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Evaluation of the anti-bacterial and anti-Biofilm activity of Lactobacillus strains isolated from milk and dairy products

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ABSTRACT

This study was carried out to assess the antagonistic ability of *Lactobacillus* obtained from raw milk and various dairy products, including "Karish cheese, fermented milk, yogurt, and ice cream." From a total of 100 samples that were examined, 74 of them comprised lactic acid bacteria, and 36 of them were related to the genus *Lactobacillus*. The isolated strains were examined for their ability to form biofilm, and most isolates showed medium-to-strong biofilm formation. Pathogenic bacteria like *Escherichia coli, Salmonella Typhimurium, Staphylococcus aureus, and Listeria monocytogenes* could not form biofilms on any of the isolated *Lactobacillus* strains. The in-vitro antimicrobial activity of the *Lactobacillus* isolates was evaluated against several pathogenic strains. The results showed that all examined isolates had a strong antibacterial effect against *Staphylococcus aureus*, with inhibition zones ranging from 10 to 17 mm. The antimicrobial activity of extracted bacteriocins from *Lactobacillus* isolates differs greatly. The bacteriocins from six *Lactobacillus* isolates demonstrated antibacterial activity against the tested pathogens, with inhibition zones ranging from 1 to 7 mm. In contrast, the bacteriocins from four isolates exhibited low activity against the tested pathogens. Molecular identification for the isolated strains (n = 5) using 16S rRNA gene sequencing revealed that three isolates were related to *Limosilactobacillus fermentum* that registered in GeneBank with accession numbers (PP784302-PP784303-PP784304), while the other two isolates were related to *Lactiplantibacillus plantarum* and registered with accession numbers (PP788561-PP788562).

1. INTRODUCTION

Lactobacillus, a Gram-positive rod-shaped, acid-tolerant, non-spore-forming microaerophilic, is closely related to lactic acid bacteria (Kusmiyati *et al.*, 2014; Du *et al.*, 2019). It may expand to encompass 23 new genera like *Limosilactobacillus, Latilactobacillus*, and *Lactiplantibacillus* (Zheng *et al.*, 2020). It represents an important contributor in the fermentation of food and the production of beneficial byproducts, including organic acids, peptides (such as bacteriocins), antimicrobial agents, and aromatic compounds (Cicenia *et al.*, 2014; and Gaenzle, 2015).

Bacteriocins, produced by ribosomal synthesis, exhibit antibacterial activity by disrupting cell walls, nucleic acid synthesis, and protein production in various bacterial species (Kumariya *et al.*, 2019). It influences both Gram-positive and Gram-negative bacteria such as *Listeria monocytogenes, Campylobacter jejuni, Enterococcus faecalis, Salmonella enterica, Enterohaemorrhagic E. coli* (EHEC), *Vibrio parahaemolyticus, and V. cholerae* (Nigatu *et al.*, 2015; Ren *et al.*, 2018).

As a result of the essential role that *lactobacilli* play during the fermentation process and the production of beneficial byproducts, the differentiation of this species must not depend on phenotypic methods alone. Hence, the detection of 16S rRNA sequences is now a vital step in the precise identification of these bacteria. This molecular method not only results in a more reliable way of differentiating *lactobacilli* species but also increases our knowledge of their functional roles in the fermentation processes, thus ensuring the production of food of acceptable quality (Awd *et al.*, 2020). In this study, we focused on isolating and identifying *lactobacillus* species from milk and dairy products, extracting bacteriocins, and evaluating their antimicrobial and antibiofilm properties against some pathogenic strains.

2. MATERIALS AND METHODS

2.1. Samples

A total of 100 random samples of raw milk and milk products (Karish cheese, yogurt, fermented milk, and ice cream)—20 samples each—were collected from different supermarkets and small retailers in Al-Qalyobia Governorate, Egypt. Each sample was kept in a clean, sterile bag and handed quickly to the laboratory for bacteriological analysis.

2.2. Isolation and Identification of Lactobacillus

Weight 10 gm from each examined dairy sample, suspended in 90 ml of peptone water in a sterile stomacher bag, and mix it probably in a stomacher (MA106402, France, 450 to 640 blows per minute) for 2 minutes, then transfer 1 ml from the mixed sample into 9 ml of De Man, Rogosa, and Sharpe broth (MRS) broth (TM media, TM 147) and incubate at 37 ^{o}C for 24 h. The enriched sample was then streaked over MRS agar (HiMedia) and incubated at 37 ^{o}C for 24 h in an anaerobic condition. according to Bhardwaj *et al.* (2012)

The developed colonies were detected by staining and differentiated by biochemical tests such as "citrate utilization, urea test, VP, lysine hydrolysis, indol test, and fermentation of different sugars" (De Vos *et al.*, 2009).

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2.3. Biofilm formation of the isolated lactobacilli.

To perform biofilm tests, ten isolates were identified using the adherence test, as follows:

The test organisms were cultured in 10 mL of trypticase-soy broth with 1% glucose in tubes. The tubes were incubated at 37 oC for a day. Then, the tubes were emptied, rinsed with phosphate-buffered saline (pH 7.3) and dried. The tubes were stained with 0.1% crystal violet and washed with deionized water. The tubes were dried upside down. The tube scoring method was based on the control strain results. Biofilm formation was positive if a film was seen on the tube wall and bottom. The biofilm amount was scored as 1-weak/none, 2-moderate, or 3-high/strong. The experiment was done three times, with three replicates each time. (Christensen *et al.,* 1995).

2.4. Antibiofilm formation of the isolated Lactobacilli.

The ability of *Lactobacilli* to remove biofilm formed by pathogenic strains "*Listeria monocytogenes* NCTC 7973, *Staphylococcus aureus* ATTC 6538, *Escherichia coli* ATCC 25922, *Klebsiella pneumonia* NCTC 9633, and *Salmonella* Typhimurium ATCC 14028" obtained from Cairo-MIRCEN (Microbiology Resource Center) Faculty of Agriculture, Ain Shams University, Cairo, Egypt, was recognized through the inoculation of pathogenic strains with strong biofilm formation with isolated *lactobacilli* in 10 mL of Tryptic Soya broth (TM media) with 1% glucose in glass test tubes and incubation at 37 oC for 48 h. Then determine the capability of *lactobacilli* to form or remove biofilm. The glass tubes were emptied, rinsed with phosphate-buffered saline (pH 7.3) and dried. The tubes were stained with 0.1% crystal violet and washed with deionized water. The tubes were dried upside down. The tube scoring method was based on the results of control strains. Biofilm formation was positive if a film was seen on the wall and bottom of the tube. The biofilm amount was scored as (1) for weak/none, (2) for moderate, or (3) for high/strong, according to Christensen *et al.* (1995). The experiment was done three times with three replicates each time (Sancineto *et al.,* 2016).

2.5. Antimicrobial effect of the isolated lactobacilli

The antagonistic effect of the isolated *lactobacilli* was measured by an agar-well diffusion assay, as described by Topisirovic *et al.* (2006) and Boulares *et al.* (2012).

A fresh culture of *lactobacilli* was prepared by inoculation of the isolated *lactobacilli* in MRS broth (TM media, TM 147) and incubated at 37 ºC for 24 h. Then inoculate a sterilized blank disc with 50 µl of each bacterial culture and let it slightly dry, then place it into Mueller Hinton agar medium (TM media) inoculated with *Listeria monocytogenes* (NCTC-7973), *Staphylococcus aureus* (ATTC-6538), *Escherichia coli* (ATCC-25922), *Klebsiella pneumonia* (NCTC-9633), and *Salmonella typhimurium* (ATCC-14028). The plates were then incubated at 37 °C for 24 hours.

2.6. Extraction of Bacteriocin.

The isolates were propagated into MRS broth (TM media, TM 147) for 24 h at 37 ºC, centrifuged at 8000 rpm for 3 min and then filtered through a 0.22 µm filter (hydrophilic PTFE, United States). The pH of the obtained Cell-Free Supernatant Containing Bacteriocin ("CFSCB") was adapted to 6.0–6.5 using 1M NaOH.

The antimicrobial effect of bacteriocin was measured by the agar-well diffusion method, as described by Topisirovic *et al.* (2006) and Boulares *et al.* (2012).

2.7. Molecular identification (DNA extraction and 16S rRNA sequencing.)

The isolated *lactobacilli were* confirmed by using polymerase chain reaction (PCR) by using a pair of universal primers for lactic acid bacteria F: TCCGGATTTATTGGGCGTAAAGCGA and R: TCGAATTAAACCACATGCTCCA for lactic acid bacteria 16S *rRNA* obtained from Midland Certified Reagent Company (Oilgos, USA) (Kim *et al*., 2015). The reaction mixture (25µl) contained 12.5µl of Emerald Amp GT PCR master mix (2x premix), 5.5µl of PCR grade water, 1µl from both forward and reverse primers, and 5µl from template DNA. The amplification was carried out in the thermocycler as follows: the primary denaturation occurs at 94 °C for 5 min, followed by 35 cycles including denaturized temperature at 94 °C for 30 s, annealing at 60 °C for 40 s, and elongation at 72 °C for 45 s, which was followed by a terminal 10-minute extension step at 72 °C.

The DNA analysis was conducted using a 1.5% agarose gel electrophoresis method, employing a BioRad system, in a 1x TBE buffer. This process was carried out at 100 volts for 30 minutes. The DNA was then visualized under UV illumination (Syukur *et al.,* 2014). Following this, a 411-base pair segment was amplified. The resulting PCR product was then sequenced from both ends using an Applied Biosystems 3130 DNA sequences. For the sequencing reaction, a Bigdye Terminator V3.1 kit was utilized, supplied by Perkin-Elmer/Applied Biosystems, based in Foster City, California.

2.8. Data analysis.

The genetic sequences were analyzed by matching them against those available in the GenBank database through the BLAST algorithm. Subsequently, data was retrieved using the MEGA6 software, which stands for Molecular Evolutionary Genetics Analysis version 6. A phylogenetic tree was then created employing the Maximum Likelihood (ML) method, which was reiterated a thousand times for robustness, and BioEdit software was utilized to examine the conserved regions. Additionally, the Clustal X feature within MEGA6 was used for sequence alignment.

3. RESULTS

3.1. Incidence of *lactobacilli* in the examined samples.

From 100 examined samples from milk and milk products, 74 isolates developed in the media, and by examining them morphologically, 36 isolates were seen to be *lactobacilli:* "12/20 from fresh milk, 5/20 from Karish cheese, 4/20 from fermented milk, 4/20 from yoghurt, and 11/20 from ice cream." They appeared as Gram-positive short rods arranged in chains or pairs, and their colonies in De Man, Rogosa, and Sharpe agar, "MRS agar," appeared as circular, low-convex, and white to gray colonies (Table 1). Also, ten isolates were examined for fermentation of 12 sugars: "Mannitol, Inulin, Rhamnose, Sucrose, Adonitol, Sorbitol, Galactose, Trehalose, Cellobiose, Arabinose, Inositol, Mannose, Ethyl-D-glucoside, Raffinose, Salicin, Xylose, Ethyl-D-Mannoside, and Melezitose" and showed diversity against sugar fermentation.

Table (1) Incidence of *lactobacilli* in examined samples.

Sample	Number of examined samples	Number of positive samples		Number of bacilli isolates		Number of other lactic acid bacteria	
		No.	%	No.	%	No.	%
Fresh milk	20	16	80.0	12	60.0	4	20.0
Karish cheese	20	15	75.0	5	25.0	10	50.0
Fermented milk	20	12	60.0	4	20.0	8	40.0
Yogurt	20	15	75.0	4	20.0	11	55.0
Ice cream	20	16	80.0	11	55.0	5	25.0
Total	100	74	74.0	36	36.0	38	38.0

%* Percentage of lactobacilli relative to the total sample in each row

3.2. Biofilm formation of the isolated *lactobacilli*.

The results of biofilm formation for the isolated *lactobacilli* (n = 36) showed that most of the isolated *lactobacilli* produced biofilm with varying degrees that was marked as the development of film lined the wall and bottom of the tubes, where 8/36 (22.2%) isolates showed strong biofilm formation while all other isolates, 28/36 (77.8%), showed medium biofilm formation.

3.3. *In-vitro* antibiofilm activity of the isolated strains against some pathogenic strains.

All the isolated *lactobacilli can* inhibit biofilm formed by pathogenic strains "*Listeria monocytogene, E. coli, Staph. aureus, and S. typhimurium,"* where no biofilms were produced after inoculation of both tested pathogenic strains with isolated *Lactobacilli* strains in the same test tubes.

3.4. *In-vitro* antimicrobial activity of the isolated *Lactobacilli*.

The results of Table 2 declared the antibacterial effect of ten lactobacilli isolates that showed different sugar fermentation against several pathogenic strains: "*Listeria monocytogenes* (NCTC-7973), *Staphylococcus aureus* (ATTC-6538), *Escherichia coli* (ATCC-25922), *Klebsiella pneumonia* (NCTC-9633), and *S. typhimurium* (ATCC-14028)." The results showed an inhibition zone formed around the blank disc inoculated with the *Lactobacillus* strain. The results showed that all the examined isolates (n = 10) showed a great antibacterial effect against *S. aureus* (the zone of inhibition varied from 10 to 17 mm), while exhibiting different antibacterial effects against other pathogenic strains (Table 2, Fig. 1).

Table (2) *In-Vitro* antimicrobial activity of the isolated *Lactobacilli*.

Lactobacillus strains	Average Inhibitory effect on pathogenic bacteria "diameter measured by mm"				
	Listeria monocytogenes (NCTC-7973)	*Staphylococcus aureus* (ATTC-6538)	*Escherichia coli* (ATCC-25922)	*Klebsiella pneumonia* (NCTC-9633)	*Salmonella typhimurium* (ATCC-14028)
L1-L6-L7	8-10	15-17	4-5	4-7	10-13
L2- L5-L10	7-9	11-13	5-7	4-6	6-8
L3-L9	3-5	10-12	2-4	2-3	2-4
L4	5-7	11-13	2-3	2-3	7-9
L8	6-8	10-12	3-5	3-6	9-11

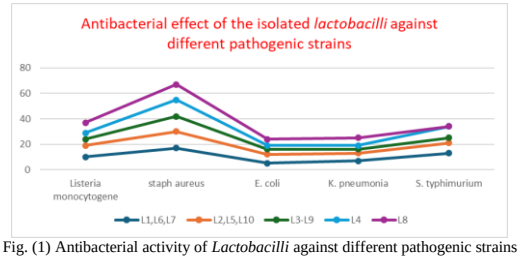


Fig. (1) Antibacterial activity of *Lactobacilli* against different pathogenic strains

3.5. *In Vitro* antimicrobial activity of the extracted bacteriocin.

The bacteriocin produced from the isolated *lactobacilli* showed varying antibacterial activity against pathogenic strains in which the bacteriocin of the isolates "*L1-L6-L7-L2- L5-L10"* showed activity varying from (1-7mm) against tested pathogenic strains while the bacteriocin of the strains "*L3-L9-L4-L8"* showed low activity against tested pathogenic strains (Table 3, Fig. 2)

Table (3) *In Vitro* antimicrobial activity of the extracted bacteriocin.

Bacteriocin extracted from	Average Inhibitory effect on pathogenic bacteria "diameter measured by mm"				
	Listeria monocytogenes (NCTC-7973)	*Staphylococcus aureus* (ATTC-6538)	*Escherichia coli* (ATCC-25922)	*Klebsiella pneumonia* (NCTC-9633)	*Salmonella typhimurium* (ATCC-14028)
L1-L6-L7	1-3	5-7	2-4	2-4	3-5
L2- L5-L10	1-3	2-3	3-4	2-3	2-3
L3-L9	0	1-2	0	1-3	1-3
L4	0	1-2	0	0	0
L8	0	2-5	1-3	1-3	0

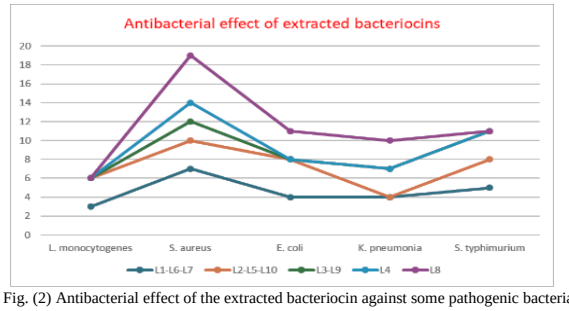
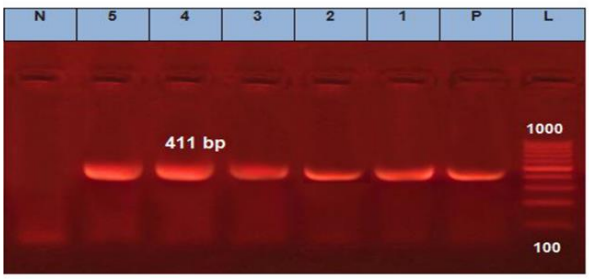


Fig. (2) Antibacterial effect of the extracted bacteriocin against some pathogenic bacteria

3.6. Molecular identification of the isolated *Lactobacilli* and their sequencing.

3.6.1. Molecular identification of 16S rRNA.

The genomic DNA of examined *lactobacilli* was tested using universal primer of Lactic acid bacteria. The findings of PCR exhibited that all the examined strains were amplified at 411bp and showed positive results (Fig. 3).



Fig/ (3): PCR amplification of specific gene of Lactic acid bacteria (16SrRNA) on agarose gel electrophoresis.
Lane L: 100-1500 bp. DNA Ladder
Lane N: Negative control (has no product)
Lan P: Positive control (Field strain previously confirmed by PCR for the related gene in the reference laboratory for veterinary quality control on poultry production, Animal health research institute).
Lan 1-5: positive at 411 bp.

3.6.2. Sequencing and phylogenetic analysis for the 16S rRNA gene.

The sequencing results showed that three strains (no. 1, 3, and 4) isolated from fresh milk and ice cream were related to *Limosilactobacillus fermentum* and registered in Gene Bank with an accession number (PP784302-PP784303-PP784304), and by blasting it in the gene bank, it seemed to be identical with 95–97% of the related *Limosilactobacillus* strains as appeared in the phylogenetic tree (Fig. 4). And the other two strains (no. 2 and 5) isolated from ice cream and yogurt were related to *Lactiplantibacillus plantarum* and registered in the gene bank with an accession number (PP788561- PP788562) and by blasting it in the gene bank, it seemed to be identical with 97.5%–96.8% of *Lactiplantibacillus* as appeared in the phylogenetic tree (Fig. 5).

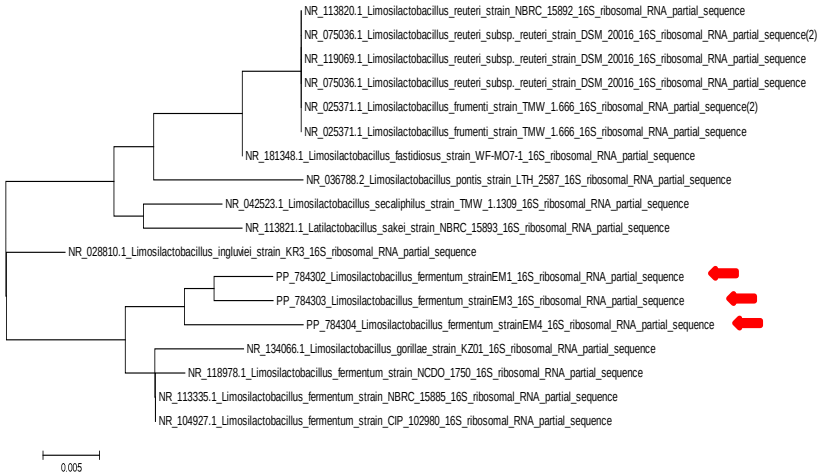


Fig. (4): The phylogenetic tree of the strains related to the isolated *Limosilactobacillus fermentum.*

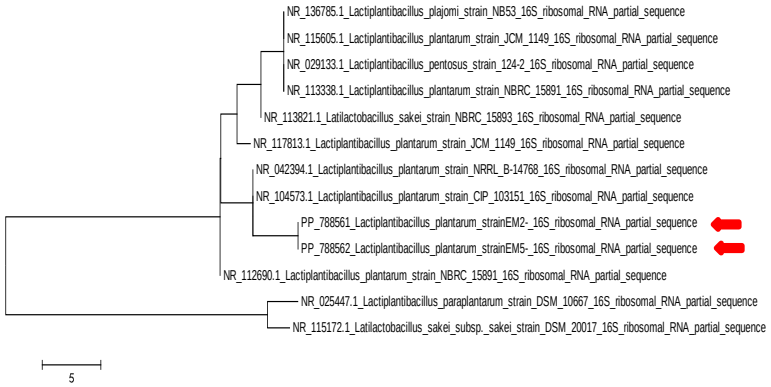


Fig. (5): The phylogenetic tree for the strains related to isolated *Lactiplantibacillus plantarum.*

4. DISCUSSION

Throughout the current study, a total of seventy-four isolates (74 %) attained from raw milk and dairy products ("Karish cheese, fermented milk, yogurt, and ice cream") were selected based on their morphological characters on MRS agar and staining to be related to lactic acid bacteria. All isolates appeared to be gram-positive, non-motile, non-spore-forming, and catalase-negative. All of them appeared as small, whitish-creamy colonies when cultured anaerobically in MRS agar. This was nearly similar to that detected by Bhardwaj *et al.* (2012), who isolated 78 isolates of LAB from a fermented milk product isolated from a region in India (DHI), while higher than Nasr and Abd Alhalim (2024), who isolated 13 isolates of LAB from 40 samples of Kashmiri cheese, Rayeb milk, local yogurt, and buttermilk, and lower than Alharbi and Alsaloom (2021), who isolated one hundred isolates of LAB from raw milk. The *Lactobacillus* isolates from the examined samples, "Karish cheese, fermented milk, yogurt, and ice cream," represent thirty-six isolates (36%), which according to Al-Rawi *et al.* (2023).

The ability of *lactobacilli* isolates to form biofilm that represented a survival mode for bacterial growth was examined with a varied degree of biofilm formation, from medium to strong. This may occur due to variations in dynamics and characteristics in biofilm formation between different *Lactobacillus* strains (Kubota *et al.*, 2008; Martinez *et al.*, 2020). The ability of the isolated *lactobacilli* to inhibit biofilm formation of the pathogenic strains was also examined, and the results declared that all isolated *lactobacilli were* able to inhibit biofilm formed by the pathogenic strains "*L.monocytogene, E. coli, Staph. aureus, and S. typhimurium," a*nd this was in agreement with Jara et al. (2020), Giordani et al. (2021), and Thuy et al. (2024).
The current results, which determined the capability of the isolated strains to inhibit the growth of the pathogenic strains "*L. monocytogenes (NCTC-7973*), *Staph. aureus (ATTC-6538*), *Escherichia coli (ATCC-25922*), *K. pneumonia (NCTC-9633), and S. typhimurium (ATCC-14028),"* in which the isolated *lactobacillus* showed varied antibacterial power against pathogenic strains with an inhibition zone ranging from 2 to 17 mm., came in accordance with Reuben *et al.* (2020) and Alharbi and Alsaloom (2021), which described that *lactobacilli* exhibited varying degrees of incompatible activity against pathogenic bacteria. And

based on the obtained antimicrobial data, it showed that it affects more Gram-positive pathogenic strains (*L. monocytogenes and S. aureus*) than Gram-negative bacteria (*E. coli, K. pneumonia, and S. typhimurium)*, mainly due to the presence of an outer cytoplasmic layer in Gram-negative bacteria that is formed from rich lipopolysaccharides (Gao *et al.,* 1999). This corroborated the results obtained by DeAlmeida J´unior *et al.* (2015), Reuben *et al.* (2020) and Alharbi and Alsaloom (2021). The antagonistic activity of *lactobacilli* mainly occurred due to the production of several inhibitory substances like organic acids, hydrogen peroxide, and bacteriocin (Hor and Liong, 2014). While the results showed the antibacterial activity of the extracted bacteriocins, in which only strains (*L1-L6-L7-L2-L5-L10)* showed antibacterial effect against the tested pathogenic strains with an inhibition zone ranging from 2–7 mm with great effect in *S. aureus*, this was nearly similar to Ha and Hoa (2016) and Nasr and Abd-Alhalim (2024). While the antagonistic effect of bacteriocin extracted from the other strains (L3-L9-L4-L8) was unable to cause inhibition in the tested pathogenic isolates, this may explain why the antagonistic effect in the *lactobacillus* strain itself occurs due to the production of other inhibitory substances like organic acid and hydrogen peroxide. This comes in line with the study of Alharbi and Alsaloom (2021), who determined that some strains were unable to make inhibition after treating their supernatant to PH 6.5. This also confirmed the theory that bacteriocin extracted from lactic acid has a varying degree of inhibition against pathogenic bacteria (Lozo *et al.*, 2021).

The partial sequence for 16S rRNA was performed on five isolated *Lactobacillus* strains in order to differentiate between the isolates at the genus and species levels, as mentioned by Sadrani *et al.* (2014). The sequences of the 16S rRNA gene for the selected isolates were compared with the sequences in the Gene-Bank database, and by similarity, about 97-95% of the examined isolates were related to *Limosilactobacillus fermentum* and those registered in Gene-Bank with an accession number (PP784302-PP784303-PP784304) and the phylogenetic tree were constructed with the neighborhood-related strains. The other two strains showed identity with 97.5%–96.8% of *Lactiplantibacillus plantarum* (previously known as *Lactobacillus plantarum*) and were registered in GeneBank with an accession number (PP788561- PP788562) and the phylogenetic tree was constructed with the related neighboring strains. This is nearly similar to Alharbi and Alsaloom (2021); Pakroo *et al.* (2022); Senjaliya and Georrge (2023); and Thuy *et al.* (2024), who isolated them from fermented milk.

5. CONCLUSIONS

This study highlights the possibility of *Lactobacillus* strains isolated from raw milk and dairy products as probiotic cultures and natural food preservatives due to their ability to inhibit biofilm formation and growth of some pathogenic strains, especially *S. aureus*. The study also emphasized the importance of identifying and characterizing the strains of *Lactiplantibacillus plantarum with* accession numbers (PP788561–PP788562) *and Limosilactobacillus fermentum* with accession numbers (PP784302-PP784303–PP784304) at the species level using molecular techniques such as 16S rRNA gene sequencing.

6. REFERENCES

1. Alharbi, N.K., Alsaloom, A.N., 2021. Characterization of Lactic Bacteria Isolated from Raw Milk and Their Antibacterial Activity against Bacteria as the Cause of Clinical Bovine Mastitis. Journal of Food Quality Volume, 6466645, 8 pages. https://doi.org/10.1155/2021/6466645
2. Al-Rawi, S.S.M., Salahdin, O.D., Al-Alaq, F.T., Abdulazeem, L., Kzar, M.H., Khattab, E.S., Naje, A.S., 2023. Antibacterial Activity of Bacteriocin - Isolated from *Lactobacillus* Spp. against Some Pathogenic Bacteria. Journal of Medicinal and Chemical Sciences, 6, 702-709.
3. Awd, A. R., Khater, K. A., Ismail, A. I., 2020. Identification and phylogenetic studies of a new probiotic *Lactobacillus* spp. Egyptian isolate based on 16s rRNA gene. Acta Scientific Nutritional Health, 4 ,3, 1–7.
4. Bhardwaj, A., Puniya, M., Sangu, K. P. S., Kumar, S., Dhewa, T., 2012. Isolation and Biochemical Characterization of *Lactobacillus* Species Isolated from Dahi. Research & Reviews: A Journal of Dairy Science and Technology, 1 ,2, 1-14.
5. Boulares, M., Aouadhi, C., Mankai, M., Ben Moussa, Essid, O., Mnasser, H., 2012. Characterisation, identification and technological properties of psychotrophic lactic acid bacteria originating from Tunisian fresh fish. Journal of Food Safety, 32, 333–344.
6. Christensen, G.D., Simpson, W.A., Younger, J.A., Baddour, L.M., Barrett, F.F., Melton, D.M., Beachey EH., 1995. Adherence of coagulase negative staphylococci to plastic tissue cultures: A quantitative model for the adherence of staphylococci to medical devices. *J Clin Microbiol.*, 22, 996–1006.
7. Cicenia, A., Scirocco, A., Carabotti, M., Pallotta, L., Marignani, M., Severi, C., 2014. Postbiotic activities of *lactobacilli*-derived factors. J. Clin. Gastroenterol., 48, 18–22.
8. De Almeida J´unior, W. L. G., Ferrari, ´I. S., De Souza, J. V., Da Silva, C. D. A., Da Costa, M. M., Dias, F. S., 2015. Characterization and evaluation of lactic acid bacteria isolated from goat milk. Food Control, 53, 96–103.
9. De Vos, p., Garrity, G.M., Jones, D., Krieg, N.R., Ludwig, W., Rainey, F.A., Schleifer, K-H., Whitman, W.B., 2009. Bergey's manual of systematic bacteriology. Second Edn.
10. Du, X., Cao, K., Tan, M., Pan, Q., 2019. *Lactobacillus futsaii* subsp. Chongqingii subsp. nov., isolated from a traditional Chinese pickle. Current Microbiology, 76 ,2, 153–158.https://doi.org/10.1007/s00284-018-1601-2.
11. Gaenzle, M.G., 2015. Lactic metabolism revisited: Metabolism of lactic acid bacteria in food fermentations and food spoilage. Curr. Opin. Food Sci., 2, 106–117.
12. Gao, Y., Van Belkum, M. J., Stiles, M. E., 1999. The outer membrane of Gram-negative bacteria inhibits antibacterial activity of brochocin-C. Applied and Environmental Microbiology, 65 ,10, 4329–4333.
13. Giordani, B., Parolin, C., Vitali, B., 2021. *Lactobacilli* as Anti-biofilm Strategy in Oral Infectious Diseases: A Mini-Review. Front. Med. Technol, 3, 769172. doi: 10.3389/fmedt.2021.769172.
14. Ha, T.Q., Hoa, T.M., 2016. Selection of lactic acid bacteria producing bacteriocin. J Viet Environ, 8, 271–276.
15. Hor, Y.Y., Liong, M.T., 2014. Use of extracellular extracts of lactic acid bacteria and *bifdobacteria* for the inhibition of dermatological pathogen *Staphylococcus aureus*. Dermatol Sin, 32, 141–147.
16. Jara, J., Pérez-Ramos, A., Solar, G., Rodríguez, J.M., Fernández, L., Orgaz, B., 2020. Role of *Lactobacillus* biofilms in *Listeria monocytogenes* adhesion to glass surfaces. International Journal of Food Microbiology, 334, 108804. https://doi.org/10.1016/j.ijfoodmicro.2020.108804.
17. Kim, D-H., Chon, J-W., Kim, H., Kim, H-S., Choi, D., Hwang, D-G., Seo, K-H., 2015. Detection and Enumeration of Lactic Acid Bacteria, Acetic Acid Bacteria and Yeast in Kefir Grain and Milk Using Quantitative Real-Time PCR. Journal of Food Safety, 35, 102–107.
18. Kubota, H., Senda, S., Nomura, N., Tokuda, H., Uchiyama, H., 2008. Biofilm Formation by Lactic Acid Bacteria and Resistance to Environmental Stress. Journal of Bioscience and Bioengineering, 106 ,4, 381-386. https://doi.org/ 10.1263/jbb.106.381.
19. Kumariya, R., Garsa, A., Rajput, Y., Sood, S., Akhtar, N., Patel, S., 2019. Bacteriocins: classification, synthesis,

mechanism of action and resistance development in food spoilage causing bacteria. Microbial. Pathogenesis, 128, 171–177.

20. Kusmiyati, N., Hidayat, N., Fatchiyah, F., 2014. Partial Analysis 16S rRNA Gene in *Lactobacillus* spp. from Natural Fermented Milk. Cukurova Medical Journal, 39 ,1, 99-104.
21. Lozo, J., Topisirovic, L., Kojic, M., 2021. Natural bacterial isolates as an inexhaustible source of new bacteriocins. Applied Microbiology and Biotechnology, 105 ,2, 477–492.
22. Martinez, S., Garcia, J.G., Williams, R., Elmassry, M., West, A., Hamood, A., Hurtado, D., Gudenkauf, B., Ventolini, G., Schlabritz-Loutsevitch, N., 2020. *Lactobacilli spp.:* real-time evaluation of biofilm growth. *BMC Microbiol* 20,1, 64. https://doi.org/10.1186/s12866-020-01753-3
23. Nasr, N.M., Abd-Alhalim, L.R., 2024. Characterization and identification of *Lactobacillus rhamnosus* and *Enterococcus durans* as probiotic potential isolated from selected dairy products in Egypt. Journal of Umm Al-Qura University for Applied Sciences,10, 168–177.
24. Nigatu, J. M., Tuji, F. A., Tefera, A. T., 2015. Evaluation of the antagonistic effect of six mixed cultures of lactic acid bacteria, isolated from the Ethiopian fermented milk ergo, against some foodborne pathogens inoculated into the Ethiopian cottage cheese ayib. Afr. J. Microbiol. Res., 9, 1789–1797.
25. Pakroo, S., Tarrah, A., Takur, R., Wu, M., Corich, V., Giacomini, A., 2022. *Limosilactobacillus fermentum* ING8, a Potential Multifunctional Non-Starter Strain with Relevant Technological Properties and Antimicrobial Activity. Foods., 11 ,5, 703. https://doi.org/10.3390/foods11050703
26. Ren, D., Zhu, J., Gong, S., Liu, H., Yu, H., 2018. Antimicrobial characteristics of lactic acid bacteria isolated from homemade fermented foods. Biomed Res. Int., 5416725. DOI: 10.1155/2018/5416725
27. Reuben, R. C., Roy, P. C., Sarkar, S. L., Rubayet Ul Alam, A. S. M., Jahid, I. K., Jahid, I. K., 2020. Characterization and evaluation of lactic acid bacteria from indigenous raw milk for potential probiotic properties. Journal of Dairy Science, 103 ,2, 1223–1237.
28. Sadrani, H., Dave, J., Vyas, B.R.M., 2014. Screening of potential probiotic *Lactobacillus* strains isolated from fermented foods, fruits and of human origin. Asian J Pharm Clin Res., 7, 216–225.
29. Sancineto, L., Piccioni, M., De Marco, S., Pagiotti, R., Nascimento, V., 2016. Diphenyl diselenide derivatives inhibit microbial biofilm formation involved in wound infection. BMC Microbiology, 16 ,1, 220. https://doi.org/10.1186/s12866-016-0837-x
30. Senjaliya, D.P., Georrge, J.J. 2023. Functional Characterization of *Lactobacillus plantarum* Isolated from Cow Milk and the Development of Fermented Coconut and Carrot Juice Mixed Beverage. Curr Microbiol., 80 ,5, 139. DOI: 10.1007/s00284-023-03258-4
31. Syukur, S., Rijal, F., Jamsari, Purwati, E., 2014. Isolation and Molecular Characterization of Lactic Acid Bacteria by Using 16s rRNA from Fermented Buffalo Milk ,Dadih in Sijunjung, West Sumatera. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 5,6, 871-876.
32. Thuy, T.T.D., Lu, H-F., Bregente, C.J.B., Huang, F-C.A., Tu, P-C. and Kao, C-Y., 2024. Characterization of the broad-spectrum antibacterial activity of bacteriocin-like inhibitory substance-producing probiotics isolated from fermented foods. BMC Microbiology, 24, 85. https://doi.org/ 10.1186/s12866-024-03245-0
33. Topisirovic, L., Kojic, M., Fira, D., Golic, N., Strahinic, I., Lozo, J., 2006. Potential of lactic acid bacteria isolated from specific natural niches in food production and preservation. International Journal of Food Microbiology, 112 ,3, 230–235.
34. Zheng, J., Wittouck, S., Salvetti, E., Franz, C.M.A.P., Harris, H.M.B., Mattarelli, P., O'toole, P.W., Pot, B., Vandamme, P., Walter, J., Watanabe, K., Wuyts, S., Felis, G.E., G€anzle, M.G., Lebeer, S., 2020. A taxonomic note on the genus *Lactobacillus*: description of 23 novel genera, emended description of the genus *Lactobacillus beijerinck* 1901, and union of Lactobacillaceae and Leuconostocaceae. Int. J. Syst. Evol. Microbiol., 70, 2782–2858.