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Probiotic Characterization of *Limosilactobacillus* and *Lactiplantibacillus* Strains Isolated from Traditional Egyptian Dairy Products

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ABSTRACT

Fermented foods, notably traditional dairy products, continue to host a diverse array of microorganisms, including probiotic lactic acid bacteria. Consequently, this study strives to assess the probiotic capacity of Lactobacillus strains obtained from artisanal dairy products and select promising candidates for potential use as innovative food preservatives or probiotic additives in functional dairy products. The *in vitro* examinations encompassed survival under acidic conditions and exposure to bile salt, as well as assessment of antimicrobial activity against *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* O157:H7, and *Salmonella typhimurium*. Tolerance to 0.4% bile salt and exposure to pH 2.0 acidity were first determined, and subsequently assessed other probiotic characteristics. From the pool of 422 isolates, 25 displayed notable resistance to both acid and bile salt, and from this group, only five isolates were selected for more detailed characterization. Phenotypic methods were used for their characterization, and through 16S rRNA gene sequencing, they were identified as two distinct species: *Limosilactobacillus fermentum* (3 isolates) and *Lactiplantibacillus plantarum* (2 isolates). The 5 isolates exhibited varying degrees of antimicrobial effectiveness against pathogenic strains. Considering all the experiments, all strains appear to be promising candidates for utilization in food preservation and/or as probiotic supplements in functional food.

Keywords: *Lactobacillus* sp., probiotic properties, antimicrobial activity, carbohydrate fermentation

INTRODUCTION

Probiotics is a rapidly expanding market for food producers, particularly in the dairy food sector (Mitropoulou *et al*., 2013). When administered in sufficient proportions, probiotics are live microbial dietary supplements that have a variety of positive effects on the health of the host (Vinderola *et al*., 2008; Dawood *et al*., 2021; Darwish *et al*., 2022a; Darwish *et al*., 2022b; Elbermawi *et al*., 2022a; Elbermawi*et al*., 2022b and Darwish *et al*., 2023a). Studies conducted earlier have documented the potential antimicrobial effects, antioxidative capacity and anti-tumor properties associated with probiotics (Feng *et al*., 2023). The frequently utilized probiotics primarily come from *Bifidobacterium* and lactic acid bacteria (Delgado *et al*., 2020). Lactic acid bacteria (LAB) encompass numerous bacterial genera, with the most well-known ones including lactococci, streptococci, lactobacilli, enterococci, pediococci and leuconostoc (; El-Sharoud *et al*., 2012; Ryssel *et al*., 2014; Delorme *et al*., 2017 and Nassib *et al*., 2018). These genera vary in terms of their appearance, tolerance to pH and salt, optimal temperature, natural habitats, and potential for causing diseases. Currently, it's challenging to clearly distinguish between beneficial and potentially harmful species, as certain problematic characteristics are often more associated with specific strains rather than entire species. However, Lactobacilli and Lactococci have been categorized as "generally recognized as safe," making them extensively applied in the food sector (Bin Masalam *et al*., 2018 and Darwish *et al*., 2023b), particularly in the dairy industry. Lactic acid bacteria (LAB) are typically recognized as crucial in the industrial sector, where they play a role in fermenting various dairy products, foods, and beverages, ultimately resulting in the production of lactic acid. Within this group, *Lactobacillus* strains are particularly notable for their promising probiotic attributes (Yadav *et al*., 2016). The market success of probiotics hinges on the efficiency of the probiotic cultures employed. Probiotics generate a range of substances, including organic acids, exopolysaccharides, antimicrobial compounds and bacteriocins (Jacobsen *et al*., 1999). These acids and bacteriocins that are produced work to inhibit the growth of harmful microorganisms in the intestinal tract (Pessione, 2012).

A probiotic that functions optimally should be live and viable, safe for consumption, capable of withstanding the harsh conditions of bile and gastric juices, able to endure the journey through the digestive system, and proficient at attaching to and establishing a presence on the surface of intestinal cells. To assess their ability to adhere to the gut surfaces and thus improve their interaction with the host, we conducted a Bacterial Adhesion to Hydrocarbons (BATH) study (Khojah *et al*., 2022). Another crucial factor to consider in the selection process is the ability to tolerate phenols. This is significant because intestinal bacteria have the capacity to deaminate amino acids from dietary sources, resulting in the production of phenolic compounds. These bacteria provide various advantages to the host, including improved serum cholesterol management, lipid control, enhanced immune function, and a range of health benefits such as anti-cancer, anti-allergic, antioxidant, and antimicrobial properties (Lee *et al*., 2014).

The inhibitory impact of bacteriocins generated by probiotics extends to pathogens. Furthermore, there is

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supporting evidence of their involvement in the treatment of conditions such as necrotizing enterocolitis, ulcerative colitis, acute infectious, pouchitis, irritable bowel syndrome, antibiotic-associated diarrhea and Crohn's disease (Chong, 2014). Probiotics have shown positive outcomes in the management of liver conditions, regulation of intestinal permeability, and mitigation of inflammation. The supplementation of *Lactobacillus paracasei* results in a decrease in *Enterococcus* species and Enterobacteriaceae, potentially fostering the proliferation of *Bifidobacteria*, *Lactobacillus* and other advantageous microorganisms in the gastrointestinal system (Chávez-Tapia *et al*., 2015). Moreover, probiotics have the capacity to enhance antioxidant function and serve as a supplementary approach in managing conditions such as allergies, *Helicobacter pylori* infection, cancer, lactose intolerance and vaginosis (Vinderola *et al*., 2008; Mitropoulou *et al*., 2013; Lynne, 2014; Touchefeu *et al*., 2014; Abdel-Aziz *et al*., 2020; Taher *et al*., 2022 and Samra *et al*., 2023).

Recent research has proposed that *Limosilactobacillus fermentum* and *Lactiplantibacillus plantarum* (formerly *Lactobacillus fermentum* and *Lactobacillus plantarum*, respectively) are versatile and prominent member of LAB, mainly because of their unique probiotic attributes. This species can withstand both bile salt and acidic environments and exhibits antagonistic effects against intestinal pathogens (Yadav *et al*., 2016).

The aim of this research was to assess the probiotic capabilities and conduct a physiological and biochemical analysis of *L. plantarum* strains obtained from traditional Egyptian dairy items.

MATERIALS AND METHODS

Materials

Chemicals and culture media

HCL, sodium hydroxide, bile salt, L cysteine, and bromophenol blue were acquired from Sigma Aldrich (St. Louis, MO, USA). De Man, Rogosa, and Sharpe (MRS), MRS broth, tryptone soya broth (TSB) and tryptone soya agar (TSA) were acquired from Thermo Fisher Scientific (Cairo, Egypt).

Bacterial strains

All bacterial strains (*Bacillus cereus* (ATCC 14579), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* O157:H7 (CICC 21530) and *Salmonella* Typhimurium (CICC 10420)) were used in this study. They were taken from stock strains collection of food microbiology laboratory (Dairy department, Faculty of Agriculture, Mansoura University, Egypt).

Collection of dairy product samples

To gauge the prevalence of probiotic lactic acid bacteria in traditional dairy product samples, 100 samples of those items were randomly chosen from local markets in Mansoura city. This set of samples included 22 yoghurt samples, 18 Laban Rayeb samples, 14 Kariesh samples, 12 Pickled Domiati samples, 18 Fresh Domiati samples, and 16 Mish samples (Table 1).

Methods

Isolation of probiotic potential of lactic acid bacteria from traditional dairy products

One hundred different dairy samples were gathered to isolate lactic acid bacteria (LAB). Table 1 provides information about these samples and their respective origins. Twenty-five grams of each sample were mixed with 225 milliliters of 0.1% peptone water. Subsequently, these samples were subjected to serial dilution in the same diluent to produce dilutions ranging from 10^{-1} to 10^{-8}. Probiotic bacteria were then isolated from these serially diluted samples using the streak-plate technique on MRS-cysteine-bromophenol blue (MRS-Cys-BPB) agar [MRS broth (55 gL^{-1}) + L cysteine (0.5 gL^{-1}) + Bromophenol blue (0.02 gL^{-1}) + agar (55 gL^{-1})]. The incubation was conducted at 38°C for 24 hours in an anaerobic condition, which was created by placing a gas pack inside anaerobic jar. Following this, microbial isolates' colonies were restreaked onto MRS-Cys-BPB agar plates to achieve pure cultures, which were subsequently preserved on MRS-Cys agar slants at 4°C or as frozen cultures (Ngamsomchat *et al*., 2022).

Identification and characterization of bacterial isolates

The isolates underwent to carbohydrate fermentation test (using API 50 CHL, bioMérieux, France), biochemical assays, including catalase, oxidase tests and Gram staining. The identification of the isolates was carried out through 16S rRNA gene sequencing, following the method described by Ngamsomchat *et al*. (2022).

Gram stain test

The Gram staining method was used to conduct morphological observations. Let the bacteria settle on the glass item for up to one, then add 1-2 drops of distilled water, fix on a Bunsen flame, dry, then apply 2-3 drops of crystal violet to the bacterial colonies. Let the crystal violet sit on the bacterial colonies for a minute before washing with distilled water, drying, and then allowing to air dry. Drop a couple of drops of the iodine solution, wait 30 seconds, then rinse with alcohol one more before drying. Following a minute of waiting, 2-3 drops of safranin solution were drip-applied to the preparations, which were then washed with distilled water and dried. This test tries to identify the test isolate's microscopic properties, including its response to gramme staining and the size, shape, and composition of its cells. Utilize a microscope to observe (Prastujati *et al*.,2022).

Catalase test

A suitable amount of growth from a single distinct LAB isolate colony was put into a clean glass slide, and then 1 drop of H2O2 (30%) was added. Instant bubbling (gas production) was seen as a successful outcome (Prastujati *et al*.,2022).

Oxidase test

The 1% Kovács oxidase reagent was applied to a tiny piece of filter paper and allowed to air dry. A well-isolated colony was selected and applied on treated filter paper from a fresh (18–24hour culture). When the color turns to dark purple within 5 to 10 seconds, microorganisms are oxidase positive. Microorganisms are oxidase negative, nevertheless, if the colour remains the same or takes more than two minutes (Ngamsomchat *et al*., 2022).

Evaluation of probiotic characteristics of isolated strains

To identify isolates for the development of a functional dairy product, we evaluated the probiotic characteristics of the isolates obtained from traditional dairy products. Figure 1 provides an overview of the probiotic property testing process.

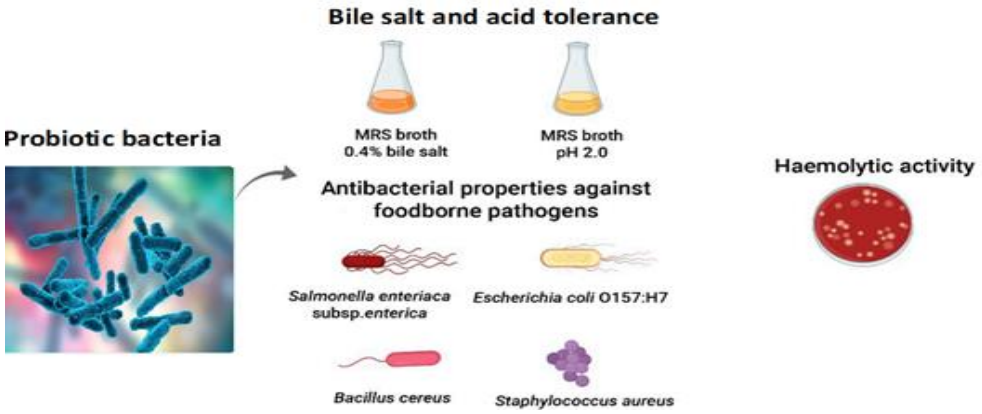

Figure 1. Assessment of the probiotic characteristics of isolates from traditional Egyptian dairy products.

Acid tolerance

The isolates were cultured in MRS broth overnight at 37°C. Actively growing cells were collected through centrifugation (6500 g, 5°C, 15 minutes). Using 1N HCl, MRS broth's pH was brought down to 2.0. As a control, MRS broth with a pH of 6.5 was used. The collected cells were placed in MRS broth with an acidic pH and incubated at 37 °C for 3 hours. Afterward, the samples were diluted using phosphate-buffered saline. Afterwards, these samples were applied onto MRS-CYS-BPB agar plates and left to incubate at 38°C for a duration of 24 hours. The plate count technique was used to measure cell viability, and the findings were reported as a survival percentage in accordance with equation 1 (Liong & Shah, 2005).

$$\text{Tolerance percentage} = \frac{\text{number of suvivors}}{\text{initial number of bacteria}} \times 100$$

Tolerance to Bile Salts

An overnight culture of LAB isolate was inoculated in 5 ml of MRS medium that had been enriched with 0.4% Oxgall. A control group without Oxgall was also prepared. Following a 4-hour incubation period at 37°C, the samples were successively diluted with saline. Counting of colonies was performed by placing 100 µl of cultures at the appropriate dilutions onto MRS agar. The tolerance percentage was then calculated as above (Ngamsomchat *et al*., 2022).

Hemolytic activity

The bacterial isolates were streaked onto blood agar plates that contained 5% sheep blood and then incubated for 48 hours at 38°C to determine if there was a hemolysis zone around the colonies (Wei *et al*., 2019).

Antimicrobial activity

The bacterial isolates were introduced into the MRS liquid medium with an initial inoculation of 1%, followed by incubation at 35°C for 24 hours. Subsequently, to obtain a cell-free supernatant, the culture was subjected to centrifugation for 5 min at 9,500 rpm. The resulting supernatant was then filtered through a 0.22 µm filter. *Bacillus cereus* (ATCC 14579), *Staphylococcus aureus* (ATCC 25923), *E. coli* O157:H7 (CICC 21530) and *Salmonella typhimurium* (CICC 10420) were employed as indicator bacteria. The antibacterial effects of cell suspension (CS) or cell-free supernatants (CFS) were assessed using the Oxford cup method. These bacterial indicators were introduced into TSA medium. Following a 24-hour incubation period at 38°C, a 100 µL suspension was uniformly spread onto separate media using sterile cotton swabs. The surface of the spread medium was gently compressed using an Oxford cup and 200 µL of either the cell suspension or cell-free supernatant was added to the cup. Non-bacterial MRS medium was used as a blank control. The plates were pre-diffused for 5 hours at 4°C and then incubated at 37°C for 18 hours to measure the diameter of the inhibition zone (Wei *et al*., 2019). As a positive control, polymyxin B (50 g/mL) and penicillin (100 IU) were utilized.

Evaluation of exopolysaccharide (EPS) synthesis

LAB isolates were grown in MRS broth medium overnight at 37°C. The cultures obtained were subsequently employed to inoculate freshly prepared sterilized skim milk (10% w/v) at a concentration of 2% (v/v), and this mixture was then incubated for 24 hours at 37°C. The coagulated cultures produced by LAB were gently stirred 5-7 times with a spoon. The existence of cohesive, uninterrupted strands of milk while extracting samples indicated the synthesis of EPS.

Statistical analysis

Every test was run in triplicate. The changes in tolerance to acid and bile salt as well as antibacterial activity were assessed using an ANOVA test with a significance threshold of p 0.05. The data was displayed as an average and standard deviation. The Duncan's multiple range tests were used to identify significant deviations between values. All statistical tests in the current study were evaluated using SPSS Statistics program. Using the XLSTAT program, a principal component analysis (PCA) and heat map analysis were performed.

RESULT AND DISCUSSION

Isolation of potential probiotic bacteria from traditional Egyptian dairy products

The prevalence of probiotic lactic acid bacteria was established by culturing the samples on MRS-Cys-BPB agar. Suspected colonies were picked up from MRS-Cys-BPB agar plates, which were then put through biochemical and physiological identity tests. From these samples, a sum of 422 suspected LAB isolates was obtained, and these isolates subsequently underwent initial identification through hemolytic response assessment, the catalase test and Gram staining (Table 1). The isolates were all catalase-negative and Gram-positive. On sheep blood agar, there was no hemolytic response from them (Table 1). Numerous dairy items, such as pickled Domiati cheese, mish, Laban

Rayeb, yoghurt, fresh Domiati cheese, and Kariesh cheese, seemed to be potential sources of probiotic lactic acid bacteria. The majority of microbial isolates discovered in these foods were Gram-positive bacteria. On a sheep blood agar plate, they did not produce catalase and did not exhibit hemolysis, which suggests that they may be LAB. One of the most important factors in choosing a probiotic strain is its nonpathogenic property (Ngamsomchat *et al*., 2022). The study evaluated the capacity of 100 LAB isolates sourced from traditional. Among the 100 LAB isolates investigated, only 10 isolates were identified as an EPS producer. Laban Rayeb and Kariesh cheese were the sources of these isolates.

Table 1. Verification of the physiological characteristics of the potential LAB isolates found in the traditional Egyptian dairy products.

Source	No. of Samples	No. of suspected LAB isolates	Cell morphology		Gram stain	Hemolytic activity	Catalase production	Oxidase Production	EPS
			Lactobacilli	Cocci					
Yogurt	22	103	30	73	+	-	-	-	0
Laban Rayeb	18	81	24	57	+	-	-	-	8
Kariesh cheese	14	61	20	41	+	-	-	-	2
Fresh Domiati cheese	18	74	35	39	+	-	-	-	0
Pickled Domiati cheese	12	43	13	30	+	-	-	-	0
Mish	16	60	15	45	+	-	-	-	0
Total	100	422	13 7	285					10

Tolerance to acidity and bile salts

To find prospective probiotics, bile salt and acid tolerance tests were performed on each isolate recovered from the dairy samples. In the initial screening, it was noted that 25 out of the 422 isolates demonstrated significant resilience to both bile salts and acidic conditions, displaying a survival rate exceeding 50%. The 25 isolates that showed tolerance to bile salts and acid were Gram-positive, with a negative reaction for oxidase and catalase, and exhibited gamma hemolysis (Table 1). Among them, only five isolates demonstrated over 80% survival after exposure to bile salts and acid conditions (Figure 2). Through 16S rRNA gene sequencing, these isolates were identified as *L. fermentum* (3 isolates) and *L. plantarum* (2 isolates) (Table 2). These five isolates were chosen for a more detailed examination of their probiotic characteristics. *L. fermentum* and *L. plantarum* were recognized as bile salt/acid-tolerant isolates. Due to their capacity to survive challenging conditions and their nontoxic nature, these species are thought to be suitable for use as probiotics (Senok *et al*., 2005; Shah, 2007). Positive effects on the human body depend on probiotic bacteria surviving. To get to the colon, they must survive in the gastrointestinal (GI) tract (Finlay *et al*., 1999; Abd El-Aziz and Darwish, 2014 and Ngamsomchat *et al*., 2022). Hence, the capacity of probiotic bacteria to endure harsh environmental conditions, such as the acidic stomach environment and the high bile salt levels in the intestine, is of paramount importance (Ouwehand and Salminen, 1998 and Abeijón Mukdsi *et al*., 2013). Such unfavorable conditions may cause cell membrane damage by disrupting the lipid bilayer, which might have a negative effect on probiotic bacteria (Pyar and Peh, 2014). In a prior study, the potentially probiotics survival, specifically those belonging to the genera *Enterococcus*, *Lactococcus*, and *Lactobacillus*, was investigated when cultured in the presence of bile salts at a concentration of 0.3% and an acidic setting with a pH of 2.5. The findings revealed that these bacteria exhibited survival rates ranging from 65% to 98% in the presence of bile salts and from 81% to 85% in the acidic environment (Haghshenas *et al*., 2017). In our research, we initially evaluated the potential probiotic characteristics of *Limosilactobacillus fermentum* and *Lactiplantibacillus plantarum* isolates by subjecting them to a challenging environment consisting of a high bile salt concentration (0.4% w/v) and strong acidity (pH 2.0) for a duration of 3 hours. These conditions were created to simulate the challenging conditions within the digestive system.

Table 2. The nearest species/strains of the selected isolates based on their 16S rRNA gene sequences.

Isolate	Source	Closet Strain	% identity
MSD21	Kariesh cheese	*Lactobacillus plantarum* strain JCM 1149	100.0
MSD24	Mish	*Lactobacillus fermentum strain* CIP 102980	99.80
MSD58	Yogurt	*Lactobacillus fermentum strain* CIP 102980	100.0
MSD73	Laban Rayeb	*Lactobacillus plantarum strain* JCM 1149	99.93
MSD101	Laban Rayeb	*Lactobacillus fermentum strain* CIP 102980	99.90

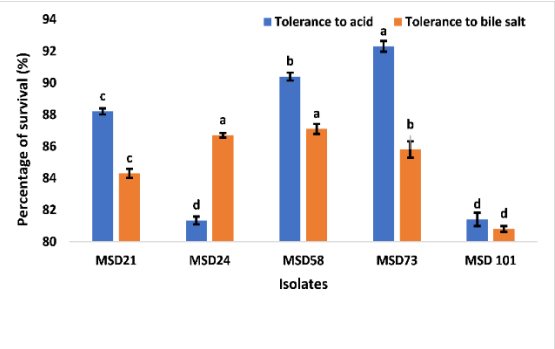

Figure 2. The percentage of survival for five potential probiotic isolates in relation to their tolerance to bile salts (0.4%) and acid (pH 2.0).

Carbohydrate Fermentation Characteristics of Potential Probiotic Isolates

The chosen strains, namely MSD21, MSD24, MSD58, MSD73, and MSD101, underwent carbohydrate fermentation analysis. The carbohydrate fermentation patterns of these strains are illustrated in Figure 3. The carbohydrate fermentation analysis revealed that all isolates were capable of fermenting D-fructose, D-glucose, D-ribose, L-arabinose, arbutin, D-mannose, sucrose and D-raffinose (Figure 3). Notably, both MSD21 and MSD73, which originate from Kariesh cheese and Laban Rayeb, respectively displayed a comparable pattern of carbohydrate fermentation. What's intriguing is that they demonstrated the capacity to use a wider range of carbohydrates compared with the other isolates (Figure 4). These results are

consistent with those of Ngamsomchat *et al*. (2022), who showed that all *Lactobacillus plantarum* and *Lactobacillus fermentum* isolates could ferment D-Ribose, L-Arabinose, D-Fructose, D-Glucose, Arbutin, D-Mannose, D-Saccharose, and D-Raffinose, according to the results of the carbohydrate fermentation.

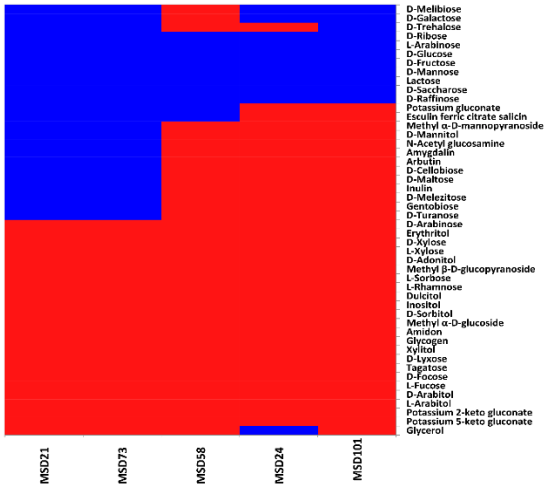

Figure 3. heat map analysis of carbohydrate fermentation for the 5 isolates, the color red denotes a lack of carbohydrate fermentation (negative), while the color blue signifies active carbohydrate fermentation (positive).

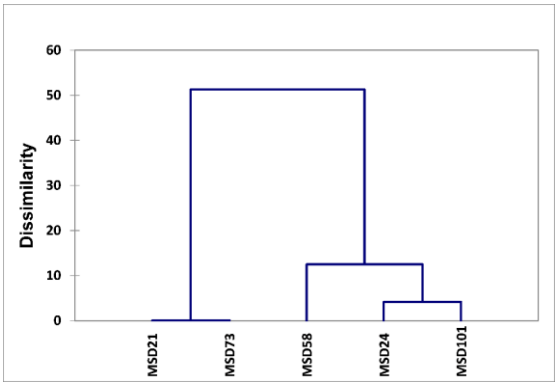

Figure 4. Agglomerative hierarchical clustering (AHC) of various bacterial isolates obtained from traditional Egyptian dairy Products.

Antibacterial activity

The *in vitro* antibacterial activity against pathogenic bacteria can be seen as a desirable trait for certain probiotics. In this study, out of the 5 strains examined using the Oxford Cup method, all of isolates (cell-free supernatant or cell suspension) exhibited notable antibacterial activity against six commonly found pathogenic bacteria (Figure 5). The findings indicated that the isolates' cell-free supernatant exhibited inhibition zones ranging from 8.4 to 21 mm, while the cell suspension of the isolates displayed inhibition zones ranging from 10.8 to 25 mm, as detailed in Figure 5. In comparison to other probiotic strains, the cell suspension of the MSD 24 strain had the strongest inhibitory zone against *B. cereus*, *S.* Typhimurium, and *E. coli* O157:H7 (Figure 5). The results indicate that each potential probiotic isolate exhibited inhibitory effects on pathogenic bacteria, including Gram-negative, Gram-positive, and spore-forming varieties, whether in the form of cell suspensions or cell-free supernatants. In general, the inhibitory effect against the pathogens observed in the cell-free supernatant was relatively weaker compared to that of the cell suspension. Selecting lactobacilli strains with probiotic potential is essential for preserving the optimal balance of gastrointestinal microbiota and providing additional health benefits. *Lactobacillus*, a gram-positive bacterium that is non-sporogenic and can thrive in both facultative anaerobic conditions and anaerobic, is a significant probiotic category present in the digestive systems of both humans and animals. Some strains even produce a unique compound called lactobacillin, which possesses natural antiseptic properties. This compound can potentially prevent and hinder the infiltration and establishment of harmful microorganisms through lowering the pH or biological antagonism (Vinderola *et al*., 2008; Zago *et al*., 2011). The capability of lactobacilli to restrain these pathogenic bacteria arises from the organic acids they generate, alongside other antimicrobial substances like diacetyl, bacteriocins and hydrogen peroxide. These compounds collectively augment the inhibitory effects of lactic acid (Hoque *et al*., 2010; Victor *et al*., 2011 and El-Gammal *et al.*, 2017).

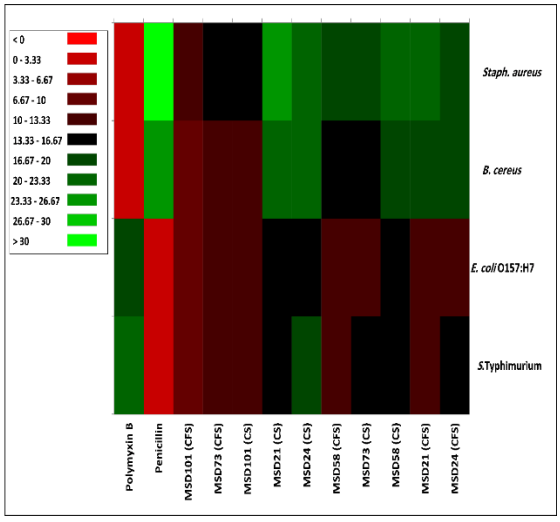

Figure 5. Heat map analysis of the antimicrobial activity of 5 lactobacilli strains with bacteriostatic properties based on the diameter of the inhibition zone. The various colors in the figure indicate the size of the diameter of the bacteriostatic zone, with green indicating the largest diameter of the inhibition zone and red representing the smallest diameter of the bacteriostatic zone. The colors transition from red to green as the diameter of the bacteriostatic zone increases.

Multivariate Analysis of Parameters Related to Lactic Acid Bacteria

Table 3 presents the Varimax rotated factor loadings, revealing the relationships between the initial characteristic measurements and the PC. Significantly influential factors are highlighted with an absolute value exceeding 0.560 (highlighted in bold text). Furthermore, PCA provides factor score values (Table 4) that indicate the positioning of each treatment concerning the Varimax rotated PC factors. Principal component analysis (PCA) of characteristics such as acid and bile salt tolerance, carbohydrate fermentation,

and antimicrobial activity in *Limosilactobacillus fermentum* (MSD-24, MSD-58, and MSD-101) and *Lactiplantibacillus plantarum* (MSD-21 and MSD-73) explained 82.44% of the variability on two principal components (2PC) (Fig. 6A). PC1 (55.88%) included attributes like D-Mannitol, Methyl α-D-mannopyranoside, N-Acetyl glucosamine, Amygdalin, Arbutin, Esculin ferric citrate, salicin, D-Cellobiose, D-Maltose, Inulin, D-Melezitose, Gentobiose, D-Turanose, Potassium gluconate, and tolerance to acid. On the other hand, the second component (26.56%) was primarily associated with antimicrobial activity against *B. cereus*, *Staph. aureus*, *S. typhimurium*, and *E. coli* O157:H7, as well as both D-Trehalose and tolerance to bile salt (Fig. 6A). The treatments were categorized into three groups: Groups 1 (MSD-24 and MSD-58) and the second group (MSD-101) were positioned on the left side of PC1, while the third group (MSD-21 and MSD-73) was situated on the positive side of PC1 (Fig. 6A). Correlations were identified among the 24 attributes under investigation (Figure 6B). The glycerol fermentation by these strains displayed a positive correlation with their antimicrobial activity, as well as the fermentation of both D-Galactose and D-Melibiose, and their tolerance to acidity and bile salts (Fig. 6B). Conversely, the glycerol fermentation by these strains exhibited a negative correlation with other attributes (Fig. 6B).

Table 3. Factor loadings for chosen attributes of probiotic lactic acid bacteria using Varimax rotation in the principal component analysis

	F1	F2	F3	F4
B. cereus	0.002	0.559	0.324	0.114
Staph. aureus	0.061	0.495	0.182	0.262
S. *typhimurium*	0.019	0.766	0.205	0.011
E. coli O157:H7	0.023	0.929	0.027	0.021
Glycerol	0.215	0.127	0.589	0.069
D-Galactose	0.106	0.324	0.553	0.016
D-Mannitol	0.992	0.001	0.007	0.000
Methyl α-D-mannopyranoside	0.992	0.001	0.007	0.000
N-Acetyl glucosamine	0.992	0.001	0.007	0.000
Amygdalin	0.992	0.001	0.007	0.000
Arbutin	0.992	0.001	0.007	0.000
Esculin ferric citrate salicin	0.532	0.184	0.276	0.008
D-Cellobiose	0.992	0.001	0.007	0.000
D-Maltose	0.992	0.001	0.007	0.000
D-Melibiose	0.106	0.324	0.553	0.016
D-Trehalose	0.416	0.571	0.000	0.012
Inulin	0.992	0.001	0.007	0.000
D-Melezitose	0.992	0.001	0.007	0.000
Gentobiose	0.992	0.001	0.007	0.000
D-Turanose	0.992	0.001	0.007	0.000
Potassium gluconate	0.532	0.184	0.276	0.008
Tolerance to acid	0.478	0.141	0.347	0.034
Tolerance to bile salt	0.005	0.877	0.000	0.118
Tolerance to bile salt	0.005	0.877	0.000	0.118

Table 4. Principal component factor scores for specific attributes of probiotic lactic acid bacteria following Varimax rotation in the principal component analysis

	F1	F2	F3	F4
MSD21	4.461	0.411	0.959	1.342
MSD24	-3.398	1.798	2.832	-0.473
MSD58	-2.390	2.876	-2.743	0.231
MSD73	4.473	-0.635	-0.591	-1.378
MSD101	-3.145	-4.449	-0.456	0.279

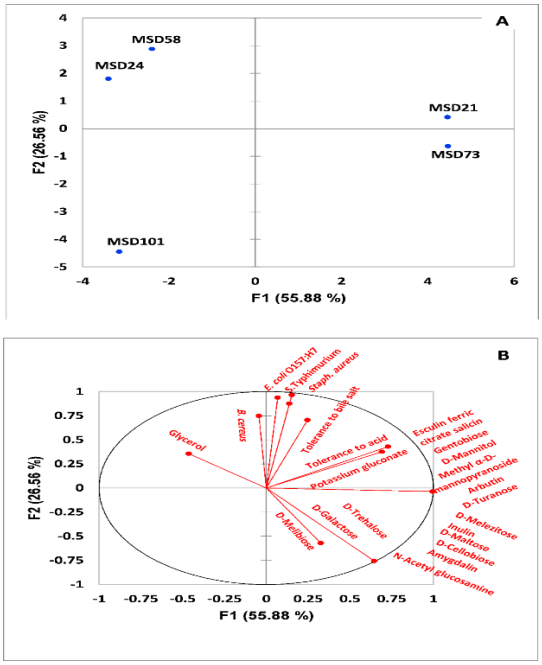

Figure 6. (A) The PCA biplot illustrates the examined attributes of probiotic lactic acid bacteria. (B) It represents the analysis of correlated variables.

CONCLUSION

Traditional dairy products, like fermented foods, continue to be rich in a diverse assortment of microorganisms, including beneficial lactic acid bacteria known as probiotics. As a result, this study's primary objective is to evaluate the probiotic potential of Lactobacillus strains extracted from artisanal dairy items and identify promising candidates that could serve as innovative food preservatives or probiotic enhancements in functional dairy products. The initial tests involved assessing the strains' ability to withstand the harsh conditions of low pH and bile exposure, followed by an exploration of various probiotic attributes. Out of 422 isolates, 25 demonstrated remarkable resilience against both acid and bile salt. Out of this subset, 6 isolates were selected for more comprehensive characterization. These isolates underwent phenotypic characterization, and their precise identification was accomplished through 16S rRNA gene sequencing, revealing two distinct species: *Limosilactobacillus fermentum* (3 isolates) and *Lactiplantibacillus plantarum* (2 isolates). These 5 isolates exhibited varying degrees of effectiveness in inhibiting the growth of 5 different pathogenic strains. Taking all the experimental findings into consideration, these 5 strains emerge as highly promising candidates for potential application in food preservation and as probiotic supplements in functional food products.

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توصيف البروبيوتيك للسلالات التابعة *Limosilactobacillus* و *Lactiplantibacillus* والتي تم عزلها من منتجات الألبان المصرية التقليدية

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الملخص

تعتبر الأغذية المتخمرة، وخاصة منتجات الألبان التقليدية، مصدر أساسي لمجموعة متنوعة من الكائنات الحية الدقيقة، بما في ذلك سلالات البروبيوتيك التابعة لجنس بكتيريا حمض اللاكتيك ونتيجة لذلك، تسعى هذه الدراسة إلى تقييم قدرة سلالات البروبيوتيك التابعة لجنس *Lactobacillus* والتي تم الحصول عليها من منتجات الألبان التقليدية واختيار السلالات الجيدة التي يمكن استخدامها كمواد حافظة غذائية مبتكرة أو كإضافات بروبيوتيك في منتجات الألبان الوظيفية. شملت الاختبارات المعملية قياس القدرة علي تحمل الظروف الحامضية و تركيزات مختلفة من أملاح الصفراء، بالإضافة إلى تقييم النشاط المضاد للميكروبات ضد *Bacillus cereus* و*Staphylococcus aureus*، و*E. coli* O157:H7، و *Salmonella typhimurium*. تم تحديد مدى تحمل العزلات لأملاح الصفراء بتركيز يصل إلي ٠,٤% وتحمل درجات من pH تصل إلى ٢,٠ ويمثل الاختبارين السابقين خطوة مبدئية لتحديد العزلات المناسبة والتي سوف يتم استكمال الاختبار عليها. من بين ٤٢٢ عزلة، أظهرت ٢٥ عزلة مقاومة ملحوظة لكل من درجات pH المنخفضة وأملاح الصفراء، ومن هذه المجموعة، تم اختيار خمس عزلات فقط لتعريفها بالطرق الجينية وتحديد قدرتها علي تخمر العديد من السكريات. ومن خلال تحديد التتابع الجيني للـ 16SrRNA، تم تصنيفها ضمن نوعين ثلاث عزلات تابعة لـ *Limosilactobacillus fermentum* و عزلتان تابعة لـ *Lactiplantibacillus plantarum* . أظهرت النتائج أن العزلات الخمس لها درجات متفاوتة من الفعالية المضادة للميكروبات المرضية الخمسة سالفة الذكر. وبالنظر إلى جميع التجارب، يبدو أن هناك جميع العزلات السابقة مرشحة بقوة للاستخدام في حفظ الأغذية و/أو كمكملات بروبيوتيك في الأغذية الوظيفية.

الكلمات الدالة : جنس اللاكتوباسيلس، خصائص البروبيوتيك، النشاط المضاد للميكروبات، تخمير الكربوهيدرات