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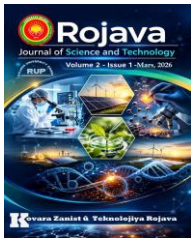


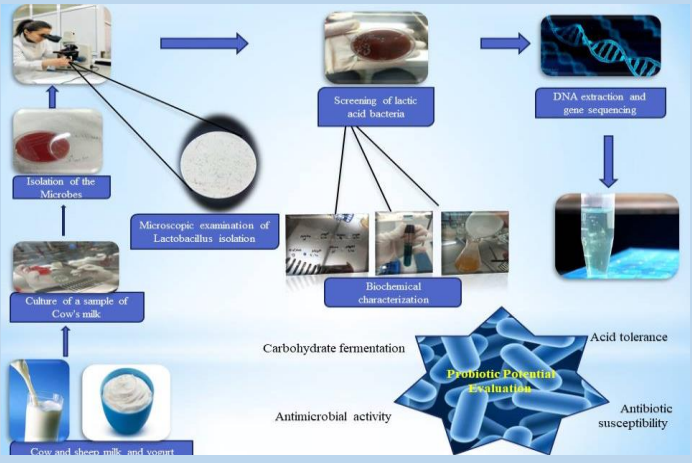
Isolation and Identification of Probiotic Microorganisms in the Regions of Northeast Syria

Zozan Ehmed[1], Haidar Saify Nabiabad*[2]

[1,2] Department of Biotechnology and Biomedicine, Institute of Science and Modern Technology-Rojava University Syria

Abstract

Probiotics are defined as live microorganisms that, when given in sufficient amounts, provide health benefits to the host by maintaining intestinal microbial balance. Among these, lactic acid bacteria (LAB), commonly found in milk and dairy products, are the most widely used. Over 100 samples of milk and its derivatives were inoculated onto De Man, Rogosa, and Sharpe (MRS) agar plates and incubated at 37°C for 48 hours under both aerobic and anaerobic conditions. From the aerobic incubation, more than 30 samples were identified as Lactobacillus bacteria using morphological and biochemical assays, including Gram staining and the catalase test. All isolates displayed either cocci or rod-shaped forms, Gram-positive and catalase-negative, confirming their classification as LAB. Finalay, PCR method with specific primers was used to confirm the isolated strains. These 30 LAB isolates were further tested for antimicrobial activity against human pathogens *Escherichia coli* and *Staphylococcus aureus*. Seven sample showed broad-spectrum antimicrobial effectiveness. The isolates tolerated acidic conditions within a pH range of 2.0 to 5.0, grew well in the presence of NaCl, and efficiently metabolized lactose. Antibiotic susceptibility testing with Ampicillin, Doxycycline, Ceftriaxone, and Amikacin revealed notable resistance to Ampicillin and Amikacin, while susceptibility was observed for Doxycycline and Ceftriaxone. Based on probiotic characterization and antibiotic resistance patterns, the seven sample were confirmed as promising probiotic LAB strains. 16S rDNA sequence analysis identified them as belonging to *Lactobacillus plantarum, L. paracasei, L. casei, L. fermentum, L. rhamnosus, L. reuteri, L. brevis, and L. acidophilus*.



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* - Corresponding Author: homan_saify@yahoo.com

1. Introduction

Currently, LAB are the focus of extensive global research due to their essential roles in fermentation processes and their ability to produce antimicrobial compounds that contribute to various probiotic effects. These effects include antitumor activity, reduction of lactose intolerance symptoms, lowering of serum cholesterol levels, stabilization of intestinal microbiota, and enhancement of the immune system. In fermented milk production, specific LAB strains are utilized for their capacity to produce exopolysaccharides, which improve product viscosity and texture. Additionally, certain LAB strains are associated with the production of mannitol, a compound that may offer health-promoting properties. Probiotics are defined as live microorganisms, including bacteria and yeasts, which, when administered in adequate amounts, confer health benefits to the host. With increasing public awareness of health and nutrition, the consumption of probiotic-enriched foods has gained significant attention. The most widely studied and utilized probiotic strains belong primarily to the genera Lactobacillus and Bifidobacterium. Other probiotic organisms include certain species of Streptococcus, Enterococcus, Bacillus, Propionibacterium, and the yeast Saccharomyces (Gareau M.G., 2010; Fesseha H., 2019).These microorganisms exhibit antimicrobial activity against enteric pathogens, stimulate both mucosal and systemic immune responses, and contribute to antitumor, anticholesterol, and anti-inflammatory effects. Furthermore, probiotics enhance nutritional status and help alleviate symptoms associated with diarrhea, lactose intolerance, and allergic responses. In studies conducted by some researchers, Neha et al. (2023) isolated probiotic strains from raw cow milk sourced from the Lahaul Valley, demonstrating their ability to produce cis-9, trans-11 conjugated linoleic acid (CLA) isomers, in addition to exhibiting antioxidant properties and potential for food formulation. Asha et al. (2024) conducted a study across various government and private dairy farms in Sylhet, where a total of 166 milk samples were collected from local and crossbred dairy cattle using simple random sampling. The Lactobacillus colonies on MRS agar appeared as white or creamy yellow, round-edged, smooth-surfaced single colonies with diameters ranging from 0.5 to 3 mm. The study also found that the Lactobacillus isolates exhibited antibacterial activity and inhibited the growth of several pathogens. These findings align with previous studies indicating Lactobacillus species as common probiotic bacteria and potential alternatives for preventing Salmonella-related diseases (Kowalska et al., 2020). In a similar vein, Djadouni and Kihal (2012) reported that LAB produce antibacterial compounds that inhibit the growth of indicator organisms, with inhibition zones ranging from 10–14 mm against *E. coli, S. aureus*, and S. *Typhimurium* using the agar spot test. The antibacterial activity demonstrated by the isolates in this study was comparable, and the broad-spectrum activity of the Lactobacillus isolates against both Gram-positive and Gram-negative organisms highlights their potential as effective probiotics. Despite the well-documented global significance of LAB and probiotics in dairy science, limited research has been conducted on the isolation and characterization of LAB from milk and milk-derived products in Northern and Eastern Syria. Given the nutritional and therapeutic importance of probiotic LAB, further investigation in these regions is warranted. This study therefore focuses on isolating and identifying LAB strains from raw cow milk and traditional dairy products sourced from these underexplored areas.

2. Materials and methods

2.1. Sample Collection

A cross-sectional study was conducted to isolate and characterize lactic acid bacteria (LAB) from 100 sample milk and dairy products collected from lactating dairy cows and sheep located at various farms in the northern and eastern regions of Syria. Samples of cheese and yogurt were obtained from local food stores and households that prepare yogurt manually. Cow milk samples were sourced from dairy farms in the city of Qamishlo. Sampling was carried out randomly, following the methodology outlined in previous studies by Hoque et al. (2010) Sterilely inside syringes and at low temperatures to protect them from damage. The sampling locations included Qamishlo, Amuda, Terbesbia, and Derik, aiming to isolate a diverse range of probiotic strains from local dairy products.

2.2. Isolation and identification of bacteria using conventional methods

Lactic acid bacteria (LAB) strains were isolated from milk, yogurt, and cheese samples by weighing 50 grams of each sample and performing serial dilutions using 2% sodium citrate solution. Following homogenization, samples were diluted up to 10^{-5} and plated on Nutrient Agar, Blood Agar, M17 Agar, and MRS (de Man, Rogosa, and Sharpe) Agar media. The inoculated plates were incubated at 37°C for 48 hours

to allow for bacterial growth, following the protocol described by Pundir et al. (2013). After incubation, the bacterial isolates from milk, cheese, and yogurt were subjected to a series of morphological and biochemical tests to assist in the identification of their genus, following standard protocols (Barrow and Feltham, 1993; Theivendrarajah, 1990; Kapilan and Arasaratnam, 2010; Karuppaija et al., 2016). These included assessments of colony morphology, growth under aerobic and anaerobic conditions, Gram staining, catalase activity, oxidase and indole production, methyl red reaction, and the ability to produce acid and gas from glucose.

2.3. Confirmation Tests of LAB Isolates

2.3.1 Gram staining test

Gram staining is a widely used differential staining technique to classify bacteria into two major groups: Gram-positive and Gram-negative, based on the composition of their cell walls and their reaction to the staining process. This method provides a rapid and preliminary diagnosis of bacterial infections. According to the manufacturer's instructions (BTC Syria), a small amount of bacterial culture was transferred onto a clean glass slide and spread into a thin smear. The smear was air-dried and then heat-fixed to adhere the bacteria to the slide surface. The four dyes were spread on the slide, taking care to wash each dye after one minute with distilled water. Finally, the slide was air-dried and examined under a light microscope to determine the Gram reaction of the bacterial cells.

2.3.2 Catalase test

The catalase test was performed to detect the presence of the enzyme catalase, which breaks down hydrogen peroxide (H_2O_2) into water and oxygen. This test helps differentiate catalase-positive bacteria from catalase-negative ones. A clean glass slide was used for the procedure. A small portion of the bacterial culture was aseptically transferred onto the slide. 1–2 drops of 3% (v/v) hydrogen peroxide solution were added directly onto the bacterial smear.

2.3.3. Oxidase Test

The microbial growth was aseptically collected from the culture medium using a platinum inoculating loop and subsequently applied to a pre-prepared oxidase test disc impregnated with a 1% (w/v) aqueous solution of tetramethyl-p-phenylenediamine dihydrochloride. A positive oxidase reaction is indicated by the appearance of a purple coloration within 10 seconds, whereas the absence of color change or delayed coloration beyond this time frame denotes a negative result.

2.3.4. Indole test:

The Lactobacillus indole test is a biochemical assay employed to assess the ability of Lactobacillus species to catabolize the amino acid tryptophan into indole. For the preparation of the test medium, 0.5 g of peptone and 0.5 g of sodium chloride (NaCl) were dissolved in 50 mL of distilled water. The resulting solution was sterilized by autoclaving at 121°C under 20 psi pressure. Following incubation of the bacterial culture in the tryptophan-enriched broth for 24 to 48 hours, the addition of an indole-detecting reagent such as Kovac's or Ehrlich's reagent facilitates result interpretation. A positive reaction is evidenced by the development of a red or red-violet coloration, whereas the absence of color change—indicated by the reagent retaining its yellow hue—denotes a negative result.

2.3.5 Co_2 production from glucose

To assess the metabolic pathway utilized by the test organism for glucose catabolism, the oxidative-fermentation (OF) test was conducted. A solution containing 1.9 g of Hugh-Leifson medium was prepared by dissolving the medium in 100 mL of distilled water, followed by sterilization via autoclaving at 121°C under 20 psi pressure. Post-sterilization, two test tubes were inoculated with Lactobacillus spp. by inserting a sterile inoculating needle approximately halfway into the medium.

To differentiate between oxidative and fermentative metabolic processes, one tube was overlaid with a 1 cm layer of sterile mineral oil to establish anaerobic conditions, while the other was left exposed to atmospheric oxygen to permit aerobic growth. Both tubes were incubated at 37°C for 48 hours. For organisms exhibiting slow growth, the incubation period was extended to 72–96 hours.

2.3.6 Growth determination at different temperatures

To assess the effect of temperature on bacterial growth, agar media were prepared in sterile Petri dishes. Two bacterial samples were inoculated onto the media under aseptic conditions. The inoculated plates were then incubated at two different temperatures: 20°C and 45°C, for a period of 7 days. Following incubation, the plates were examined for bacterial growth.

2.3.7 Growth at Different Nacl Concentrations

The tolerance of lactic acid bacteria (LAB) isolates to varying concentrations of sodium chloride (NaCl) was evaluated using modified MRS broth supplemented with bromocresol purple as a pH indicator. Two NaCl concentrations were tested: 2.5- 8.5%. For each concentration, four test tubes containing 5 mL of the respective NaCl-modified MRS broth were inoculated with 50 µL of 1% (v/v) overnight LAB cultures. The inoculated tubes were incubated at 37°C for 7 days.

2.3.8 Arginine Hydrolysis Test

The arginine test medium was prepared by mixing 1 gram of LB medium with 1 gram of MRS agar, along with a 1000-milliliter arginine capsule and 100 ml of distilled water. The pH of the mixture was initially measured and found to be neutral at 7.0. Subsequently, the mixture was sterilized in an autoclave for two hours. After autoclaving, the pH was measured again and recorded at 6.6, indicating a slight shift toward acidity likely due to the sterilization process. A sample of Lactobacillus bacteria was then inoculated into the prepared medium, which was placed on a vibrating device to ensure proper mixing and aeration, and incubated for 24 hours.

2.3.9 DNA Extraction and PCR Amplification

Gene sequences corresponding to the target bacterial species were retrieved from the GenBank database. Primer design was carried out with precision using Gene Runner software and the Primer3 tool provided by the National Center for Biotechnology Information (NCBI), ensuring high specificity and accuracy in subsequent identification procedures. Polymerase chain reaction (PCR) mixtures were prepared in a final volume of 25 µL, comprising 8 µL of 2× PCR Master Mix (containing 1.5 mM $MgCl_2$), 11 µL of DNase-free water, 0.5 µL of each primer (as detailed in Table 1), and 5 µL of DNA template. Reaction conditions were optimized to enhance both amplification efficiency and specificity. PCR amplification was conducted using a Qiagen Rotor-Gene Q thermocycler under the following cycling conditions: an initial denaturation step at 95°C for 10 minutes; followed by 35 cycles of denaturation at 95°C for 1 minute, annealing at primer-specific temperatures (Table 1), and extension at 72°C for 1 minute; concluding with a final extension at 72°C for 10 minutes. The PCR products were separated on a 2% (w/v) agarose gel via electrophoresis using the Fisherbrand™ Real-Time Electrophoresis System in Tris-EDTA (TE) buffer. DNA bands were visualized by staining with ethidium bromide, allowing for clear and distinct resolution of amplicons suitable for subsequent analysis.

Table 1: PCR Primers Used for Bacterial Typing

Bacteria	**Primer-gene**	**Nucleotide sequence (5'->3')**	**Product size(bp)**	**Annealing temperature**
L. paracase	Paracasei-F	CAA TGC CGT GGT TGT TGG AA	106	58°C
	Paracasei-R	GCC AAT CAC CGC ATT AAT CG		
L. fermentum	Fermentum-F	GAC CAG CGC ACC AAG TGA TA	129	57°C
	Fermentum-R	AGC GTA GCG TTC GTG GTA AT		
L. reuteri				
	Reuteri-F	GAT TGA CGA TGG ATC ACC AGT	161	59°C
	Reuteri-R	CAT CCC AGA GTG ATA GCC AA		
L. casei	Casei-F	CCA CAA TCC TTG GCT GTT CT	115	56°C
	Casei-R	GCT TGA GGC GAT TGT AAT CC		
L. rhamnosus	Rhamnosus-F	GCC GAT CGT TGA CGT TAG TTG G	137	58°C
	Rhamnosus-R	CAG CGG TTA TGC GAT GCG AAT		
L. brevis	Brevis-F	GGG CAA CGA AGC AAG ATC GC	260	59°C
	Brevis-R	TTC CAA TCG TGT GCA CAC CA		
L. acidophilic	Acido-F	TCATGTTGGGATGCAATGAG	828	54.3
	Acido-R	TTTCAAAACTTGTCCTGCTG		

2.3.10 Acid tolerance

Isolated Lactobacillus species were evaluated for their acid tolerance by inoculating them into MRS broth adjusted to varying pH levels (ranging from pH 2 to 5). The cultures were incubated at 37 °C for 48 hours. Following incubation, 0.1 mL of each culture was transferred onto MRS agar plates using the pour plate technique. The plates were then incubated at 37 °C for an additional 48 hours.

2.3.11 Antibiotic susceptibility testing

To prepare the antibiotic susceptibility test, a measured amount of Mueller-Hinton agar was weighed and transferred into a container, followed by the addition of 100 mL of distilled water. The mixture was then sterilized by autoclaving. Upon completion of the autoclaving process, the medium was allowed to cool before being aseptically poured into sterile Petri dishes. Once the agar solidified, a bacterial suspension of Lactobacillus spp. was evenly spread over the entire surface of each agar plate using a sterile cotton swab, employing a circular motion to ensure uniform distribution. Subsequently, wells were aseptically created in the agar, into which a specified concentration of an antibiotic, such as Doxycycline, Cefatrixone, Amikacin and Ampicillin was introduced. The inoculated plates were then incubated at 37°C for 48 hours to allow for bacterial growth and antibiotic activity assessment.

2.3.12 Antimicrobial activity

To evaluate the impact of Lactobacillus on harmful bacteria, A measured quantity of Mueller-Hinton agar was prepared by adding 100 mL of distilled water, followed by sterilization in an autoclave for a minimum of two hours. After sterilization, the medium was poured aseptically into sterile Petri dishes and allowed to solidify. Separately, a solution was prepared by dissolving 1 gram of LB medium in 100 mL of distilled water. This solution was sterilized by autoclaving to 30°C. A small aliquot of Lactobacillus culture was then inoculated into the cooled solution and incubated on a shaker for 24 hours to promote bacterial growth. For the assay, a Petri dish was inoculated uniformly with a lawn of pathogenic bacteria, specifically Staphylococcus species. Wells were aseptically created in the agar surface, into which the Lactobacillus suspension was introduced. The plates were then incubated at 37°C for 48 hours to observe the interaction between the pathogenic bacteria and Lactobacillus.

3. Results

3.1 Gram staining test:

After performing the Gram stain on a Lactobacillus sample, the slide was allowed to air dry before being examined under the microscope. Various lens magnifications were used to investigate the finer details of the sample. During the examination, it became evident that the bacterial cells displayed a deep purple coloration, indicating that they were Gram-positive(Figure1).

Figure 1: Lactobacillus bacteria after applying Gram staining test under the microscope

3.2 Catalase test:

A sample of Lactobacillus was subjected to the catalase test. The bacterial sample was inoculated directly onto a clean microscope slide, followed by the addition of one or two drops of 3% hydrogen peroxide solution (v/v). Upon observation, no bubble formation was detected, indicating a negative result for catalase activity (Figure 2).

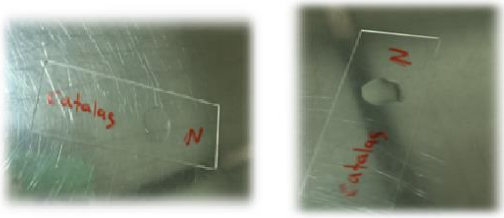

Figure 2: Catalase test after placing drops of the hydrogen peroxide solutionon raw bacteria

3.3. Oxidase Test

Upon completion of the 24–48-hour incubation period, the bacterial sample was removed from the incubator and applied to an oxidase test disc using gentle rubbing for approximately 10 seconds. The absence of any visible color change on the disc following application indicated a negative oxidase reaction, confirming that the Lactobacillus strain tested does not possess cytochrome c oxidase activity (Figure 3).

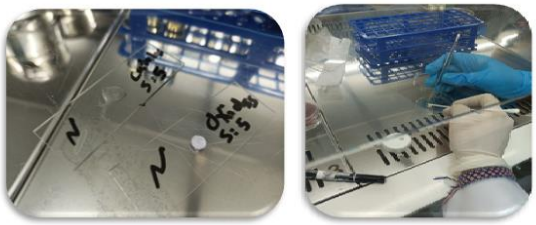

Figure 3. Placing the bacteria sample on the oxidase disk and displaying the final result.

3.4. Indole test:

Following a 24–48-hour incubation period, several drops of Kovac's reagent were introduced to the Lactobacillus culture grown in an indole-containing medium. The absence of any observable color change upon reagent addition indicated a negative result for the indole test, suggesting that the organism did not produce indole from tryptophan degradation(Figure 4).

Figure 4. Indole sample after bacteria were cultured and the final result was obtained.

3.5. Co_2 production from glucose

Following 48 hours of incubation, a distinct color change from green to yellow was observed in the test medium, accompanied by the formation of gas bubbles within the tube. This outcome is indicative of a positive oxidative-fermentation (OF) test, confirming that Lactobacillus spp. metabolized glucose via fermentation. Subsequent literature review and experimental validation revealed that the majority of Lactobacillus strains possess the metabolic capability to ferment glucose, thereby supporting the observed results (Figure 5).

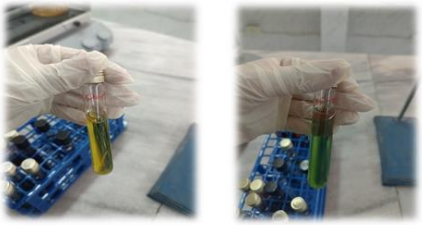

Figure 5: Testing CO2 production from glucose after planting a sample of Lactobacillus bacteria inside it and incubating it

3.6. Growth determination at different temperatures

Lactobacillus samples were cultured on agar media with a mildly acidic pH, which was adjusted to create a slightly acidic environment. The cultures were incubated at varying temperatures ranging from 20°C to 45°C for a period of one week (Figure 6). Following the incubation period, microscopic examination revealed that the bacteria remained viable, with no apparent signs of damage from the temperature fluctuations, whether induced by heat or cold (Figure 7).

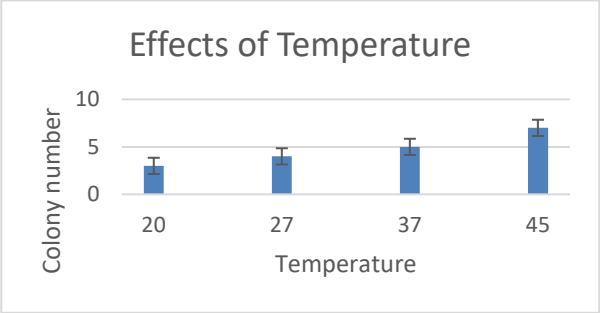

Figure 6 :Effect of Lactobacillus growth at different temperatures:

Figure 7 : A sample of bacteria incubated at different temperatures in an incubator.

3.7. Resistance to low pH

M17 agar samples were prepared at various low pH levels (2, 3, 4, 5). The bacterial samples were cultured on these acidic media and incubated at 37°C for 24 to 48 hours (Figure 8). After the incubation period, the samples were examined under a light microscope, and it was observed that the bacteria remained alive and viable (Figure 9).

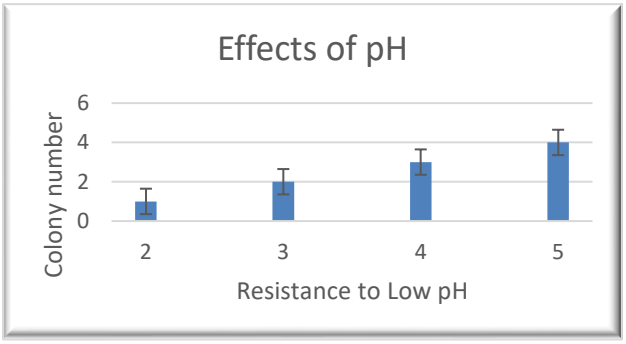

Figure 8 : Effect of Low pH on the growth of Lactobacillus bacteria.

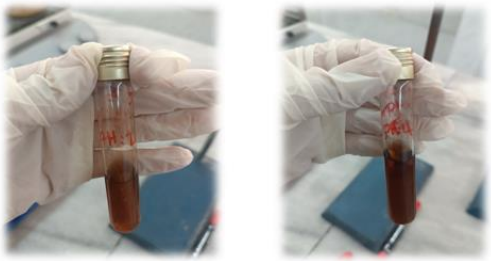

Figure 9: Samples of bacteria in agar with low acidity and different degrees

3.8 Growth at Different Nacl Concentrations

The sample was tested in media with varying salinity levels (2.5%, 4.5%, 6.5%, 8.5%) and incubated at 37°C for 7 days (Figure 10). After the incubation period, the sample was examined under a microscope, revealing that Lactobacillus bacteria thrived in moderate salinity conditions, while they did not survive in both high and low salinity environments (Figure 11).

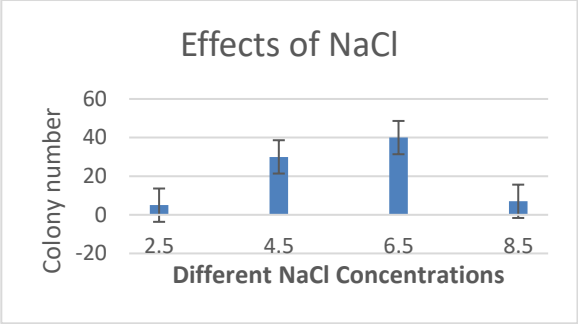

Figure 10: Effect of different concentrations of NaCl on the growth of Lactobacillus bacteria.

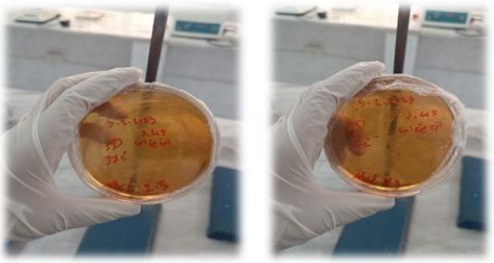

Figure 11: Samples of bacteria on agar of different salinity levels after incubation for 3 days

3.9 Arginine Hydrolysis Test

Observation of pH change indicating arginine hydrolysis by lactobacillus prior to inoculation, the sample exhibited an acidic pH of approximately 6.0. However, after 24 hours of incubation on a shaker, the sample was retrieved and the pH was measured again, revealing an increase to 8.6. This substantial rise in pH suggests the occurrence of arginine hydrolysis, indicating that Lactobacillus bacteria actively metabolize arginine, leading to the production of alkaline compounds (Figure 12).

Figure 12: Agar solution containing arginine and probiotics on a shaker and pH meter to measure pH

3.10 PCR Analysis

Molecular identification through PCR confirmed the findings obtained via traditional methodologies for the identification of probiotic bacteria. The application of species-specific primers enabled the successful detection of various bacterial species. The amplification and subsequent identification of bacterial DNA in this study are consistent with previously published research, further supporting the reliability and accuracy of PCR as a method for bacterial identification. PCR-based molecular screening revealed that certain Lactobacillus species were more commonly detected among the analyzed samples. Identified species included *L. acidophilus, L. brevis, L. rhamnosus, L. casei, L. reuteri, L. fermentum, and L. paracasei*. Notably, several of these, such as L. acidophilus, are well-recognized for their probiotic properties and health benefits. In conclusion, the successful molecular identification of multiple bacterial species using PCR, as demonstrated by the clear band patterns on the electrophoresis gel (Figure 13), is in strong agreement with conventional identification techniques. These findings validate both the specificity of the primers and the robustness of the PCR conditions employed, underscoring the utility of PCR as a reliable method for the identification of probiotic bacteria.

Figure 13: Electrophoresis gel of PCR products, Lane M, DNA Ladder. Lane 1, L. paracasei. Lane 2, L. casei. Lane 3, L. fermentum. Lane 4, L. rhamnosus. Lane 5, L. reuteri. Lane 6, L. brevis. Lane 7, L. acidophilus.

3.11 Antibiotic susceptibility testing

Following incubation, distinct zones of inhibition measuring approximately 1 cm in diameter were observed around wells containing the antibiotics doxycycline and ceftriaxone. These transparent halos, consistent with findings reported in prior international studies, indicate that Lactobacillus spp. exhibit susceptibility to these antimicrobial agents (Figure 14). In contrast, no inhibition zones were detected around wells containing other antibiotics, including ampicillin and amikacin, suggesting resistance of Lactobacillus to these compounds. These results demonstrate that Lactobacillus strains may possess selective resistance profiles, being sensitive to specific antibiotics while resistant to others.

Figure 14: Probiotic testing on antibiotic-containing medium

3.12 Antimicrobial activity

Prior to incubation, three wells were created on the agar plate: the first was filled with a suspension of Lactobacillus bacteria, the second with a selected antibiotic, and the third served as a negative control. Following incubation, clear inhibition zones ranging from 0.5 to 1 cm in diameter were observed surrounding the well containing Lactobacillus. This finding demonstrates the antibacterial activity of Lactobacillus against Staphylococcus species. These results suggest that administration of Lactobacillus may have therapeutic potential in treating certain gastrointestinal infections caused by pathogenic bacteria in humans (Figure 15).

Figure 15: Testing probiotics on a medium containing harmful bacteria and measuring the degree of probiotic expansion and its effect on harmful bacteria

4.Discussion

According to the results of this study, Lactobacillus colonies produced a diameter of 0.5–2 mm with a white to yellowish white colour and round edges on MRS agar when incubated at 37◦C, which is consistent with the findings of a previous study where milky white round colonies were also observed on MRS agar (Jose et al.,2015). In a separate study (Hoque et al., 2010), Lactobacillus from yogurt samples was examined for morphology, as well as various biochemical and physiological properties. Under the microscope, gram-positive rods or bacilli shaped bacteria were observed. The isolated bacteria were catalase negative. Fermentation of lactose, glucose and sucrose was also observed in Lactobacillus isolates. Similar morphological and biochemical characteristics of LAB isolated from dahi samples were reported in the study by Harun-Ur-Rashid et al. (2007). The use of MRS medium for initial identification of the Lactobacillus genus was chosen due to the inability of other species to grow in this medium, which is in agree ment with another report suggesting the prevalence of the Lactobacillus genus in MRS medium (López-Díaz et al., 2000; Vasudha & Gayathri, 2023). In the present investigation, the prevalence of Lactobacillus isolates in cow's yogurt was assessed using both cultural and biochemical methods, revealing a rate of 25%. However, when employing PCR, the prevalence was slightly lower at 15%. These results align with those reported by Abdullah and Osman, who conducted a study on the prevalence of the Lactobacillus genus in Sudanese fermented milk (rob), raw milk and white cheese. In their research, the prevalence, determined through cultural, physiological and biochemical tests, was reported to be 69.23%(Abdullah &Osman,2010). In a separate investigation (Saeed et al., 2020), the prevalence of Lactobacillus in goat yogurt was determined to be 10% through PCR analysis. This suggests a higher incidence of probiotic Lactobacillus strains in cow yogurt when compared to goat yogurt. Furthermore, when examining the prevalence of the Lactobacillus genus in raw milk, cheese and yogurt using cultural, physiological and biochemical tests, the reported rate was 24.38% (Taye et al., 2021). It is noteworthy that this prevalence is comparatively lower than the findings observed in the current study. Moreover, the prevalence of the bacteriocin protein plnEF gene in this research was determined to be 4.96%, which stands in stark contrast to a prior study in Egypt (Refayetal.,2020). In their investigation, the

prevalence of the plnEF gene was reported to be substantially higher at 32.35% (22/68). Lactobacillus produces antimicrobial components, such as organic acids, hydrogen peroxide, diacetyl and bacteriocins– low molecular weight antimicrobial com pounds– that exhibit inhibitory effects against pathogens (Santos et al., 2003). In this study, the Lactobacillus isolates demonstrated antibacterial activity and inhibited the growth of the tested pathogens. These findings are consistent with other studies that suggest Lactobacillus species as a common probiotic bacteria used as an alternative measure to prevent Salmonella-related diseases (Kowalska et al., 2020). Similarly, Djadouni and Kihal (2012) reported that LAB produce antibacterial compounds that inhibit the growth of indicator organisms, resulting in inhibition zones of 10–14mm in diameter against *E. coli* and *S. aureus* using the agar spot test. The antagonistic activity of LAB isolates against *S. aureus* observed in this study was similar to the findings of (Prabhurajeshwar & Chandrakanth, 2017). The antibacterial capability of the isolates in this study was comparable, and the fact that Lactobacillus isolates exhibited antibacterial activity against both gram-positive and gram-negative organisms demonstrates their broad-spectrumactivity.

5. Conclusions

The present study provides comprehensive evidence about the presence, diversity, and potential health benefits of Lactobacillus species found in dairy products from several cities across Jazira Province. Over three years, more than one hundred samples—including milk, yogurt, and cheese—were systematically collected and analyzed using microbiological, biochemical, and molecular (PCR) methods. These findings significantly improve understanding of naturally occurring probiotics in both traditional and industrial dairy products in the region. The results showed that yogurt is the most consistent and abundant source of Lactobacillus species, while cheese and specific milk samples exhibited limited or negligible levels of these bacteria. Laboratory tests further demonstrated that these Lactobacillus isolates have significant inhibitory effects against both gram-positive and gram-negative pathogenic bacteria. These antibacterial properties strongly suggest their involvement in promoting gut immunity, restoring intestinal balance, and preventing colonization by harmful microbes.

And Based on this study's findings, the following suggestions are made:

Public health authorities should promote daily consumption of yogurt containing naturally occurring Lactobacillus species. Public awareness campaigns could highlight the importance of maintaining gut health and reducing the risk of gastrointestinal disorders.

Dairy manufacturers and food technologists should standardize fermentation processes to ensure the survival and stability of beneficial Lactobacillus strains. Additionally, incorporating selected probiotic strains into commercial yogurt could improve its function as a health-promoting food with therapeutic benefits.

Finally, this study confirms the probiotic richness of yogurt in Jazira Province and highlights its broader implications for nutrition, medicine, and food technology. These findings should serve as a foundation for practical applications and future scientific research aimed at maximizing Lactobacillus species' health benefits for the general population.

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Reference

1. Abdullah, Sawasan Abdelaziz, and Maymouna Mubarak Osman. "Isolation and identification of lactic acid bacteria from raw cow milk, white cheese and Rob in Sudan." (2010): 1203-1206.

2. Asha, S. A., C. Sudalaimani, P. Devanand, G. Alexander, V. Thomas Sanjeev, and N. Menon Ramshekhar. "Analysis of EEG microstates as biomarkers in neuropsychological processes–Review." Computers in biology and medicine 173 (2024): 108266.
3. Barrow, G. I. and Feltham, R. K. A. (1993). Staining of Bacteria. In: Cowan and Steel's Manual for the identification of medical bacteria. Eds. Barrow, G. I. and Feltham, R. K. A. Cambridge University Press, Great Britain. ISBN 0-521-32611-07. 51-93.
4. Djadouni, Fatima, and Mebrouk Kihal. "Antimicrobial activity of lactic acid bacteria and the spectrum of their biopeptides against spoiling germs in foods." Brazilian Archives of Biology and Technology 55 (2012): 435-444.
5. Djadouni, Fatima, and Mebrouk Kihal. "Antimicrobial activity of lactic acid bacteria and the spectrum of their biopeptides against spoiling germs in foods." Brazilian Archives of Biology and Technology 55 (2012): 435-444.
6. Fesseha, Haben. "Probiotics and its potential role in poultry production: a review." Veterinary Medicine-Open Journal 4, no. 2 (2019): 69-76.
7. Gareau, Mélanie G., Philip M. Sherman, and W. Allan Walker. "Probiotics and the gut microbiota in intestinal health and disease." Nature reviews Gastroenterology & hepatology 7, no. 9 (2010): 503-514.
8. Harun-ur-Rashid, Md, Kaname Togo, Minoru Ueda, and Taku Miyamoto. "Identification and characterization of dominant lactic acid bacteria isolated from traditional fermented milk Dahi in Bangladesh." World Journal of Microbiology and Biotechnology 23, no. 1 (2007): 125-133.
9. Hoque, M. Z., F. Akter, K. M. Hossain, M. S. M. Rahman, M. M. Billah, and K. M. D. Islam. "Isolation, identification and analysis of probiotic properties of Lactobacillus spp. from selective regional yoghurts." (2010): 39-46.
10. Jose, Neethu Maria, Craig R. Bunt, and Malik Altaf Hussain. "Comparison of microbiological and probiotic characteristics of lactobacilli isolates from dairy food products and animal rumen contents." Microorganisms 3, no. 2 (2015): 198-212.
11. Kapilan, R., and Vasanthy Arasaratnam. "A novel Bacillus pumilus strain for alkaline xylanase production at and above 40° C." Ceylon Journal of Science (Biological Sciences) 39, no. 1 (2010.(
12. Karuppaija, Sinthuja, Ranganathan Kapilan, and Vasantharuba Seevaratnam. "Characterization of best naringinase producing fungus strain isolated from palmyrah (Borrasus flabellifer) fruit pulp." (2016.(
13. Kowalska, Justyna D., Adriana Nowak, Katarzyna Śliżewska, Małgorzata Stańczyk, Magdalena Łukasiak, and Jarosław Dastych. "Anti-Salmonella Potential of New Lactobacillus Strains with the Application in the Poultry Industry." Polish Journal of Microbiology 69, no. 1 (2020.(
14. Kowalska, Justyna D., Adriana Nowak, Katarzyna Śliżewska, Małgorzata Stańczyk, Magdalena Łukasiak, and Jarosław Dastych. "Anti-Salmonella Potential of New Lactobacillus Strains with the Application in the Poultry Industry." Polish Journal of Microbiology 69, no. 1 (2020.(
15. Lopez-Dıaz, T. M., C. Alonso, C. Roman, M. L. Garcıa-Lopez, and B. Moreno. "Lactic acid bacteria isolated from a hand-made blue cheese." Food Microbiology 17, no. 1 (2000): 23-32.
16. Neha, Neha, Yogita Sharma, Anshu Yadav, Ramnarayan Ramnarayan, and G. Swapna. "Sustainability and role conflict: A bibliometric analysis of research themes, trends, and future research directions: 2013-2022." In E3S Web of Conferences, vol. 430, p. 01186. EDP Sciences, 2023.
17. Prabhurajeshwar, Chidre, and Revanasiddappa Kelmani Chandrakanth. "Probiotic potential of Lactobacilli with antagonistic activity against pathogenic strains: An in vitro validation for the

production of inhibitory substances." Biomedical journal 40, no. 5 (2017): 270-283.

18. Pundir, Ram Kumar, Satish Rana Neha Kashyap, and Amandeep Kaur. "Probiotic potential of lactic acid bacteria isolated from food samples: an in vitro study." Journal of Applied Pharmaceutical Science 3, no. 3 (2013): 085-093.
19. Refay, Rasha Mohamed, Hala Mohamed Abushady, Sara Adel Amer, and Mahmoud Ahmed Mailam. "Determination of bacteriocin-encoding genes of lactic acid bacteria isolated from traditional dairy products of Luxor province, Egypt." Future Journal of Pharmaceutical Sciences 6, no. 1 (2020): 22.
20. Saeed, Z. K., B. A. Abbas, and R. M. Othman. "Molecular identification and phylogenetic analysis of lactic acid bacteria isolated from goat raw milk." Iraqi Journal of Veterinary Sciences 34, no. 2 (2020.(
21. Santos, A., M. San Mauro, A. Sanchez, J. M. Torres, and D. Marquina. "The antimicrobial properties of different strains of Lactobacillus spp. isolated from kefir." Systematic and applied Microbiology 26, no. 3 (2003): 434-437.
22. Taye, Yeshambel, Tadesse Degu, Haben Fesseha, and Mesfin Mathewos. "Isolation and identification of lactic acid bacteria from cow milk and milk products." The Scientific World Journal 2021, no. 1 (2021): 4697445.
23. Theivendrarajah, K. "Microbiology Laboratory Manual: Department of Botany, University of Jaffna." University Publication (1990): 1-33.
24. Vasudha, Mahantesh. "Metabolism and functional heterogeneity of fermented milk origin lactic acid bacteria for lactose intolerance." Journal of microbiology, biotechnology and food sciences 13, no. 3 (2023): e9654-e9654.