:15 hours and with the technique of ready-made extraction kit from The Korean Company Microgen.**

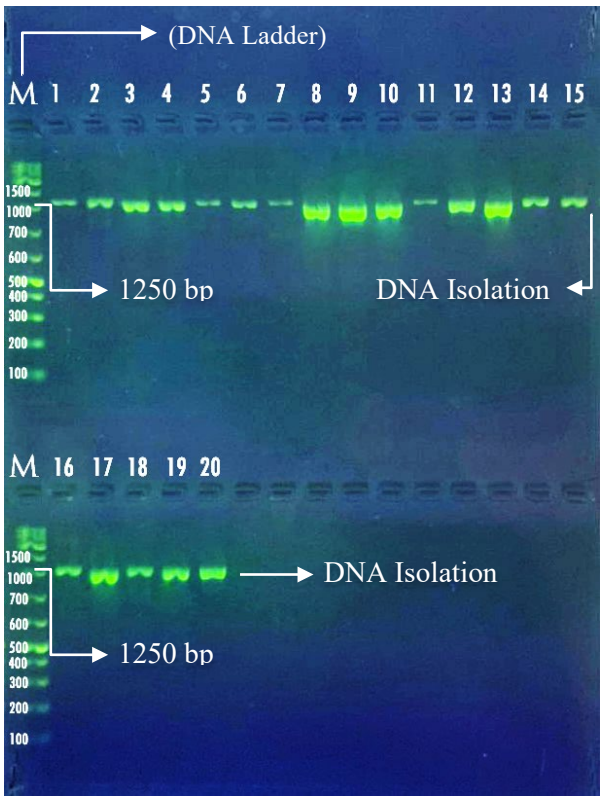



**Fig. (5): Electrophoresis product bands for genome 16sRNA (1250bp) for PCR Isolated Bacteria on Agarose gel 1.5%, 5 Volt/Cm²**

**Registration of lactic acid bacteria isolates from the jejunum**

After boycotting the results of the isolations mentioned in table (6), the screening of isolates was carried out by excluding repeated isolates, where seven pure bacterial isolations were obtained from lactic acid bacteria strains and registered at the National Center for Biotechnology Information (NCBI). A new record, some at the level of Iraq and others worldwide, with the frequency ratio for each bacterial isolation recorded and by table (6).

The phylogenetic tree of each strain was made separately for the said strains. A general evolutionary genetic tree between the studied species, and the following figure: (6), (7), (8), (9), (10), (11), (12) and (13) This is illustrated, with the calculation of the genetic distance between local and global strains and according to the following tables: (7), (8), (9), (10), (11), (12), (13) and (14).

**Table (6): bacterial strains recorded in NCBI with frequency ratio.**

| No. | Bacterial Strain | Accession no | Freq. | % |
|---|---|---|---|---|
| 1 | *Lactobacillus gasseri* strain Al-Salhi-1 | MW848596 | 5 | 25 |
| 2 | *Lactobacillus helveticus* strain Al-Salhi-2 | MW848597 | 6 | 30 |
| 3 | *Lactiplantibacillus plantarum* strain Al-Salhi-3 | MW848598 | 1 | 5 |
| 4 | *Limosilactobacillus reuteri* strain AhQuSa-1 | MW764103 | 4 | 20 |
| 5 | *Limosilactobacillus* sp. strain AhQuSa-2 | MW764104 | 1 | 5 |
| 6 | *Ligilactobacillus salivarius* strain AhQuSa-3 | MW764105 | 1 | 5 |
| 7 | *Lactobacillus johnsonii* strain AhQuSa-4 | MW764106 | 2 | 10 |
| **Total** | | | 20 | 100 |

The attached titles of bacterial strains (Al-Salhi-3, Al-Salhi-2, Al-Salhi-1) refer to the new label of bacterial strain, which is adapted from the title of the first researcher Ahmed Ali Kadhem Al-Salhi.

As well as for other right-1(AhQuSa-4, AhQuSa-3, AhQuSa-2, AhQuSa-1), attached to the names of bacterial insulation, they are adapted from the first two letters, for each of the three researchers below, respectively.

1-Ahmed Ali Kadhem Al-Salhi.

2-Qutaiba Jasim Gheni Al Khfaji.

3-Sabah Malik Habeeb Al-Shatty.

Fig. (6) shows the Phylogenetic tree for the first bacterial strain registered at the National Center for Biotechnology Information (Lactobacillus gasseri strain Al-salhi-1) is illustrated by the phylogenetic tree of Lactobacillus gasseri bacteria, with four main branches, including six strains, including the local Iraqi strains registered with MW848596.1. In addition to other Chinese strains and strains from South Korea, a match rate of 100%, while the other three branches included one strain each, all strains from china by a match of 99 %.

Fig. (7) shows the phylogenetic tree for the second bacterial strain. It was registered at the National Center for Biotechnology Information *Lactobacillus helveticus* strain Al-Salhi-2. The phylogenetic tree of these bacteria, the presence of two main branches of it, including branch and sixteen global strains from different countries including (America, France, Japan, Kazakhstan, China, Bulgaria, Germany, Switzerland, India, and Korea) with a match rate of 100%. While the local Iraqi strain *Lactobacillus helveticus* strain Al-Salhi-2, registered with MW848597.1 in its branch, meaning that it was registered for the first time in Iraq and the world, it is of Iraqi origin and origin and is 100% identical.

Fig. (8) explains the phylogenetic tree of the third bacterial strain registered at the National Center for Biotechnology Information: *L. plantarum* strain Al-Salhi-3. It shows the presence of four main branches of it. The first branch included the presence of sixteen strains, including the local Iraqi strain registered with the aforementioned name, with a percentage of 100% match. As for the other three branches, each included one strain, distributed in Nigeria, Brazil, and Thailand with a percentage of 99%.

Fig. (9) shows the presence of three main branches of it. The first branch included the presence of 17 global strains from different countries (France, Poland, Iran, India, America, Bangladesh, China, Germany, South Korea, and Thailand) with a percentage of 100% match. In contrast, the branch The second included Chinese strain with a matching rate of 99%, while the local Iraqi strain was unique to a special branch alone and was registered with the number MW764103.1 with a matching rate of 99%, and this means that this strain is of Iraqi origin and was isolated for the first time in Iraq and the world.

Fig. (10) explaines the presence of three main branches of strains. The first branch included the presence of three Canadian strains with an identical percentage of 99%, while the second branch included the local and recorded Iraqi strain *Limosilactobacillus* sp. strain AhQuSa-2 with a matching percentage of 99%. In contrast, the third section included one Portuguese strain with a percentage of 94%.

Fig. (11) demostrates the phylogenetic tree of the local strain registered in the National Center for Biotechnology Information *Ligilactobacillus salivarius* strain AhQuSa-3 shows the presence of three main branches. The first branch included five strains from China and Poland, with a percentage of 100% match. The second section included three strains from India and Ireland with a percentage of 100 %, while the local Iraqi strain has its branch, and it was registered with the number MW764105.1 with a matching percentage of 99%, which indicates that this local origin is Iraq and has nothing to do with other global strains.

The phylogenetic tree of the local strain in fig. (12) exhibits the presence of four branches. The first branch included eight strains of lactic acid bacteria previously isolated from different countries (France, Spain, China, Poland, and Japan) with a percentage of 100% match, while the second branch included one strain from In South Korea with a matching ratio of 99%. The local Iraqi strain was isolated to the third branch with a matching percentage of 99%, which was recorded by the name and number *Lactobacillus johnsonii* strain AhQuSa-4 MW764106.1, while the fourth branch of the tree included one Chinese strain with a matching rate of 99%.

Fig. (13) displays the presence of four main branches of the recorded local Iraqi bacterial strains, where the first branch included the presence of three strains of *Lactobacillus helveticus* with a percentage of 100% matching. In contrast, the second section included the presence of one local strain with a percentage of 85% matching *Limosilactobacillus* sp. strain AhQuSa-2, and the third branch included the presence of three local strains with a percentage of 90%, 91%, and 89% of each of *L. johnsonii*, *Limosilactobacillus reuteri* and *Ligilactobacillus salivarius*, respectively. The fourth section included two strains of each of *Lactobacillus gasseri* and *Lactobacillus Plantarum* with a percentage of 91% for both of them.

These bacterial strains: *L. johnsonii*, *Lactobacillus gasseri*, *Ligilactobacillus salivarius,* and *Limosilactobacillus reuteri* that were reported in NCBI, are consistent with other international studies (Dec *et al*., 2018; Markowiak & Śliżewska, 2018; Wang *et al*., 2020). While the bacterial strains *Lactobacillus helveticus* and *Lactiplantibacillus plantarum* agreed with the study (Jha *et al*., 2020). As for the bacterial strain *Limosilactobacillus* sp., it is Novel Lactobacillaceae strans (Vieco-Saiz *et al.,* 2022).

Table (7) shows the genetic distance of local strain *Lactobacillus gasseri* Strain Al-Salhi-1 with Global strains, showing no difference between a local strain and other global strains, except three strains in Wuhan, China, with a genetic distance of 0.0010.

It is clear from table (8) that the genetic distance of the local strain registered in the National Center for Biotechnology Information *Lactobacillus helveticus* strain Al-Salhi-2 with global strains. The highest genetic distance between it and all global strains was 7.0326, and there is no affinity between it and global strains. It does not grow except in the Iraqi environment, a new record.

It is clear from table (9) the genetic distance of the local strain *Lactiplantibacillus plantarum* strain Al-Salhi-3 with the global strains. The highest genetic distance between the local strain and each of the Thai, Brazilian and Nigerian strains was 0.0021, while there is no genetic distance between it and the other strains. This means that they are completely identical and close to it.

Table (10) shows the genetic distance of the local isolate registered in the National Center for Biotechnology Information *Limosilactobacillus reuteri* strain AhQuSa-1 with global strains, where the lowest genetic distance recorded between it and the Chinese isolate with a genetic distance of 0.0021. While the longest genetic distance between the local strain and other global strains. It reached 0.0032 for Chinese, Korean, German, Bangladeshi, American, Japanese, Iranian, French, and Polish strains.

Table (11) shows the genetic distance of the local strain *Limosilactobacillus* sp. strain

AhQuSa-2 with global strains. The lowest genetic distance was 0.0041 with Canadian strains, and the largest distance was 0.0339 with Portuguese strains.

Table (12) shows the values of the genetic distance of the local strain *Ligilactobacillus salivarius* strain AhQuSa-3 with global strains, where the lowest distance was with the Indian and Irish strains reached 0.0022, while the largest distance was with Chinese and Polish strains, which was 0.0033.

Table (13) shows the genetic distance of the local strain *Lactobacillus johnsonii* strain AhQuSa-4 with global strains, where the lowest genetic distance was 0.0011 with eight global strains from China, Japan, Poland, France, and Spain, while the highest genetic distance was with the Chinese strain, which amounted to 0.0033. It is known that the closer the genetic distance, the more similar the strain is to its counterpart and vice versa.

Table (14) shows the genetic distance of the seven local strains, where the farthest genetic distance was 11.4613. It was between the two local strains, *Limosilactobacillus reuteri* strain AhQuSa-1 and *L. plantarum* strain Al-Salhi-3. In contrast, the lowest genetic distance between the two local isolates was *Limosilactobacillus reuteri* strain AhQuSa -1 and *Ligilactobacillus salivarius* strain AhQuSa-3, amounted to 5.6368, which indicates the presence of genetic affinity between the two local strains.

**Table (7): Genetic distance of local strain: *L. gasseri* strain Al-Salhi-1 with global strains.**

| Global Bacterial Strains | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| MW848596.1 *Lactobacillus gasseri* strain Al-Salhi-1 IRAQ | 0 | | | | | | | |
| 1CP071801. 1:497743-498720 *L. gasseri* South Korea | 0 | 0 | | | | | | |
| 2MW692472. 1:68-1045 *L. gasseri* China | 0 | 0 | 0 | | | | | |
| 3W692468.1:65-1042 *L. gasseri* China: Wuhan | 0 | 0 | 0 | 0 | | | | |
| 5W674513.1:65-1042 *L. gasseri* China: Gaoxin Avenue | 0 | 0 | 0 | 0 | 0 | | | |
| 6W674381.1:65-1042 *L. gasseri* China: Wuhan | 0 | 0 | 0 | 0 | 0 | 0.001 | | |
| 7W527197.1:68-1045 *L. gasseri* China: Gaoxin Avenue | 0 | 0 | 0 | 0 | 0 | 0.002 | 0 | |
| 8W494815.1:62-1039 *L. gasseri* China: Wuhan | 0 | 0 | 0 | 0 | 0 | 0.002 | 0 | 0.002 |

**Table (8): Genetic distance of local strain: *Lactobacillus helveticus* Strain Al-Salhi-2 with global strains.**

| Global Bacterial Strains | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| W848597.1 *L. helveticus* strain Al-Salhi-2 Iraq | | | | | | | | | | | | | | | | |
| 1MT545102. 1: 158-986 *L. helveticus* China: Wuhan | 7.0326 | | | | | | | | | | | | | | | |
| 2MT459358. 1:158-986 *L. helveticus* China: Gaoxin Avenue | 7.0326 | 0 | | | | | | | | | | | | | | |
| 3CP045642. 1:71731-72559 *L. helveticus* China: Jiangsu | 7.0326 | 0 | 0 | | | | | | | | | | | | | |
| 4MN435581. 1:135-963 *L. helveticus* Korea | 7.0326 | 0 | 0 | 0 | | | | | | | | | | | | |
| 5MN326668.1: 136-964 *L. helveticus* India | 7.0326 | 0 | 0 | 0 | 0 | | | | | | | | | | | |
| 6LC463253.1: 115-943 *L. helveticus* Japan:Hokkaido | 7.0326 | 0 | 0 | 0 | 0 | 0 | | | | | | | | | | |
| 7MG827270.2:186-1014 *L. helveticus* India | 7.0326 | 0 | 0 | 0 | 0 | 0 | 0 | | | | | | | | | |
| 8CP031016. 1:472184-473012 *L. helveticus* Switzerland | 7.0326 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | | | | | | |
| 9CP017982. 1:70635-71463 *L. helveticus* Bulgaria | 7.0326 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | | | | | |
| 10MF108208.1: 131-959 *L. helveticus* China: Beijing | 7.0326 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | | | | |
| 11MG551115. 1:136-964 *L. helveticus* China: Qinghai Province | 7.0326 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | | | |
| 12KY 465642. 1: 110-938 *L. helveticus* Germany | 7.0326 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | | |
| 13KU555474. 1: 109-937 *L. helveticus* Kazakhstan | 7.0326 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | |
| 14LC062899. 1:186-1014 *L. helveticus* Japan | 7.0326 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| 15PO02081.1:81506-82334 *L. helveticus* USA | 7.0326 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 16NR 117060. 1: 154-982 *L. helveticus* FRance | 7.0326 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

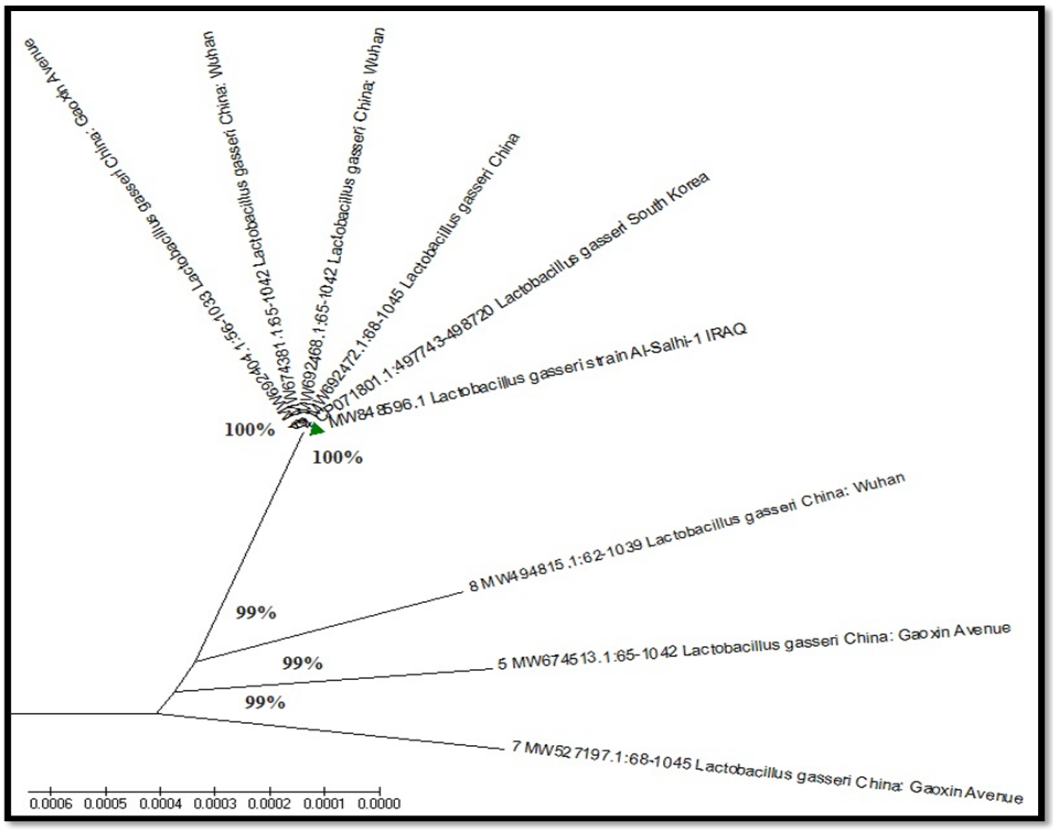



**Fig. (6): phylogenetic tree for the first bacterial strain registered at the National Center for Biotechnology Information: *Lactobacillus gasseri* strain Al-Salhi-1**

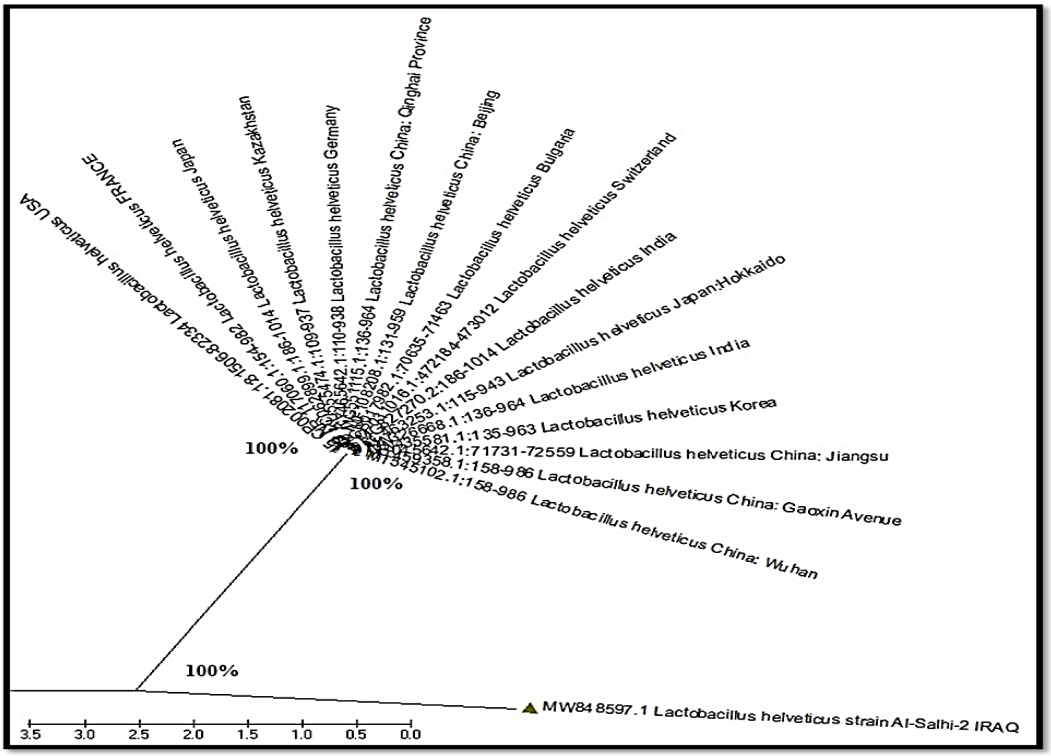



**Fig. (7): phylogenetic tree for the second bacterial strain registered at the National Center for Biotechnology Information: *Lactobacillus helveticus* strain Al-Salhi-2**

**Table (9): Genetic distance of local strain *Lactiplantibacillus plantarum* strain Al-Salhi-3 with global strains**

| Global Bacterial Strains | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MW848598.1 *Lactiplantibacillus plantarum* strain Al-Salhi-3 IRAQ | | | | | | | | | | | | | | | | | | |
| 1MT613627. 1:24-*985 Lactobacillus plantarum* China: Wuhan | 0 | | | | | | | | | | | | | | | | | |
| 2MT604646. 1:22-983 *Lactobacillus plantarum* China: Gaoxin Avenue | 0 | 0 | | | | | | | | | | | | | | | | |
| 3MT473424. 1:29-990 *Lactobacillus plantarum* China: huhhot | 0 | 0 | 0 | | | | | | | | | | | | | | | |
| 4CP052869.1:2905861-2906822 *Lactiplantibacillus plantarum* South Korea | 0 | 0 | 0 | 0 | | | | | | | | | | | | | | |
| 5CP050805. 1:1822226-1823187 *Lactiplantibacillus plantarum* South Korea: Seoul | 0 | 0 | 0 | 0 | 0 | | | | | | | | | | | | | |
| 6MT 196920.1:21-982 *Lactobacillus plantarum* Egypt | 0 | 0 | 0 | 0 | 0 | 0 | | | | | | | | | | | | |
| 7MN994357. 1:34-995 *Lactobacillus plantarum* China: Hubei | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | | | | | | | | | |
| 8MN242002. 1:39-1000 *Lactobacillus plantarum* Burkina Faso | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | | | | | | | | |
| 9MN833002. 1:10-971 *Lactobacillus plantarum* Korea | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | | | | | | | |
| 11. 10 MN826737.1:5-966 *Lactobacillus plantarum* Turkey | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | | | | | | |
| 11CP046669. 1: 1066958-1067919 *Lactiplantibacillus plantarum* Russia | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | | | | | |
| 12LC512751. 1:34-995 *Lactobacillus plantarum* Japan:Yamagata | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | | | | |
| 13CPO21929. 1:1975734-1976695 *Lactiplantibacillus plantarum* Germany | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | | | |
| 14CPO26505. 1:2985248-2986209 *Lactiplantibacillus plantarum* USA: Davis | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | | |
| 15PO23490.1:2030095-2031056 *Lactiplantibacillus plantarum* New Zealand | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | |
| 16MH973186.1:4-965 *Lactobacillus plantarum* Thailand | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| 17MH899381. 1:15-976 *Lactobacillus plantarum* Brazil | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.002 | |
| 18MK490966. 1:40-1001 *Lactobacillus plantarum* Nigeria | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.002 | 0.002 |

**Table (10): Genetic distance of local strain *Limosilactobacillus reuteri* strain AhQuSa-1 with Global strains**

| Global Bacterial Strains | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MW 764103.1 *Lim. reuteri* strain AhQuSa-1 IRAQ | | | | | | | | | | | | | | | | | | |
| 1MF850249.1:30-981 *Lactobacilus reuteri* China: Jiangsu | 0.0021 | | | | | | | | | | | | | | | | | |
| 2CP054657.1: 1466496-1467447 *Lim. reuteri* South Korea:Jeollabuk-do | 0.0032 | 0.0011 | | | | | | | | | | | | | | | | |
| 3MT585536. 1:51-1002 *L. reuteri* China: Wuhan | 0.0032 | 0.0011 | 0 | | | | | | | | | | | | | | | |
| 4MT585429.1: 16-967 *L. reuteri* China: Gaoxin Avenue | 0.0032 | 0.0011 | 0 | 0 | | | | | | | | | | | | | | |
| 5MT433817. 1:16-967 *L. reuteri* China: HENAN | 0.0032 | 0.0011 | 0 | 0 | 0 | | | | | | | | | | | | | |
| 6MT355446.1:68-10 19 *L. reuteri* Thailand: Chonburi | 0.0032 | 0.0011 | 0 | 0 | 0 | 0 | | | | | | | | | | | | |
| 7MT322927. 1:63-1014 *L. reuteri* South Korea | 0.0032 | 0.0011 | 0 | 0 | 0 | 0 | 0 | | | | | | | | | | | |
| 8N865789.1:31-982 *L. reuteri* China: Gansu | 0.0032 | 0.0011 | 0 | 0 | 0 | 0 | 0 | 0 | | | | | | | | | | |
| 9MN537548.1:84-1035 *L. reuteri* Germany | 0.0032 | 0.0011 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | | | | | | | |
| 10MN508966. 1:15-966 *L. reuteri* China: Nanjing | 0.0032 | 0.0011 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | | | | | | |
| 11MK572792. 1:35-986 *L. reuteri* Bangladesh | 0.0032 | 0.0011 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | | | | | |
| 12CP041676. 1:361127-362078 Lim. reuteri USA:CA | 0.0032 | 0.0011 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | | | | |
| 13MN128548.1:59-1010 *L. reuteri* India | 0.0032 | 0.0011 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | | | |
| 14LC485282. 1:55-1006 *L. reuteri* Japan: Okinawa | 0.0032 | 0.0011 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | | |
| 15MG547734. 1:7-958 *L. reuteri* Iran | 0.0032 | 0.0011 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | |
| 16KY930476. 1:47-998 *L. reuteri* Korea | 0.0032 | 0.0011 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| 18. 17 CP065318. 1:352732-353683 *Lim. reuteri* France: Paris | 0.0032 | 0.0011 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 18W040803. 1:12-963 *L. reuteri* Poland | 0.0032 | 0.0011 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 |

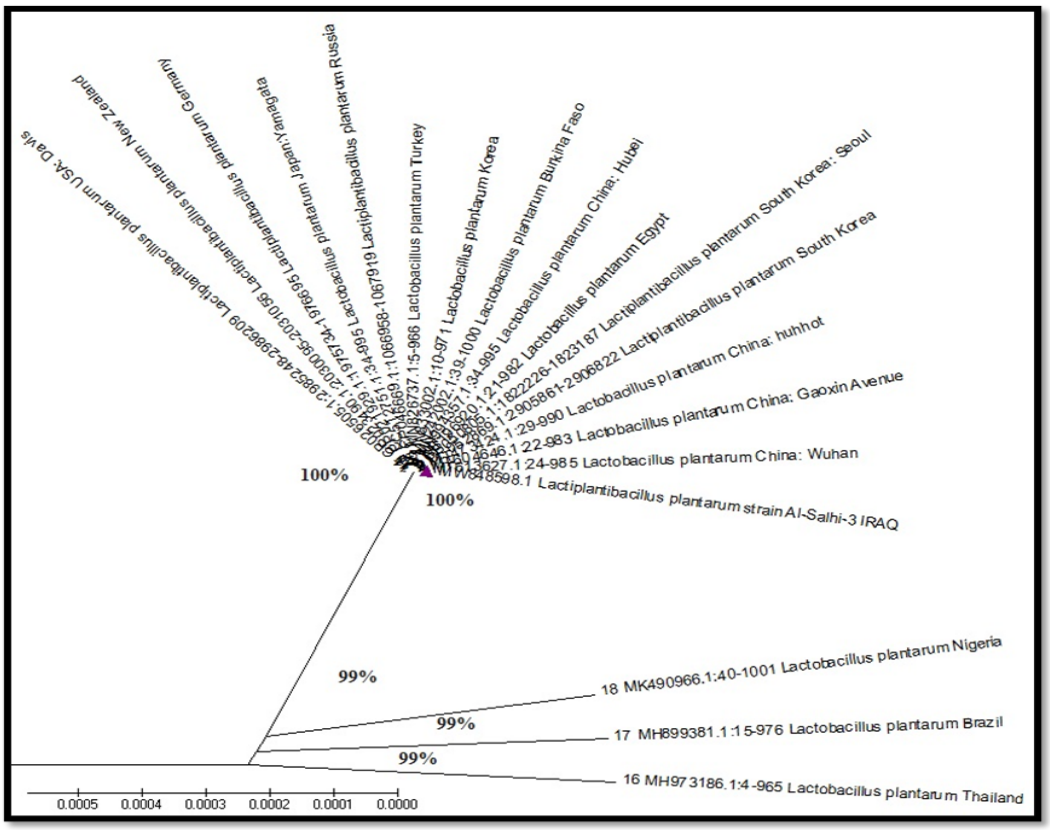



**Fig. (8): phylogenetic tree for bacterial Strain third registered at the National Center for Biotechnology Information: *Lactiplantibacillus plantarum* strain Al-Salhi-3**

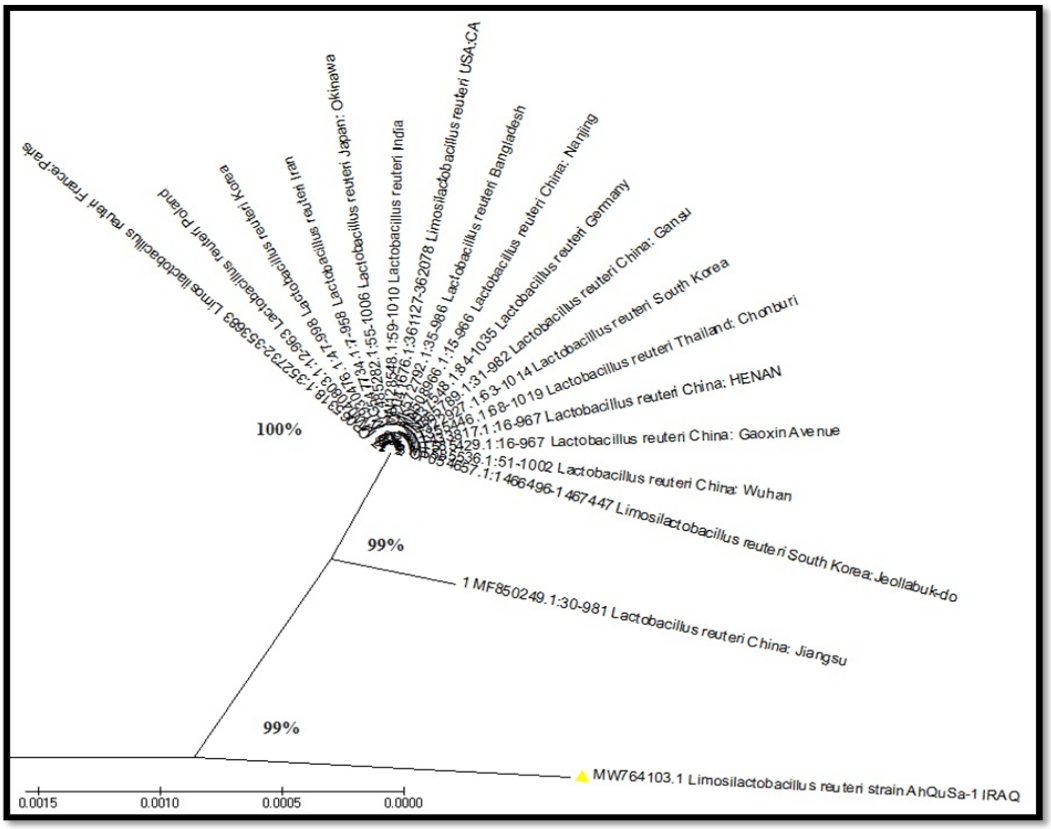



**Fig. (9): phylogenetic tree for the fourth bacterial strain registered at the National Center for Biotechnology Information: *Limosilactobacillus reuteri* strain AhQuSa-1**

**Table (11): Genetic distance of the fifth local strain *Limosilactobacillus* sp. strain AhQuSa-2 with global strains.**

| Global Bacterial Strains | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| MW764104.1 *Limosilactobacillus* sp. IRAQ | | | | |
| 1MT823179. 1:111-479 *Limosilactobacillus* sp. Canada | 0.0041 | | | |
| 2MT823192.1:111-479 *Limosilactobacillus* sp. Canada: Alberta | 0.0055 | 0.0014 | | |
| 3MT823154.1:111-479 *Limosilactobacillus* sp. Canada: Edmonton | 0.0055 | 0.0014 | 0.0027 | |
| 4MWO16036.1:66-434 *Limosilactobacillus* sp. Portugal | 0.0339 | 0.0291 | 0.0276 | 0.0306 |

**Table (12): Genetic distance of local strain *Ligilactobacillus salivarius* strain AhQuSa-3 with global strains**

| Global Bacterial Strains | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| MW764105.1 *Lig. salivarius* IRAQ | | | | | | | | |
| 1MG966327.1:46-955 *Lac. salivarius* India | 0.0022 | | | | | | | |
| 2CP000233.1: 1410977-1411886 *Lac. salivarius* Ireland | 0.0022 | 0 | | | | | | |
| 3MW714767.1:54-963 *Lig. salivarius* China: Wuhan | 0.0033 | 0.0011 | 0.0011 | | | | | |
| 4MW 709882. 1:64-973 *Lig. salivarius* China: HENAN | 0.0033 | 0.0011 | 0.0011 | 0 | | | | |
| 5MW642195. 1:72-981 *Lig. salivarius* Poland | 0.0033 | 0.0011 | 0.0011 | 0.0011 | 0 | | | |
| 6MW450426. 1:57-966 *Lig. salivarius* China: Gaoxin Avenue | 0.0033 | 0.0011 | 0.0011 | 0.0011 | 0 | 0 | | |
| 7MW 709870.1:68-977 *Lig. salivarius* China: Zhengzhou | 0.0033 | 0.0011 | 0.0011 | 0.0011 | 0 | 0 | 0 | |
| 8MG966325.1:47-956 *Lac. salivarius* India: Haryana | 0.0022 | 0 | 0 | 0.0011 | 0.0011 | 0.0011 | 0.0011 | 0.0011 |

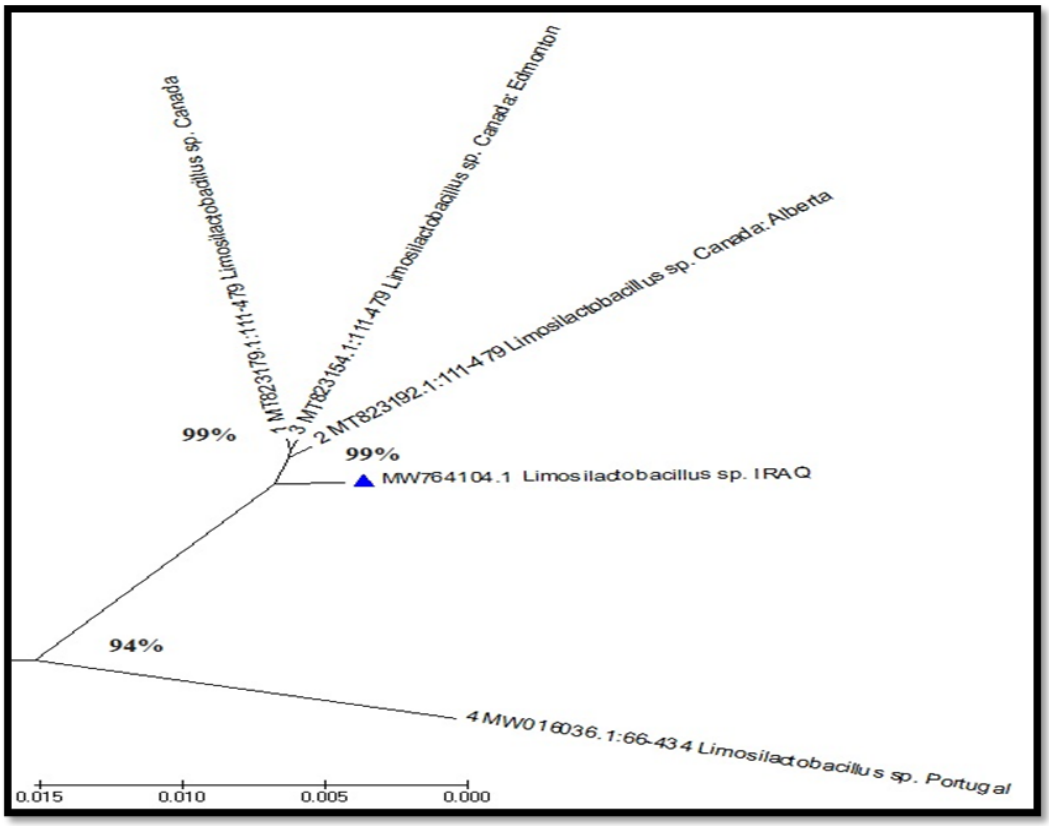



**Fig. (10): The fifth bacterial strain phylogenetic tree registered at the National Center for Biotechnology Information: *Limosilactobacillus* sp. strain AhQuSa-2.**

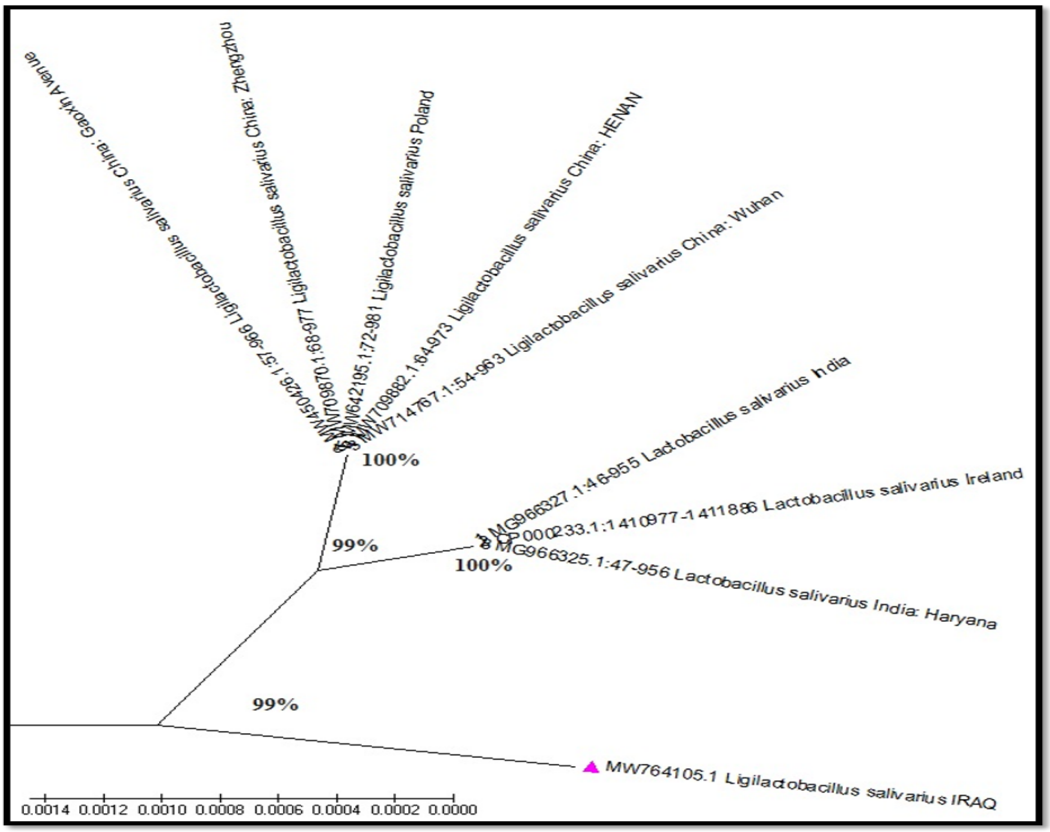



**Fig. (11): phylogenetic tree for the sixth bacterial strain registered at the National Center for Biotechnology Information: *Ligilactobacillus salivarius* strain AhQuSa-3**

**Table (13): Genetic distance of local strain *Lactobacillus johnsonii* strain AhQuSa-4 with global strains**

| Global Bacterial Strains | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| MW764106.1 *Lactobacillus johnsonii* strain AhQuSa-4 IRAQ | | | | | | | | | | |
| 1MT 597454. 1:79-988 *Lactobacillus johnsonii* China | 0.0011 | | | | | | | | | |
| 2MH819641. 1:86-995 *Lactobacillus johnsonii* China: Xiangyang | 0.0011 | 0 | | | | | | | | |
| 3LC071811. 1:95-1004 *Lactobacillus johnsonii* Japan | 0.0011 | 0 | 0 | | | | | | | |
| 4KP 1646 10.1:54-963 *Lactobacillus johnsonii* Poland | 0.0011 | 0 | 0 | 0 | | | | | | |
| 5MW714774. 1:58-967 *Lactobacillus johnsonii* China: Wuhan | 0.0011 | 0 | 0 | 0 | 0 | | | | | |
| 6MW368555. 1:58-967 *Lactobacillus johnsonii* China: HENAN0. | 0.0011 | 0 | 0 | 0 | 0 | 0 | | | | |
| 7NR 117574.1:92-1001 *Lactobacillus johnsonii* FRANCE | 0.0011 | 0 | 0 | 0 | 0 | 0 | 0 | | | |
| 8F1557013. 1:93-1002 *Lactobacillus johnsonii* Spain | 0.0011 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| 9CP031701. 1:847020-847929 *Lactobacillus johnsonii* South Korea: Gyeonggi | 0.0022 | 0.0011 | 0.0011 | 0.0011 | 0.0011 | 0.0011 | 0.0011 | 0.0011 | 0.0011 | |
| 10368636.1:66-975 *Lactobacillus johnsonii* China: Zhengzho | 0.0033 | 0.0022 | 0.0022 | 0.0022 | 0.0022 | 0.0022 | 0.0022 | 0.0022 | 0.0022 | 0.0033 |

**Table (14): Genetic distance of the seven local strains**

| Global Bacterial Strains | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| 1MW 764103.1 *Lim. reuteri* IRAQ | | | | | | | | |
| 2MW 764104.1 Lim. sp. IRAQ | 6.2430 | | | | | | | |
| 3MW 764105.1 *Lig. salivarius* IRAQ | 5.6368 | 11.7072 | | | | | | |
| 4MW 764106.1 *L. johnsonii* IRAQ | 6.3266 | 7.5504 | 6.3149 | | | | | |
| 5MW848596.1 *L. gasseri* IRAQ | 8.0009 | 11.1698 | 7.8181 | 8.1509 | | | | |
| 6MW848597.1 *L. helveticus* IRAQ | 6.4708 | 8.1183 | 10.4910 | 7.8323 | 8.1914 | | | |
| 7MW848598.1 Lactiplantibacillus plantarum IRAQ | 11.4613 | 8.5010 | 7.7015 | 7.7548 | 7.9770 | 8.0660 | | |
| 8MW989740.1 Lactobacillus helveticus IRAQ | 6.4708 | 8.1183 | 10.4910 | 7.8323 | 8.1914 | 0 | 8.0660 | |
| 9MW989741.1 Lactobacillus helveticus IRAQ | 6.4708 | 8.1183 | 10.4910 | 7.8323 | 8.1914 | 0 | 8.0660 | 0 |

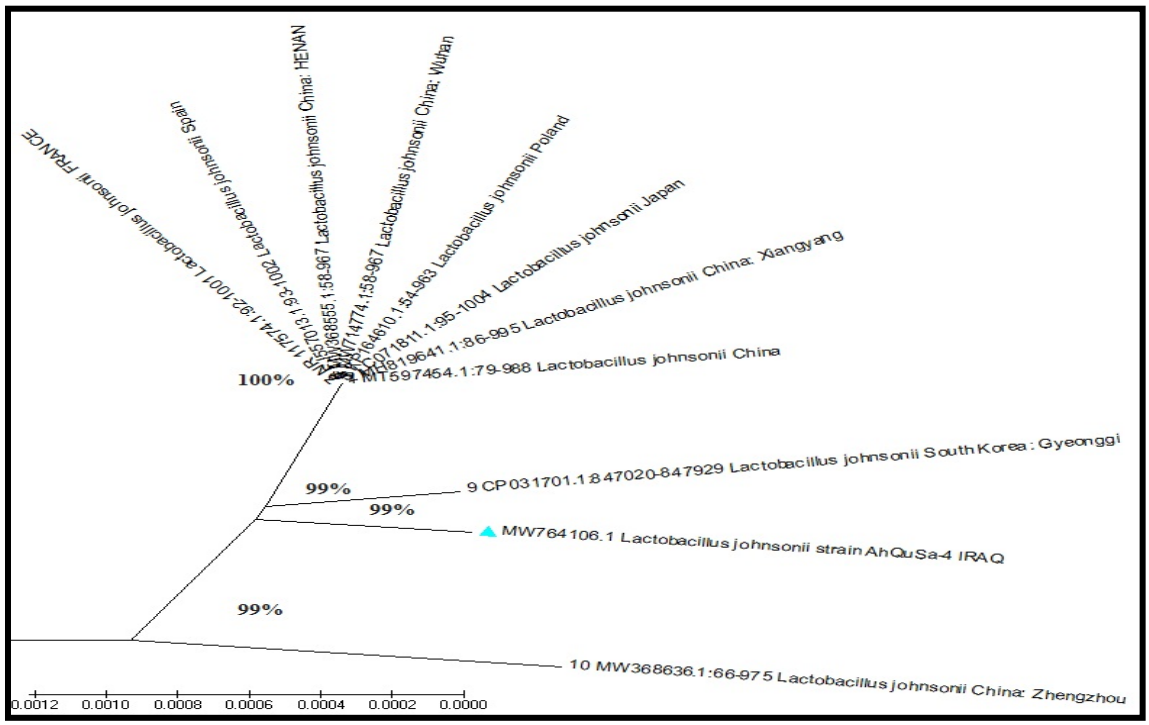



**Fig. (12): phylogenetic tree for the seventh bacterial strain registered at the National Center for Biotechnology Information: *Lactobacillus johnsonii* strain AhQuSa-4**

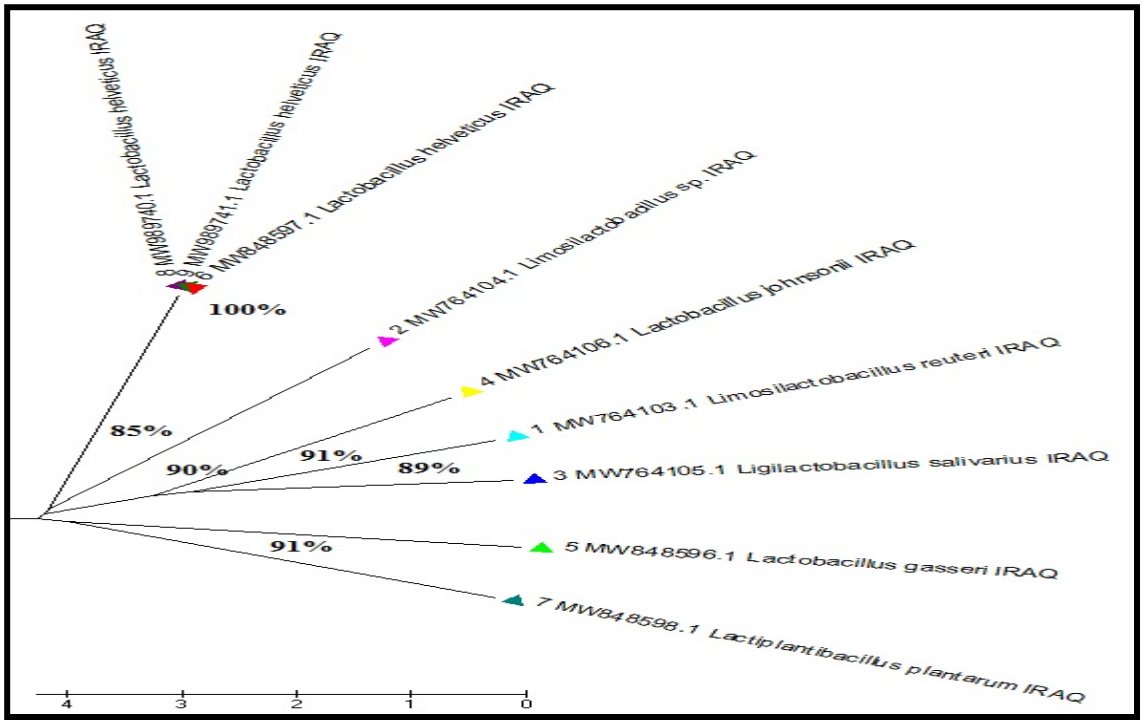



**Fig. (13): The general phylogenetic tree of the seven local bacterial strains, registered at the National Center for Biotechnology Information.**

## Conclusions

Seven pure bacterial strains of lactic acid bacteria strains were obtained and registered in the National Center for Biotechnology Information (NCBI) as new strains, some at the level of Iraq and others at the world level.

Significant superiority ($P \leq 0.05$) in the logarithmic numbers of lactic acid bacteria in the jejunum region compared to the ileum and

cecum regions, as the microbial content of lactic acid bacteria in the group of birds raised in local environments was: 6.52, 5.21, and 4.15 cfu.g$^{-1}$ for each of the jejunum, Ileum, and caecum, respectively, the results do not differ much in the group of birds raised in commercial fields in terms of the presence of lactic acid bacteria in the parts of the digestive system (jejunum, Ileum, and Ceca). It reached: 6.35, 5.02, and 3.92 cfu.g$^{-1}$ for each part over straight.

The results of the total bacteria in the jejunum region for both groups indicated that the results of the total bacteria are close to the results of the lactic acid bacteria, and this indicates that the total bacteria in the jejunum region are mostly (beneficial) lactic acid bacteria, unlike the other parts (the Ileum and Ceca) in which they are less concentrated; Because of the presence of other types of different bacteria, specifically in the cecum, and according to the indicators of the total bacteria for both groups, and through the results we reached, the jejunum area in the small intestine was determined; Because it occupies a large number of lactic acid bacteria and is mostly pure .

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## Ethical approval

All ethical guidelines related to poultry breeding and care issued by national and international organizations were implemented in this report.

## Conflict to interest

The authors declare that they have no conflict of interest.

## Contribution of authors

**A.A.A:** Sample and data collection and statistical analysis.

**Q. J. A.** and **S. M. A.:** Proposed the subject of the work and write the manuscript

**E.A.A.:** Read and revise the manuscript and assist in the isolation and identification of bacterial isolates

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# تَسجيل جَديد لسلالات بكتيريا حامض اللاكتيك من مُحتويات أمعاء الدجاج البالغ

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**المستخلص:** أُجريت هذه الدراسة في مختبر الأحياء المجهرية التابع لقسم علوم الأغذية في كلية الزراعة بجامعة البصرة للمُدة من 2020/11/15 ولغاية 2020/12/26 ، بهدف دراسة المجتمع البكتيري لأجزاء الجهاز الهضمي (الصائم، اللفائفي والأعورين) للدجاج البالغ السليم وتحديد الجُزء المُستهدف الذي يحتوي أكبر عدد من بكتيريا حامض اللاكتيك، لأنواعٍ مختلفة من الطيورالداجنة، وكذلك لعزل المستعمرات البكتيرية بعد تنقيتها ثلاث مرات، وتشخيصها جينياً بتقنية PCR، إذ تم الحصول على سبع سلالات جديدة من بكتيريا حامض اللاكتيك، وتم تسجيلها في المركز الوطني لمعلومات التقانة الحيوية(NCBI) كسلالات محلية جديدة، على مستوى العراق والعالم، وهي كالتالي:*Lactobacillus gasseri* strain Al-Salhi-1 ، *Lactobacillus helveticus* strain AhQuSa-1 ، *Limosilactobacillus reuteri* strain Al-Salhi-2، *Lactiplantibacillus plantarum* strain Al-Salhi-3، *Ligilactobacillus salivarius* strain AhQuSa-2 ، *Limosilactobacillus* sp. strain AhQuSa-3 ، *Lactobacillus johnsonii* strain AhQuSa-4. وأظهرت النتائج أيضاً حصول تفوق معنوي ($P\leq0.05$) في الأعداد اللوغارتيمية لبكتيريا حامض اللاكتيك في منطقة الصائم لكل من مجموعة الطيورالمرباة في بيئات محلية، وكذلك في مجموعة الطيورالمرباة في الحقول التجارية بالمقارنة مع منطقتي اللفائقي والأعورين، إذ بَلَغَ المحتوى البكتيري في مجموعة الطيور المحلية: 6.52، 5.21، 4.15 (cfu/g) لكل من الصائم واللفائفي والأعوررين على التوالي، بينما بلغ في مجموعة الطيور التجارية: 6.35، 5.02، 3.92 (cfu/g) لكل من الصائم واللفائفي والأعوررين على التوالي.

**الكلمات المفتاحية:** الفلورا المعوية، العصيات اللبنية، العد البكتيري، الدواجن ، السلالات.