



The Prospects of *Lactobacillus oris* as a Potential Probiotic With Cholesterol-Reducing Property From Mother's Milk

Sadia Afrin¹, Suraiya Akter², Shamima Begum² and Md Nur Hossain^{1*}

¹ Institute of Food Science and Technology, Bangladesh Council of Scientific and Industrial Research, Dhaka, Bangladesh,

² Department of Microbiology, Jagannath University, Dhaka, Bangladesh

OPEN ACCESS

Edited by:

Zhaojun Wei,
Hefei University of Technology, China

Reviewed by:

Ramasamy Ramasubburayan,
Manonmaniam Sundaranar
University, India
Rosita Jamaluddin,
Putra Malaysia University, Malaysia
Kiran Thakur,
Hefei University of Technology, China

*Correspondence:

Md Nur Hossain
mnhossain84@bcsir.gov.bd;
mnhossain84@gmail.com
orcid.org/0000-0001-8079-5170

Specialty section:

This article was submitted to
Nutrition and Food Science
Technology,
a section of the journal
Frontiers in Nutrition

Received: 20 October 2020

Accepted: 08 February 2021

Published: 04 March 2021

Citation:

Afrin S, Akter S, Begum S and
Hossain MN (2021) The Prospects of
Lactobacillus oris as a Potential
Probiotic With Cholesterol-Reducing
Property From Mother's Milk.
Front. Nutr. 8:619506.
doi: 10.3389/fnut.2021.619506

This experiment was conducted to characterize potential *Lactobacillus* spp. isolated from mother's milk and infant feces to obtain new and specific probiotic strains. In this study, seven ascendant strains were identified as *Lactobacillus* spp. based on their morphological characteristics and biochemical properties. Among them, only one (C-1) isolate was identified as *Lactobacillus oris* through BioLog™ identification. The study further investigated the isolate through probiotic potentiality tests such as pH and bile tolerance, NaCl tolerance test, gastric juice tolerance, antioxidant activity, resistance to hydrogen, reduction of sodium nitrate, antimicrobial activity, and antibiotic susceptibility test. The result showed that the strain is a potential probiotic based on probiotic capability. The identified strain was most acid-tolerant and retained around 80% viability for up to 4 h at pH 1.0 and 2.0. The isolate showed tolerance against up to 1.50% bile concentration and gastric juice and was able to grow 1–6% NaCl concentrations. *Lactobacillus oris* showed resistance to most antibiotics as well as antagonistic activity against the tested pathogen, good antioxidant properties, reduction of sodium nitrate and H₂O₂. The isolate exhibited good intestinal epithelial adhesion properties, and SDS page was performed for secreted protein analysis. Moreover, the strain showed promising cholesterol-lowering properties based on the cholesterol level. This present result indicates that *L. oris* has superior probiotic properties and can be regarded as a potential probiotic candidate.

Keywords: *L. oris*, BioLog™ identification, probiotic, breast milk, cholesterol-lowering

INTRODUCTION

Probiotics are alive, non-pathogenic microorganisms (bacteria or yeasts), which when administered in adequate amounts, reach the intestines in sufficient numbers to confer health benefits to the host (1). When selecting probiotics, a number of criteria have to be met by the probiotic organisms, for instance the phenotype and genotype stability, protein and carbohydrate utilization patterns, acid and bile tolerance, intestinal epithelial adhesion properties, production of antimicrobial substances, ability to inhibit pathogens, antibiotic resistance capability, and immunogenicity. Since adhesion to the intestinal mucosa is considered to be a prerequisite for colonization, the ability to adhere to intestinal mucosa is one of the additional important criteria for probiotics (2, 3).

Before being employed as probiotics, microorganisms must have been granted Generally Regarded as Safe (GRAS) status. In addition, they must have a number of good technological properties such as simple propagation, incorporation into foods, long-term survival, safety in food products, and have clinically validated health effects (1, 4). The majority of probiotics are lactic acid bacteria such as *Lactobacillus* and *Bifidobacterium* species that are part of human and animal intestinal flora (5, 6). In the previous study, a range of lactic acid bacteria were isolated from different sources, especially fermented food products (7, 8). *Lactobacillus* species from Mother's milk deserve significant attention, however, to date, very little information has been reported about probiotics from human breast milk (9–11).

Mother's milk is a vital nutritional factor for the initiation, development, and composition of a child's neonatal gut microbiota. Human breast milk is comprised of a high level of compulsory nutrients for infants and has a valuable effect on the intestinal immune system (12–14). Moreover, it plays a vital role in supporting the survival and development of infants not only for nutrient supply but also for the transfer of microflora originated in breast milk (11). Mother's milk is considered the main source of bacteria. Various studies have reported a different number of live bacteria in mother's milk ranging up to 1×10^7 CFU/mL. The infant takes approximately 300–800 mL of milk per day and thus receives a large number of bacteria within this time (9, 10). The gastrointestinal (GI) microbiota of formula-fed and breastfed infants is considerably different, signifying the importance of the mother's milk microbiota. In recent years, above 200 different species have been expressed in human milk (15). Martin *et al.* isolated *L. gasseri*, *L. rhamnosus* and *L. fermentum* from breast milk, which have considerable probiotic capabilities (9, 10). A high amount of LAB in milk from a healthy mother plays an essential biological role during the first month of life (10, 16). Studies on this biological fluid specify that human milk is a promising source of potential probiotic bacteria. If these bacteria can protect the host from harmful organisms, they could be attractive probiotic candidates. Recently, research has focused on identifying new strains of *Lactobacillus* with health-promoting properties. Hence, the present study aimed to isolate and identify potential *Lactobacillus* sp., with cholesterol-reducing properties from mother's milk and infant feces for the development of effective probiotics.

MATERIALS AND METHODS

Collection of Milk and Infant Feces Samples

Maternal supplementation with different probiotics may result in an increased occurrence of administered probiotics in breast milk. The infant consumes breast milk directly so the same microbiota can be found in infant feces. Based upon this rationale, mother's milk and infant's feces may be a potential natural source to isolate effective strains of *Lactobacillus* sp. In the present study, mother's milk and infant facial samples were collected from Dhaka Medical College Hospital, Bangladesh. Fifteen (15) healthy mothers and infants were targeted for

TABLE 1 | Samples collected from mothers and their infants.

Sample ID	Sample type	Age (Mother and Infant)
S-1	M	25 years & 2 days
S-2	M	28 years & 3 days
S-3	M	23 years & 5 days
S-4	F	20 years & 3 days
S-5	F	28 years & 3 days
S-6	M	27 years & 2 days
S-7	F	27 years & 5 days
S-8	M	21 years & 3 days
S-9	M	30 years & 2 days
S-10	F	19 years & 2 days
S-11	F	25 years & 3 days
S-12	F	24 years & 3 days
S-13	F	34 years & 2 days
S-14	M	25 years & 2 days
S-15	M	35 years & 3 days

M, Milk; F, Feces.

a period of 1–5 days after birth, between the ages of 24–35 years, and the feces samples were taken from neonatal within the age range from 2 to 5 days (Table 1). We collected neonatal samples within this timeframe because fecal microbiota undergoes progressive succession before the maturation period of 1–5 days (17). The microbiological analysis were performed in triplicate. The studies concerning human participants were reviewed and approved by the BCSIR Institutional Ethical Review Committee. The participants or their legal guardians (sample donors) provided written consent to take part in this study. Samples were taken and collected in sterile tubes and immediately transported to the Industrial Microbiology Laboratory, IFST, BCSIR, Dhaka. It is necessary to mention that all the sample donors were healthy, using a normal diet, and did not take antibiotic treatments during pregnancy or after birth.

Chemicals and Reagents

SDS-polyacrylamide gel (Invitrogen, Germany), Coomassie Brilliant Blue (Promega, USA), Biochemical reagent, Microbiological media, antibiotic disks (Hi-Media, India), and all other chemicals and enzymes used from Sigma, USA.

Sample Preparation

The samples were preserved at 4°C before and after processing. Approximately, 1 g of fecal material or 1 mL of milk sample was added to 100 mL of buffered peptone water, thus homogenized thoroughly and incubate at 37°C for 30 min. Both of the samples were serially diluted in phosphate buffer (0.1 M, pH 6.2). The sample preparations were executed according to Lackey *et al.* and Gharbi *et al.* (18, 19).

Isolation of Lactic Acid Bacteria

The prepared inoculum was plated on a selective medium up to 10^{15} dilution using pour plate technique to isolate potential *Lactobacillus* species. The plates were incubated anaerobically

for 24–48 h at 37°C. Individual colonies were selected and transferred into a sterile MRS broth medium. The preferred colonies were purified with a streak plate technique based on colony morphology, gram staining, catalase, and oxidase reaction as well as different carbohydrate utilization. Based on biochemical properties, only the selected strains were identified through BioLog™.

BioLog™ Identification System

BioLog™ (BIOLOG™, USA) is an advanced microbial identification system that can swiftly identify species of aerobic and anaerobic bacteria, yeasts, and fungi. The latest generation redox chemistry of the BioLog™ enables testing and identification of aerobic gram-negative and gram-positive bacteria in the same test panel (20). For the species-level identification of predicted *Lactobacillus* sp. BioLog™ identification system was used based on 23 Chemical Sensitivity Assays with 71 carbon sources in GEN-III microplate. The isolates were cultured on BUA, which is a universal growth media for anaerobic bacteria. The microplates and inoculating fluids were pre-warmed at 37°C for 30 min. After 18 h incubation, the inoculum of bacterial culture was added to the inoculating fluid-A (21). The cell suspension was then poured into the multichannel pipette and all 96 wells were inoculated with exactly 100 µL of the bacterial suspension. The microplate was then incubated anaerobically for 24–48 h at 37°C. All the wells start out colorless when inoculated. During incubation, there is increased respiration in the wells where cells can use a carbon source. Increased respiration causes a reduction of the tetrazolium redox dye, forming a purple color. The negative well (A-1) remained colorless, as it contains no carbon source. There is also a positive control well (A-10) used as a reference for the chemical sensitivity assays. After incubation, the microplate was placed into the Micro Station Reader and the result was given by comparing with the database using the software program MicroLog 4.20.05 (BIOLOG™, USA) (22).

Probiotic Properties of Isolate

According to the FAO and the WHO, before demonstrating an organism is probiotic, that microorganism must rely on some technological properties such as surviving in acidic situations, persisting even though digestive enzymes occur in the stomach, resistance to bile salts at the commencement of small intestine, and antimicrobial activity as well as antibiotic susceptibility (1, 23).

Growth at Different Temperatures

Modified MRS broth was prepared and transferred to 5 mL tubes. After that, 50 µL of overnight cultures were inoculated to tubes and incubated anaerobically for 24–48 h at 10, 20, 30, 35, 37, 40, and 45°C. Cell growth was observed and measured by capturing absorbance by a spectrophotometer at 660 nm (Thermo Multiskan EX).

Viability at Different pH

The acid tolerance of the bacterial isolate was observed by incubating the organisms with different pH in MRS broth.

Resistance to pH 3 is often used *in vitro* assays to determine the resistance to stomach pH as foods remain at the stomach for 3 h (24). To determine the growth in diverse pH, 100 µL overnight cultures of the isolate were inoculated into MRS broth, with varying pH ranging from 1.0–7.0, adjusted with 1 M HCl by pH meter (Hanna Instruments, Italy). The inoculated broths were incubated anaerobically for 4 h at 37°C and growth was observed at OD 600 nm.

Growth at Different Concentration of NaCl

The designated isolate was examined for tolerance against different levels of NaCl concentrations (1.0–7.0%). The inoculated broths were anaerobically incubated at 37°C for 24 h and growth was monitored at OD 600 nm.

Tolerance to Bile Salt

The growth rate of bacterial culture was determined in MRS broth at pH 3.0 containing different levels of bile salt concentration (0.3, 0.5, 1.0, 1.5, and 2.0%). An inoculum of probiotic bacteria was prepared in MRS broth by overnight incubation and 1% (v/v) fresh culture was inoculated to the tube containing bile salt after sterilization. The broth was incubated anaerobically overnight at 37°C and the growth of the probiotic bacteria was compared with that of MRS broth as control (25). The survival rates of the isolates were dignified by taking absorbance at 600 nm by a spectrophotometer.

Resistance to Simulated Gastrointestinal Digestion

The solutions for simulated gastrointestinal digestion were organized according to Minekus et al. (26). During gastrointestinal digestion, the survivability of probiotic isolate was evaluated using an *in-vitro* digestion method. The *in vitro* gastrointestinal digestion procedure applied here consisted of a three-step model, which sequentially simulated the digestion in the mouth, stomach, and the small intestine as expressed by Minekus et al. and Gut et al. (26, 27) with few modifications. In this process, simulated Salivary Fluid (SSF) was arranged by dissolving 0.1126 g KCL, 0.0503 g KH₂PO₄, 0.1142 g NaHCO₃, 0.0030 g MgCl₂(H₂O)₆ and 0.0006 g (NH₄)₂CO₃ in 70 mL Milli-Q water, and finally, pH was adjusted to 7.0. Simulated Gastric Fluid (SGF) was prepared by dissolving 0.0514 g KCL, 0.0122 g KH₂PO₄, 0.210 g NaHCO₃, 0.2761 g NaCl, 0.0024 g MgCl₂(H₂O)₆ and 0.0048 g (NH₄)₂CO₃ in 70 mL Milli-Q water and adjust pH to 3.0. Simulated Intestinal Fluid (SIF) was organized by dissolving 0.0507 g KCL, 0.0108 g KH₂PO₄, 0.714 g NaHCO₃, 0.2246 g NaCl, and 0.0067 g MgCl₂(H₂O)₆ in a 70 mL Milli-Q water thus adjust pH to 7.0. The samples were incubated anaerobically at 37°C in a shaking incubator and the colonies were counted accordingly. α-amylase from human saliva, porcine pepsin, and porcine pancreatic lipase enzymes were employed for mouth mastication, gastric and intestinal digestion, respectively.

Hydrophobicity

The cell surface hydrophobicity was assessed with n-hexadecane and chloroform. Cells were suspended in 3 mL of 50 mM

potassium phosphate buffer at pH 7.0 (28, 29). The suspensions were centrifuged (3-18KS centrifuge, Sigma, Germany) at 10,000×g for 5 min at 4°C. Pellets were collected, washed twice, and then resuspended with the same buffer. Absorbance at 600 nm was measured and considered as A₀. One milliliter of the suspension was mixed with 200 μL of n-hexadecane and chloroform by vortexing for 120 s. The two phases were allowed to separate for 1 h at room temperature. The lower aqueous layer was cautiously shifted to hygienic tubes and absorbance was determined as A. Changes in the absorbance of probiotic bacterial suspension were recorded at 600 nm by a spectrophotometer.

Surface hydrophobicity (SHb%) was determined using the following formula:

$$SHb\% = \frac{A_0 - A}{A_0} \times 100$$

Here, A₀ and A are the absorbances before and after extraction with chloroform and n-hexadecane, respectively. For statistical analysis, all SHb experiments were repeated three times.

Autoaggregation Ability

The culture was harvested from broth medium by centrifugation (5,000×g, 5 min), washed, and resuspended with normal saline solution (28, 30). The absorbance was measured at 0, 2, and 24 h by a spectrophotometer at 600 nm without shaking the cell suspension. The autoaggregation was estimated as follows:

$$\text{Autoaggregation (\%)} = \left(1 - \frac{A_t}{A_0}\right) \times 100$$

A₀ is the initial absorbance at 600 nm and A_t is the absorbance at 600 nm.

Co-aggregation

The co-aggregation capacity of the *L. oris* probiotic stains with other pathogenic and non-pathogenic bacteria was assessed following the method of Vlková et al. and Keller et al. (31, 32) with some modifications. The colon intestine contains millions of both pathogenic and non-pathogenic bacteria. The *L. oris* along with *L. rhamnosus* LGG, *L. casei* 431, *B. animalis* subsp. lactis 12, *Escherichia coli* ATCC 11303, *Salmonella typhi* ATCC 13311, *Shigella flexneri* ATCC 12022, *Enterobacter faecalis* ATCC 29212, *Vibrio parahemolyticus* ATCC 17802, *Pseudomonas aeruginosa* ATCC 27853, and human fecal culture (collected from Industrial Microbiology lab stock, IFST, BCSIR) were used for the co-aggregation activity. The bacterial cell suspension was prepared with the final density of 9.0 log CFU/mL. The mix of pathogenic bacteria and probiotic cells of 3.0 mL each were dispensed into sterile tubes. The tubes were thoroughly mixed and incubated for 60 min at 37°C. The absorbance was taken at 600 nm by a spectrophotometer. Pathogenic and probiotic bacteria were prepared separately and absorbance was taken as control. The percentage of co-aggregation was calculated according to the formula:

$$\text{Co-aggregation (\%)} = \frac{(A_x + A_y)/2 - A(x+y)}{(A_x + A_y)/2} \times 100$$

where A_x–represents the absorbance of the probiotic strain, A_y–represents the absorbance of the pathogenic bacteria under study; A (x + y)–represents the absorbance of the mixture of both.

Assessment of Antimicrobial Activity

The selected isolate was investigated for antimicrobial activity against a variety of indicator organisms (pathogens) by the modified method of Arques et al. (33). The plates were poured with 20 mL Muller Hinton Agar (MHA) medium. Nine different human pathogens belonging to both gram-positive and gram-negative groups such as *Bacillus cereus* ATCC 10876, *Bacillus subtilis* ATCC 11774, *Staphylococcus aureus* ATCC 9144, *Escherichia coli* ATCC 11303, *Salmonella typhi* ATCC 13311, *Shigella flexneri* ATCC 12022, *Enterobacter faecalis* ATCC 29212, *Vibrio parahemolyticus* ATCC 17802, *Pseudomonas aeruginosa* ATCC 27853 were used in this study. The pathogenic strains were grown in nutrient broth (NB) for 24 h and spread on an MHA agar plate. Three wells (6 mm) of each plate were prepared through a sterile borer and the supernatant of the isolate was placed into the well. The plates were pre-inoculated at room temperature for the diffusion and incubated anaerobically overnight at 37°C and observed for zones of inhibition.

Antibiotic Susceptibility Test

The selected strain was examined for the antibiotic resistance pattern as recommended by the Clinical and Laboratory Standards Institute (CLSI; Wayne, PA, USA) (34). The isolates were grown overnight in MRS broth at 37°C. Petri dishes containing 20 mL of MRS were allowed to solidify at room temperature and swabbed with organisms. In the present study, 10 different types of antibiotic disks viz ampicillin (10 μg), erythromycin (15 μg), gentamicin (10 μg), kanamycin (30 μg), neomycin (10 μg), vancomycin (30 μg), penicillin G (10 μg), nalidixic acid (30 μg), nitrofurantoin (300 μg) and doxycycline (30 μg) were used. The plates were incubated anaerobically for 24 h at 37°C. The diameter (mm) of the inhibition zone was measured with an antibiotic zone scale. Results were expressed in terms of resistance, moderate susceptibility, or susceptibility by comparing with the zone diameters provided in the performance standards for antimicrobial disc susceptibility tests.

Antioxidant Activity Determination

To prepare intracellular extracts, the overnight cultured bacterial isolate was centrifuged at 12,000 rpm at 4°C for 3 min. The pellet of the cell was washed twice and re-suspended in water (pre-autoclaved) and ultrasonicated at 30 KHZ for 3 min, with an intermission of 1 min. Subsequently, the cellular debris was removed by centrifugation at 200×g at 4°C for 15 min. The cell-free supernatant was used as an intracellular extract to assess the antioxidant activity (3). The antioxidant activity of intact cells and intracellular extracts were determined by the DPPH (2, 2-Diphenyl-1-picrylhydrazyl) free radical scavenging assay method. The control was prepared with PBS buffer at pH 7.4. The absorbance was measured by UV spectrophotometer at 517 nm, the readings were recorded in triplicate and the average absorbance value was calculated. The antioxidant activity

of the samples was expressed as the percentage (%) of radical scavenging as follows:

$$\text{DPPH activity (\%)} = [(Ac - As)/Ac] \times 100$$

Where, Ac = absorbance of the control and As = absorbance of the sample.

Resistance to Hydrogen Peroxide

Resistance to hydrogen peroxide was determined according to Serata et al. (35). Freshly prepared bacterial culture (10 mL) was supplemented with 0.85% (w/v) NaCl (10 mL), thus added variable concentrations (0.5, 1.0, and 1.5 mM) of H_2O_2 . The viability of the isolate was resolute after 1 h of incubation.

Sodium Nitrite Depletion

Sodium nitrite depletion of the selected isolate was carried out according to Wu et al. (36). Newly prepared bacterial culture (100 μL) was inoculated and incubated anaerobically for 48 h at 37°C. Sterilized sodium nitrite (1 mL) was combined with 9 mL of MRS broth. For control samples, sterile water was applied as a substitute for inoculum. The colorimetric nitrite method was employed to measure initial and final absorbance at 538 nm against a blank. The depletion of sodium nitrite was calculated as follows:

$$\text{Nitrite depletion (\%)} = (1 - C_i/C_f) \times 100$$

Whereas, C_i = Amount of nitrite present in MRS broth at 0 h; C_f = Amount of nitrite present in MRS broth after 48 h.

Test for Safety Evaluation

Hemolytic Activity

The hemolytic activity was determined according to the method of Yadav et al. (37). The tested isolate was streaked in a blood agar medium and incubated at 37°C for 48 h. The plates were observed for β -hemolysis, α -hemolysis, and non-hemolytic activities after the incubation period.

DNase Activity

To test for DNase enzyme production the selected isolates were streaked onto a deoxyribonuclease (DNase) agar medium. Thus, the plates were incubated at 37°C for 48 h and examined for the zone of DNase activity. An understandable zone (pinkish) around the colonies was determined as positive DNase activity (38).

Partial Analysis of Bacterial Protein by SDS-PAGE

The isolates were grown in MRS broth at 37°C for 48 h anaerobically. The culture broth was sonicated for cell disruption and centrifuged at 10,000 rpm for 15 min at 4°C. The supernatant was collected and cold acetone (−20°C) was added to the supernatant and mixed vigorously. The supernatant was incubated at −20°C for 1 h and centrifuged at 10,000 rpm for 15 min at 4°C. Cell pellets were collected, disposing of supernatant and allowing the uncapped tube to evaporate acetone completely. After adding 2% SDS, the whole-cell protein extract was stored at 4°C until use (39). Whole-cell protein extract was mixed with an equal volume of sample loading

buffer and heated for 10 min at 80°C. The processed samples were then loaded onto 7.5% SDS-polyacrylamide gel wells as illustrated by Paul (40). Electrophoresis was performed at 30 mA until the tracker dye (Bromophenol blue) reached the bottom of the gel. Gels were stained for protein with Coomassie Brilliant Blue (CBB) destaining with a solution containing 6.75% (v/v) glacial acetic acid and 9.45% (v/v) methanol. The molecular weights were determined by SDS-PAGE protein molecular markers.

In vitro Cholesterol-Lowering Activity

Water-soluble cholesterol (polyoxyethanyl-cholesterylsebacate, Sigma) was used for *in-vitro* cholesterol reduction assay. The *L. oris* was grown in modified MRS broth at 37°C anaerobically. The cholesterol was added to milk to make yogurt (41) with *Lactobacillus delbrueckii* sub sp. *bulgaricus* and *Streptococcus thermophilus* at a final concentration of 25–100 mg/100 mL, inoculated with 2% of *L. oris* (pellets) (42), and incubated at 39°C for 6–7 h. The cholesterol content was assessed by a modified colorimetric method as described by Gilliland et al. (43). The activity of cholesterol-lowering (mg/mL) was calculated as follows:

$$\text{Cholesterol lowering activity, \%} = \frac{\text{cholesterol added} - \text{cholesterol recovered}}{\text{cholesterol added}} \times 100$$

Statistical Analysis

All the experiments were executed in triplicate. The diversity index data were statistically analyzed by one-way analysis of variance (ANOVA). Data were presented as the mean \pm standard deviation (SD) for the indicated number of separately completed experiments. Data analyses were finalized by SPSS software (version 20.0; SPSS Inc., Chicago, USA).

RESULTS

Isolation of *Lactobacillus* sp.

The properly diluted samples were incubated anaerobically for 48 h at 37°C and the small bacterial colony with entire margin, creamy white shiny color, convex elevation, and opaque opacity were preliminarily screened as *Lactobacillus* spp. The presumed colonies were chosen for further study. To determine the morphological characteristics, the selected strains were examined by an optical microscope (Olympus, Japan). *Lactobacillus* spp. are the genus of gram-positive rod-shaped and non-spore-forming bacteria. The isolates that exhibited similar characters as *Lactobacillus* sp. were selected for further characterization.

Biochemical Characterization

To recognize the characteristics of *Lactobacillus* sp. some essential biochemical tests were accomplished. The results of biochemical tests were listed in **Table 2**. The carbohydrate fermentation of the isolate was determined on carbohydrate broth supplemented with 1% (w/v) of the following carbohydrates: arabinose, ribose, lactose, fructose, sucrose, maltose, glucose, rhamnose, galactose, and raffinose.

TABLE 2 | Biochemical characterization of potential *Lactobacillus* isolates.

Test	Result of the biochemical test						
	C-1	C-2	C-3	C-4	C-5	C-6	C-7
Catalase	-	-	-	+	-	-	+
Oxidase	-	-	-	-	-	-	-
Gas from Glucose	+	+	+	-	+	-	+
Gelatin hydrolysis	-	-	-	+	-	+	-
H ₂ S production	-	-	-	-	-	-	-
Indole production	-	-	-	-	-	-	-
Motility	-	-	-	+	-	-	-
Methyl Red	-	-	-	-	-	+	-
VP (Voges)	-	-	-	-	-	+	-
Urease	-	-	-	-	-	+	-
Result of the fermentation test							
Arabinose	+	+	+	-	+	-	+
Ribose	+	+	+	+	+	+	+
Lactose	*	*	-	-	*	-	+
Fructose	+	-	+	+	+	-	+
Sucrose	+	+	-	+	-	+	+
Maltose	+	+	-	+	+	-	+
Starch	+	+	+	+	+	+	+
Rhamnose	+	+	+	-	+	+	+
Galactose	+	+	+	+	+	+	+
Raffinose	*	*	*	*	+	*	*

Readings were recorded anaerobically after 24 h at 37°C; Key: +, Positive reaction; -, Negative reaction; *, Weak reaction.

TABLE 3 | Microorganism identification with BIOLOG™ system.

Strain	ID (identification)	PROB (probability)	SIM (similarity index)	DIST (distance)
C-1	<i>Lactobacillus oris</i>	0.582	0.582	6.160

Identified by Biolog™ System

For species-level identification of the selected bacterial isolate BIOLOG™ system was carried out. This system intends to give a quick, suitable approach to bacteria identification with 3,000 species databases. The result specifies that only one bacterial strain identified as *L. oris* (**Supplementary Figure 1**). **Table 3** illustrates the outcome of the BIOLOG™ identification system. For additional verification, this isolate was examined using three replications. This isolate was finally chosen for detailed observation.

Assessment of *In-vitro* Probiotic Activities of *Lactobacillus* spp.

Growth at Different Temperatures

Since the characterization of probiotics is determined by their capability to survive the upper digestive tract and colonize in the intestinal lumen and colon, the temperature is a vital criterion.

Figure 1A shows the growth of *L. oris* at different temperatures. The results indicate that the isolated organism grew best at 37°C, was also able to grow at 45°C, but unable to grow at 10°C.

pH Tolerance Assay

The pH condition of the stomach is acidic. The pH tolerance of the isolate was measured by growing the isolate in a varying pH environment. The capacity of the strain to survive in the acidic pH at 0 h and 4 h of incubation at 37°C is presented in **Figure 1B**. The results revealed that *L. oris* retained around 80% viability for up to 4 h at pH 1 and 2. No considerable differences were obtained between the viability after 4 h, indicating acid resistance-capacity to intestinal pH conditions. The result also indicated that the strain could stay alive up to pH 8.0.

Measurement of Salt (NaCl) Tolerance

Sodium chloride tolerance was measured by testing the ability to grow in the presence of various concentrations of NaCl. According to the test results, *L. oris* can grow up to 7.0% NaCl concentration but there was a rapid decrease in survival rate after 5.0% of NaCl concentration (**Figure 1C**).

Measurement of Bile Salt Tolerance

Bile salt tolerance assists in the *in vitro* assessment of metabolic activity and colonization of isolates in the small intestine. The effect of bile salt on the growth and survival rate of the identified isolates is shown in **Figure 1D**. The bile salt tolerance of *L. oris* was measured by growing isolates in a varying bile salt concentration at 37°C. *L. oris* was found to be the resistant strain, it showed viability at 1.5% of bile concentration after 5 h exposure.

Gastric Juice Tolerance

The capability to adhere to intestinal surfaces is a vital selection criterion for potent probiotics because adhesion to the intestinal epithelial tissue is considered to be a criterion for colonization and thus producing health benefits by killing the detrimental pathogens and balancing the microbiota balance in the Gastrointestinal Tract. The effect of simulated gastric juice (pH 3.0 and 6.0) on the identified isolate is presented in **Figure 2**. *L. oris* showed better viability in gastric juice for 3 h at pH 3.0. After 3 h the viability decreased. The viability of the *L. oris* on gastric juice at pH 6.0 was used as a control.

Cell Hydrophobicity, Auto, and Co-aggregation Activity

The hydrophobic properties of *L. oris* displayed a superior affinity to chloroform compare to n-hexadecane that are $91.5 \pm 2.43\%$ and $78.32 \pm 1.92\%$, respectively. The autoaggregation capacity of *L. oris* increases over time. The fast autoaggregation obtained showing percentages of $48.93 \pm 1.20\%$ at 2 h and $92.75 \pm 3.78\%$ at 24 h. **Figure 3** demonstrated the co-aggregation activity of the probiotic *L. oris* strain.

Antagonistic Activity Measurement

It has been well-documented that *Lactobacillus* spp. showed very good antagonistic properties by producing numerous antimicrobial compounds. *L. oris* was observed for their

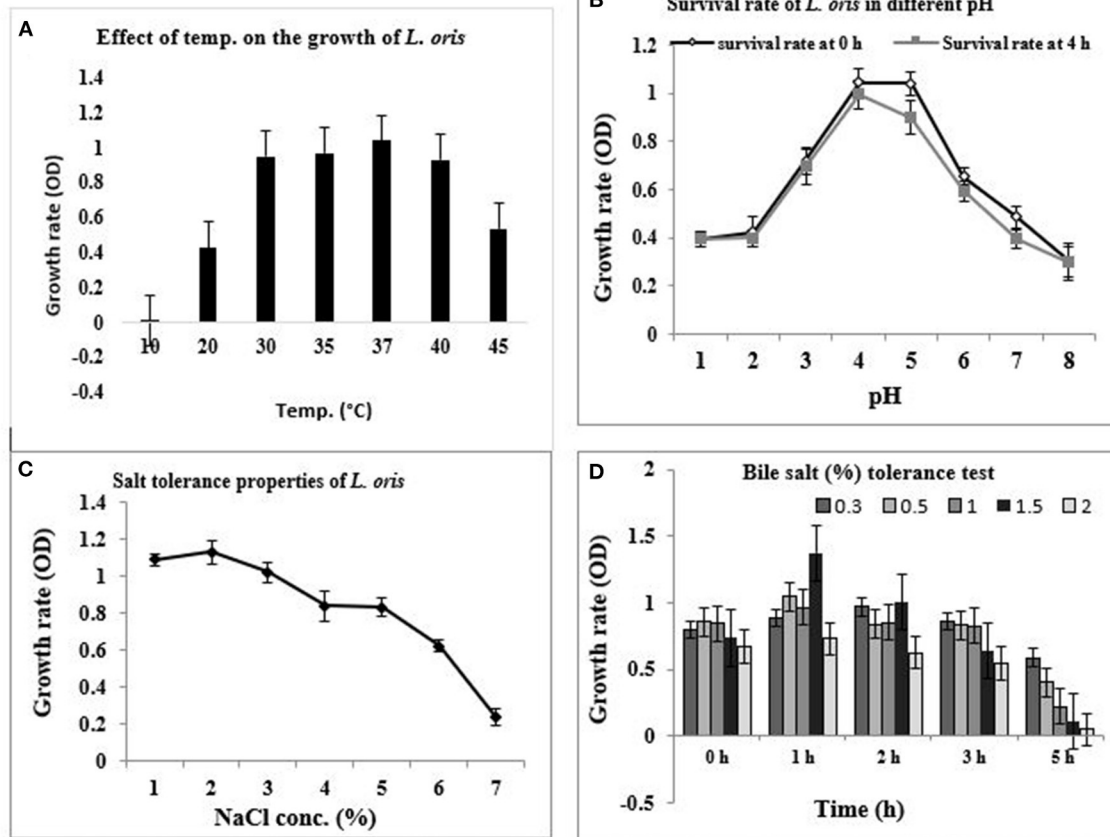


FIGURE 1 | *In-vitro* probiotic attributes of isolated strain *L. oris*. **(A)** Growth rate at different temperatures, **(B)** Survival rate with different pH at 37°C for 0 h and 4 h, **(C)** Effect of NaCl concentration, **(D)** Effect of bile salt (%) concentration under variable periods of time at 37°C.

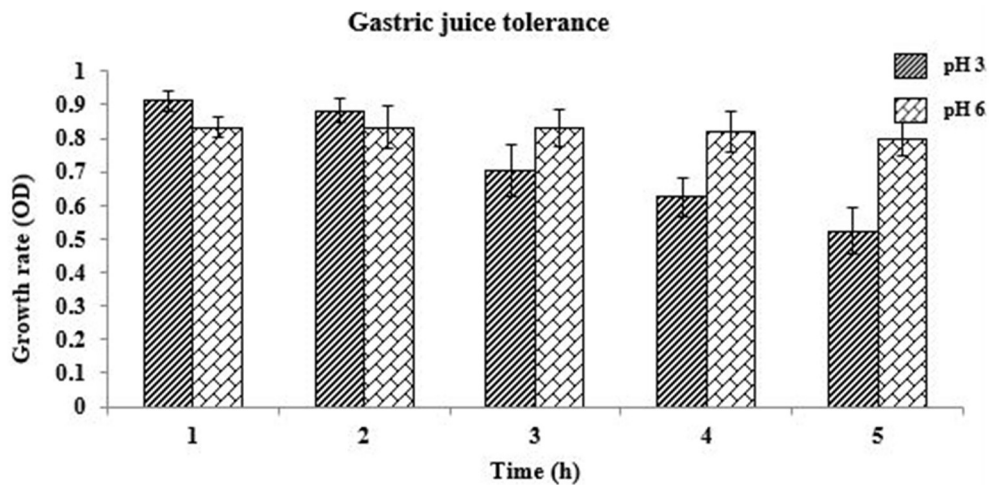


FIGURE 2 | Gastric juice tolerance of *L. oris* at pH 3.0 and 6.0.

antimicrobial activity against the indicator microorganisms. Nine human pathogens belonging to both gram-positive and gram-negative were employed in this study. The result proved *L.*

oris has significant antibacterial activity against all the tested pathogens with significant variations. The *L. oris* exhibited the highest inhibition activity against *V. parahaemolyticus*, *S.*

enteritidis, and *S. aureus* and the lowest inhibition activity against *E. faecalis*. The diameters of zones of inhibition were given in Figure 4A.

Antibiotic Susceptibility Profile

The *L. oris* was screened for susceptibility against various antibiotics. For appropriate selection generally, two groups of antibiotics are suggested in EFSA guidelines including inhibitors of cell wall synthesis (ampicillin and vancomycin) and inhibitors of protein synthesis (gentamycin, neomycin, erythromycin, kanamycin, and others). The results were compared with

the zone size interpretative chart. In the present study, the tested isolate was sensitive to doxycycline, erythromycin, and kanamycin. A variable antibiotic sensitivity was observed and reported in Figure 4B.

Antioxidant Activity

The antioxidant activity of intact cells and intracellular extracts of *L. oris* was estimated by DPPH free radical scavenging assay. The potential isolate *L. oris* exhibited a higher ability to scavenge the radical DPPH and was determined as 31.14%. This finding agreed with the previous study for *Lactobacillus* isolated from fermented foods (44). The intracellular cell-free extracts of *L. oris* showed 30.32% scavenging ability.

Resistance to Hydrogen Peroxide

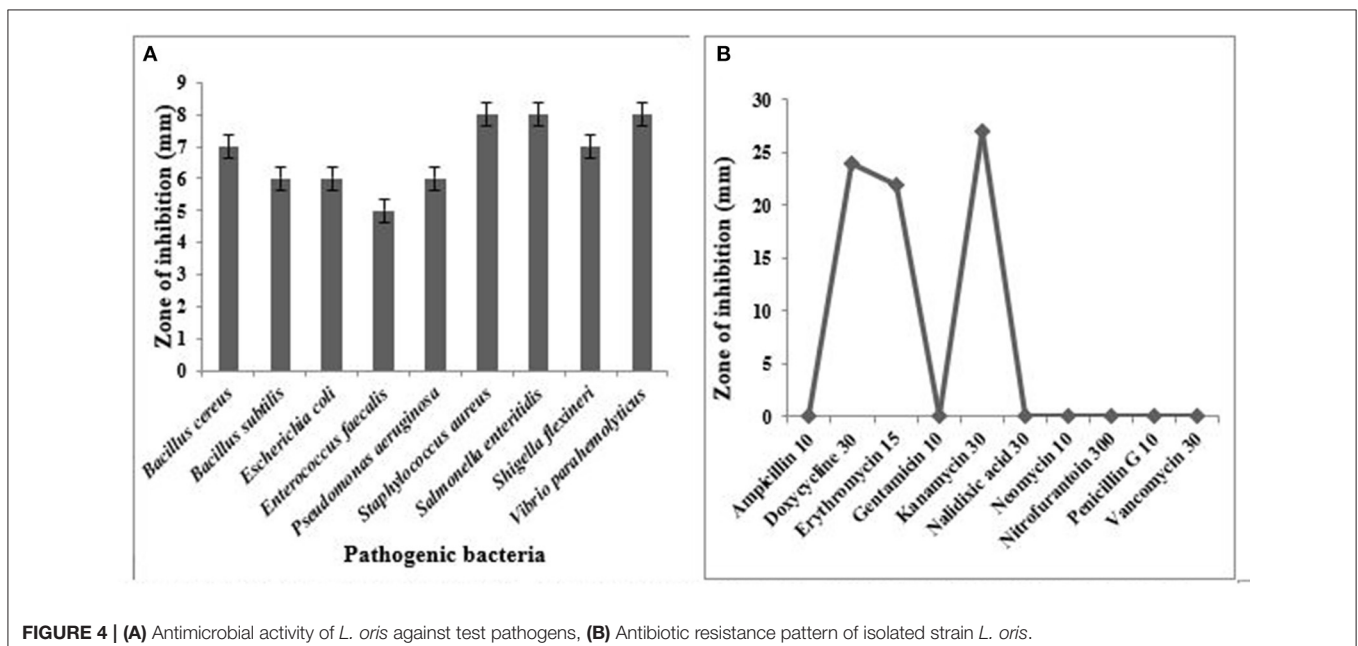
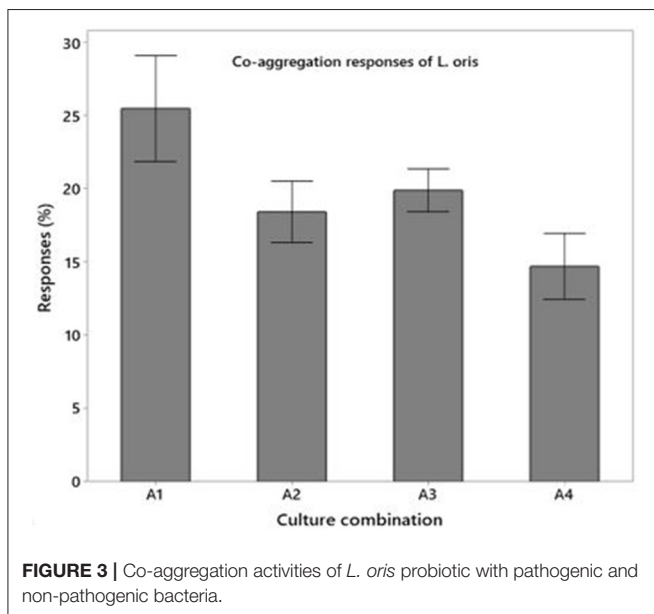
The potential isolate *L. oris* can resist H_2O_2 . Results exposed a considerable impact on the viability of the isolate were observed at the concentration of H_2O_2 increases, exhibiting a decreasing trend. The isolate showed the maximum viable cell count at 0.5 mM of H_2O_2 (8.3 logs CFU/mL) while the least was observed at 1.5 mM H_2O_2 (5.0 log CFU/mL).

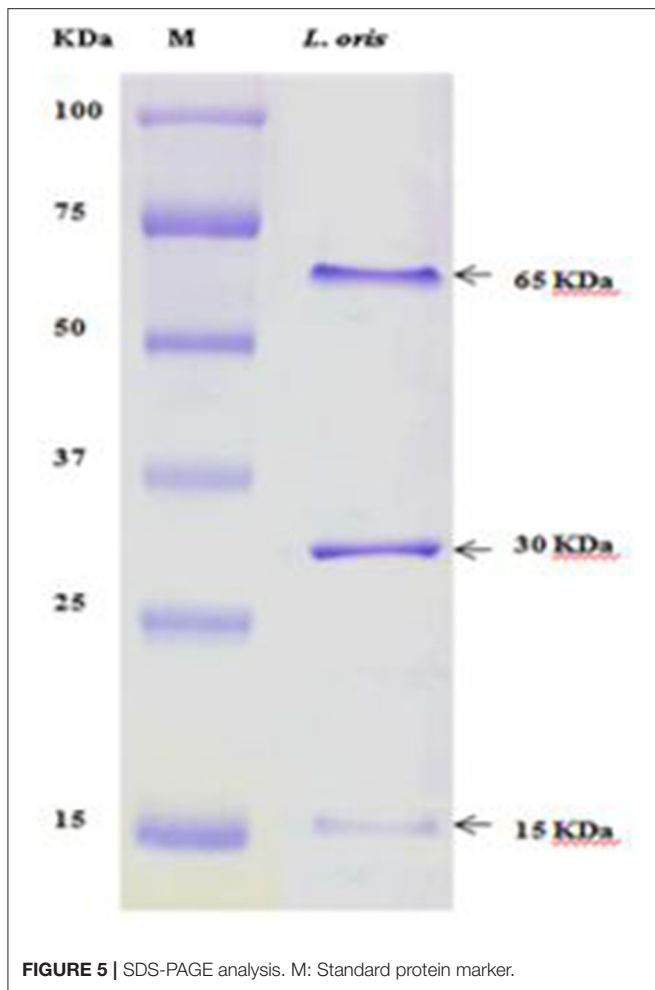
Depletion of Sodium Nitrite

Sodium nitrite is a salt that is used as an additive in food products especially in meats to keep characteristic color and flavor. Due to safety issues, sodium nitrite is considered a carcinogenic substance for foods. Therefore, controlling their concentrations is important for maintaining a safe food supply. The obtained result revealed that *L. oris* had the ability to deplete nitrite as of 85.0%.

Safety Evaluation of Isolate

The safety assessment of *L. oris* was decided by their hemolytic and DNase activities that demonstrated the non-pathogenic category of the probiotic isolate. The results of the current study





revealed “no zone” in the test plates inoculated with the isolate, thus confirmed no hemolytic or DNase activities.

Partial Analysis of Bacterial Metabolites

The partially purified protein of *L. oris* was assessed through SDS-PAGE (10% gel) and evaluated with a standard protein marker (Figure 5). Analysis of the differentially accumulated proteins of *L. oris* demonstrated molecular weights of approximately 65, 30, and 15 kDa. The molecular weights of isolated proteins were compared with a protein ladder and also a standard protein solution (albumin-68 kDa, casein-24 kDa, and β -lactoglobulin-18 kDa).

Cholesterol Reduction Assay

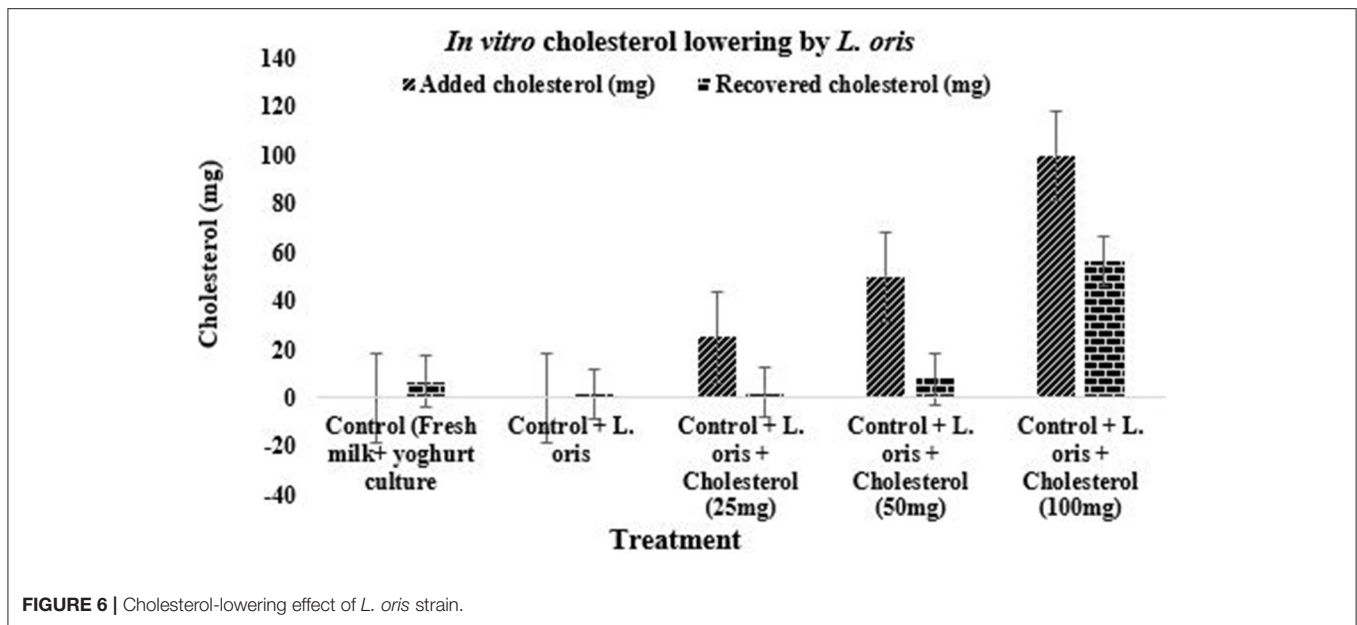
Figure 6 indicated the cholesterol-lowering effect of the newly isolated probiotic strain *L. oris*. As a larger amount of cholesterol is the common physiological problem among people and the findings demonstrated very promising cholesterol-lowering activities. The probiotic *L. oris* can reduce cholesterol up to 90% depending on the concentration of cholesterol.

DISCUSSION

Probiotics are live microorganisms that have immense benefits for health. Until recently, a number of probiotic organisms were isolated from raw or processed milk, but in the case of mother’s milk limited data have been documented. Breast milk is a vital factor for the development and composition of the neonatal gut microbiota aiming at assisting the suitable growth of the gut microbiota and the immune development of infants and an attractive source of new and specific probiotic strains. Earlier reports have indicated that the importance of milk microbiota is drastically different between breastfed and non-breastfed infants (44, 45). These previous reports indicated that *Enterococcus* sp., *Streptococcus* sp., *Micrococcus* sp., and *Staphylococcus* sp. were commonly found in human breast milk. Martin *et al* reported *Lactobacilli* such as *L. gasseri*, *L. rhamnosus*, *L. acidophilus*, *L. plantarum*, and *L. fermentum* from mother’s milk (10, 11). The results of this study indicated that a mother’s milk can be used as a potential natural source for new and specific probiotic strains from *Lactobacillus* spp.

Among the seven potential *Lactobacillus* sp. isolates in this study, only C-1 was identified as *L. oris*. The identification of *Lactobacillus* to the species level was done by the BioLogTM identification system (22). The isolated *L. oris* strain was able to grow optimally at 37°C under anaerobic conditions (46) but unable to grow at 10°C. The strain was heat stable and able to grow at 45°C. Resistance to bile concentration and low pH are both characteristics of the identified isolate, and are considered to be vital properties of probiotics (46). To reach the small intestine, they have to pass through the stressful surroundings of the stomach. Although in the stomach, pH can be as low as 1.0, in most *in vitro* assays pH 3.0 has been preferred (46). The identified strain was resistant to acid at pH 3.5 for 90 min at 37°C. Also, the isolate was able to grow at pH 3.5 containing 0.2% mixed bile salts. The optimal salt tolerance capacities of *L. oris* were up to 2% and followed by growth rate declining. The bile salt tolerance test specified that the isolated *L. oris* probiotic was capable of surviving proficiently at up to 1.5% bile salt concentration at 2 h but survivability declined drastically after 5 h.

In a research study, 29 *Lactobacillus* strains of dairy origin were examined *in vitro* as prospective probiotics. All of the examined strains were resistant to pH 3 during 3 h, but most of them lost their viability in 1 h at pH 1.0. Also, all of them were tolerated by 0.3% bile salts concentration for 4 h (47). They were identified as *L. casei*, *L. plantarum*, *L. paracasei* subsp. (48). This study indicated that *L. oris* was able to grow and colonize in high bile concentration in the intestine, even at pH 1.0 in the stressful surroundings of the stomach. The isolate can retain a viability of approximately 80% at pH 1.0 after 5 h. A similar result was also found by Anandharaj *et al.* (16). Resistance to bile salt is one of the criteria used to select probiotic strains that would be potentially able to perform effectively in the human gastrointestinal tract (49). Gastric juice tolerance at pH 3.0 was examined. *L. oris* had better viability in gastric juice for 3 h at pH 3.0. After 3 h the viability was decreased. The viability of gastric



juice at pH 6.0 was examined as a control. Additional reports designated that the growth rates of *L. gasseri*, *L. sakei*, and *L. acidophilus* decreased to 11–91% in simulated intestinal juices with 0.2% oxgall (50). The cell hydrophobic, autoaggregation and co-aggregation properties in the intestinal epithelial cells are considered to be probiotic criterion. The *L. oris* showed a good hydrophobic affinity and autoaggregation ability increases over time which matches the previous study result (30, 51). The co-aggregation ability of the isolated *L. oris* probiotic was acceptable in comparison to other fecal pathogenic and non-pathogenic microbes.

The production of antimicrobial compounds against pathogens is an imperative property of probiotics. *Lactobacillus* spp. contained antimicrobial substances that can inhibit the growth of pathogens. Darsanaki *et al* showed that *L. plantarum* (3 isolates), *L. casei* (2 isolates), and *L. brevis* (1 isolate) produced the highest tolerance against acid and bile salts as well as antimicrobial activities against *Staphylococcus aureus* PTCC 1431, *S. typhimurium* PTCC 1639 and *E. coli* PTCC 1399 (52). In the present study, *L. oris* rendered pronounced growth and inhibitory zones ranged between 7 and 8 mm against *S. aureus*, *S. enteritidis*, *V. parahaemolyticus*, and *B. cereus*. Nevertheless, *L. oris* exhibited a consistent reduction in inhibitory zones against the other bacterial pathogens tested. Savadogo *et al.* (53), confirmed the antimicrobial activity of other *Lactobacillus* strains. *L. fermentum* formed the maximum inhibition zone (12 mm) against *Enterococcus faecalis* at the same time the minimal inhibition was shown by *Leuconostoc mesenteroides* subsp. *mesenteroides* on the same strain. In an additional study, *L. salivarius* FC113 created an inhibition zone of 11.5 ± 0.7 mm and 14.3 ± 0.6 mm against *S. aureus* strains KCCM 11335 and KCCM 11593, respectively (54). This inhibitory effect may be due to the production of organic acids and other antimicrobial substances (16).

Lactobacillus spp. can act as an antioxidant by quenching free oxygen radicals. These free radical scavenging properties of *L. oris* possibly indicate small amount of redox potential in the intestine. Synthetic antioxidants have serious carcinogenic health effects in terms of safety and long term utilization (55). Therefore, it is vital to explore non-toxic, natural, and low-cost antioxidants as a substitute for a synthetic antioxidant. In the present study, the cell-free extracts of *L. oris* exhibited strong antioxidant activity. Lactic acid bacteria have a diversity of mechanisms to tolerate oxygen and reactive oxygen species, and the mechanisms change among species. Some evidence confirmed that *L. casei*, *L. acidophilus*, and *L. plantarum* have been used as model probiotics (24, 37, 56). In a previous study, this *Lactobacillus* sp. exhibited strong resistance to hydrogen peroxide (35). Likely, the potential isolates *L. oris* can resist H_2O_2 . Nitrite is usually used as a food additive and is widely found in a variety of food products. It is a vital N-nitrosamines precursor that actively causes cancer and other diseases. Moreover, nitrites also react with amines to form N-nitroso compounds (57). It is, therefore, an essential safety issue to limit the use of nitrite in foodstuff. In this circumstance, the tested isolate can deplete nitrite and restrict the conversion of nitrite into nitrosamines.

The isolated probiotic must be safe for human consumption as an important criterion for selecting an organism as a probiotic. For this purpose, the antibiotic resistance profile of the isolated organism was determined. The Agar well-diffusion method is used to detect the antimicrobial susceptibility of intestinal microorganisms. According to the European Food Safety Authority (EFSA), the assessment of hemolytic activity is mightily recommended if the isolated bacteria are intended for use in food, even if they have GRAS or QPS (Quality Presumption of Safety) status. Moreover, the organism *L. oris* also passed safety evaluation examination. In this study, the hemolytic activity

of the tested isolate evaluated the strains *L. oris* and showed no hemolytic activity. The present results were similar to the findings of Oh and Jung who determined the hemolytic activity of five *Lactobacillus* species isolated from traditionally fermented beverages (58). The present findings were also consistent with the results of Wang et al., who estimated the probiotic potential of lactic acid bacteria from Chinese spontaneously fermented non-dairy food products, which revealed no hemolytic activity of probiotics (59).

The electrophoretic pattern of the proteins from the isolate showed a different band size. The partially purified proteins of *L. oris* on SDS-PAGE displayed molecular weights of approximately 65, 30, and 15 kDa have also been reported. The molecular weights of isolated enzymes were compared with a protein ladder and also a standard protein solution (albumin-68 kDa, casein-24 kDa, and β -lactoglobulin-18 kDa). Ghazi et al. (60) prepared a standard protein solution and compared it with *Lactobacillus* strains. The SDS-PAGE technique generated multifaceted and constant patterns that were easy to interpret and compare the profiles of the strains.

The *L. oris* strain was found to reduce cholesterol by 43.78 ± 6.97 to $90.50 \pm 2.32\%$ depending on the different concentrations of cholesterol in the *in vitro* test. The cholesterol assimilated by the *L. oris* showed wide variation within 6/7 h in anaerobic incubation. The amount of cholesterol decomposition or assimilation was higher in the low concentration of cholesterol followed by increments. The highest amount of cholesterol reduction was found in 25 mg/100 mL yogurt which was $90.50 \pm 2.32\%$ and followed by $84.60 \pm 6.03\%$, and $43.78 \pm 6.94\%$ for 50 mg and 100 mg of cholesterol in the same amount, respectively. There was a significant difference ($P < 0.05$) in the reduction of cholesterol in different concentrations. The recovered amounts of cholesterol in the control sample with yogurt culture were 6.80 ± 1.02 mg and the control sample with *L. oris* culture was 1.32 ± 0.73 mg. Some of the previously published results were directly agreed with the results of this study (16, 42, 61). The above results indicated that *L. oris* had a clear effect on *in vitro* cholesterol reduction which was dependent on *L. oris* counts and cholesterol amount. Development of various probiotic products, such as fermented milk, drinks, yogurt, cheese, ice-cream etc. with the help of *L. oris* culture, could confer health benefits for people in Bangladesh and other countries. Based on the results of the present study, *L. oris* has great probiotic properties and the ability to survive in human gastrointestinal conditions. *L. oris* needs to be further investigated and could be incorporated into food supplements or developed as a new probiotic.

CONCLUSION

The use of probiotics as food or dietary supplement is increasing gradually with increasing awareness of its beneficial effects. In this study, one isolate was identified as *L. oris* and the experiment

observation of probiotic capability revealed that the strain was a probiotic with excellent potential. The cell hydrophobicity, auto and co-aggregation properties in the intestinal epithelial cells were promising. *L. oris* indicates resistance to the majority of antibiotics as well as antagonistic activity against tested pathogen. Furthermore, the strain demonstrated promising cholesterol-lowering properties based on cholesterol level and its ability to survive in the gastrointestinal environment. These results reveal that *L. oris* has superior probiotic properties and can be regarded as a probiotic candidate, potentially opening new directions for future research into enhanced effectiveness and advancing biotechnological research in the food and dairy industries.

DATA AVAILABILITY STATEMENT

The original contributions generated for this study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the BCSIR Institutional Ethical Review Committee. The participants or their legal guardian (sample donors) provided written consent to participate in this study.

AUTHOR CONTRIBUTIONS

MH designed the study. Saf and SAk executed the experiments. MH and SB provided advice and guidelines on conducting this research. MH, Saf, and SAk wrote the draft. All authors read and approved the final version of the manuscript.

FUNDING

The present study was funded by the R & D project of the Institute of Food Science and Technology (IFST), Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka.

ACKNOWLEDGMENTS

The authors are grateful to THE IFST and BCSIR.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fnut.2021.619506/full#supplementary-material>

Supplementary Figure 1 | Identification of *L. oris* by BioLog™ Identification system.

REFERENCES

1. FAO/WHO. *Guidelines for the Evaluation of Probiotics in Food. Report of a Joint FAO/WHO Working Group on Drafting Guidelines for the Evaluation of Probiotics in Food.* (2002). Available online at: https://www.who.int/foodsafety/fs_management/en/probiotic_guidelines.pdf (accessed February 19, 2021).
2. Sanders ME, Merenstein D, Merrifield CA, Hutkins R. Probiotics for human use. *Nutri Bull.* (2018) 43:212–25. doi: 10.1111/nbu.12334
3. Hossain MN, Afrin S, Humayun S, Ahmed MM, Saha BK. Identification and growth characterization of a novel strain of *Saccharomyces boulardii* isolated from soya paste. *Front Nutr.* (2020) 7:27 doi: 10.3389/fnut.2020.00027
4. Nagpal R, Kumar A, Kumar M, Behare PV, Jain S, Yadav H. Probiotics, their health benefits and applications for developing healthier foods: a review. *FEMS Microbiol Lett.* (2012) 334:1–5. doi: 10.1111/j.1574-6968.2012.02593.x
5. Aizawa E, Tsuji H, Asahara T, Takahashi T, Teraishi T, Yoshida S, et al. *Bifidobacterium* and *Lactobacillus* counts in the gut microbiota of patients with bipolar disorder and healthy control. *Front Psy.* (2019) 9:730–8. doi: 10.3389/fpsy.2018.00730
6. O'Hara AM, O'Regan P, Fanning Á, O'Mahony C, MacSharry J, Lyons A, et al. Functional modulation of human intestinal epithelial cell responses by *Bifidobacterium infantis* and *Lactobacillus salivarius*. *Immunology.* (2006) 118:202–15. doi: 10.1111/j.1365-2567.2006.02358.x
7. Garriga M, Pascual M, Monfort JM, Hugas M. Selection of *Lactobacilli* for chicken probiotic adjuncts. *J Appl Microbiol.* (1998) 84:125–32. doi: 10.1046/j.1365-2672.1997.00329.x
8. Bao Y, Zhang Y, Zhang Y, Liu Y, Wang S, Dong X, et al. Screening of potential probiotic properties of *Lactobacillus fermentum* isolated from traditional dairy products. *Food Cont.* (2010) 21:695–701. doi: 10.1016/j.foodcont.2009.10.010
9. Heikkilä MP, Saris PE. Inhibition of *Staphylococcus aureus* by the commensal bacteria of human milk. *J Appl Microbiol.* (2003) 95:471–78. doi: 10.1046/j.1365-2672.2003.02002.x
10. Martín R, Olivares M, Marín ML, Fernández L, Xaus J, Rodríguez JM. Probiotic potential of 3 lactobacilli strains isolated from breast milk. *J Hum Lactat.* (2005) 21:8–17. doi: 10.1177/0890334404272393
11. Martín V, Maldonado BA, Moles L, Rodríguez BM, Campo RD, Fernández L, et al. Sharing of bacterial strains between breast milk and infant feces. *J Hum Lactat.* (2012) 28:36–44. doi: 10.1177/0890334411424729
12. Fernández L, Delgado S, Herrero H, Maldonado A, Rodríguez JM. The bacteriocin nisin, an effective agent for the treatment of *Staphylococcus mastitis* during lactation. *J Hum Lactat.* (2008) 24:311–16. doi: 10.1177/0890334408317435
13. Lim YM, Barnes MB, Gras SL, McSweeney C, Lockett T, Augustin MA, et al. Esterification of high amylose starch with short chain fatty acids modulates degradation by *Bifidobacterium* sp. *J Funct Foods.* (2014) 6:137–46. doi: 10.1016/j.jff.2013.09.027
14. Sherman PM, Johnson KC, Yeung HP, Ngo PS, Goulet J, Tompkins TA. Probiotics reduce enterohemorrhagic *Escherichia coli* O157:H7-and enteropathogenic *E. coli* O127:H6-induced changes in polarized T84 epithelial cell monolayers by reducing bacterial adhesion and cytoskeletal rearrangements. *Infec Immun.* (2005) 73:5183–8. doi: 10.1128/IAI.73.8.5183-5188.2005
15. Hunt KM, Foster JA, Forney LJ, Schütte UM, Beck DL, Abdo Z, et al. Characterization of the diversity and temporal stability of bacterial communities in human milk. *PLoS ONE.* (2011) 6:e21313. doi: 10.1371/journal.pone.0021313
16. Anandharaj M, Sivasankari B. Isolation of potential probiotic *Lactobacillus oris* HMI68 from mother's milk with cholesterol-reducing property. *J Biosci Bioeng.* (2014) 118:153–9. doi: 10.1016/j.jbiosc.2014.01.015
17. Yassour M, Vatanen T, Siljander H, Hamalainen AM, Harkonen T, Ryhanen SJ, et al. Natural history of the infant gut microbiome and impact of antibiotic treatment on bacterial strain diversity and stability. *Sci Transl Med.* (2016) 8:343ra81. doi: 10.1126/scitranslmed.aad0917
18. Lackey KA, Williams JE, Meehan CL, Zachek JA, Benda ED, Price WJ, et al. What's normal? Microbiomes in human milk and infant feces are related to each other but vary geographically: the INSPIRE study. *Front Nutr.* (2019) 6:45. doi: 10.3389/fnut.2019.00045
19. Gharbi Y, Fhoula I, Ruas-Madiedo P, Afef N, Boudabous A, Gueimonde M, et al. *In-vitro* characterization of potentially probiotic *Lactobacillus* strains isolated from human microbiota: interaction with pathogenic bacteria and the enteric cell line HT29. *Ann Microbiol.* (2018) 69:61–72. doi: 10.1007/s13213-018-1396-1
20. Kostas ET, Cooper M, Shepherd BJ, Robinson JP. Identification of bio-oil compound utilizing yeasts through phenotypic microarray screening. *Waste Biom Valor.* (2019) 26:1–3. doi: 10.1007/s12649-019-00636-7
21. Kostas ET, White DA, Du C, Cook DJ. Selection of yeast strains for bioethanol production from UK seaweeds. *J Appl Phycol.* (2016) 28:1427–34. doi: 10.1007/s10811-015-0633-2
22. Banik A, Mondal J, Rakshit S, Ghosh K, Sha SP, Halder SK, et al. Amelioration of cold-induced gastric injury by a yeast probiotic isolated from traditional fermented foods. *J Funct Foods.* (2019) 9:164–73. doi: 10.1016/j.jff.2019.05.039
23. Sanders ME, Akkermans LM, Haller D, Hammerman C, Heimbach JT, Hörmannspurger G, et al. Safety assessment of probiotics for human use. *Gut Microbes.* (2010) 1:164–85. doi: 10.4161/gmic.1.3.12127
24. Tyagi AK, Prasad S. Commentary: probiotic and technological properties of *Lactobacillus* sp. strains from the human stomach in the search for potential candidates against gastric microbial dysbiosis. *Front Microbiol.* (2015) 6:433. doi: 10.3389/fmicb.2015.00433
25. Ruiz L, Margolles A, Sánchez B. Bile resistance mechanisms in *Lactobacillus* and *Bifidobacterium*. *Front Microbiol.* (2013) 4:396. doi: 10.3389/fmicb.2013.00396
26. Minekus M, Alminger M, Alvito P, Ballance S, Bohn TO, Bourlieu C, et al. A standardised static *in vitro* digestion method suitable for food—an international consensus. *Food Funct.* (2014) 5:1113–24. doi: 10.1039/C3FO60702J
27. Gut AM, Vasiljevic T, Yeager T, Donkor ON. Salmonella infection prevention and treatment by antibiotics and probiotic yeasts: a review. *Microbiology.* (2018) 164:1327–44. doi: 10.1099/mic.0.000709
28. Tiago FD, Martins FD, Souza EL, Pimenta PF, Araújo HR, Castro ID, et al. Adhesion to the yeast cell surface as a mechanism for trapping pathogenic bacteria by *Saccharomyces* probiotics. *J Medic Microbiol.* (2012) 61:1194–207. doi: 10.1099/jmm.0.042283-0
29. Dianawati D, Shah NP. Survival, acid and bile tolerance, and surface hydrophobicity of microencapsulated *B. animalis* ssp. lactis Bb12 during storage at room temperature. *J Food Sci.* (2011) 76:592–99. doi: 10.1111/j.1750-3841.2011.02422.x
30. Romero Luna HE, Hernández Sánchez H, Ribas Aparicio RM, Cauich Sánchez PI, Dávila Ortiz G. Evaluation of the probiotic potential of *Saccharomyces cerevisiae* Strain (C41) isolated from Tibicos by *in vitro* studies. *Prob Antimicrobiol Prot.* (2019) 11:794–800. doi: 10.1007/s12602-018-9471-2
31. Vlková E, Rada V, Šmehilová M, Killer J. Auto-aggregation and co-aggregation ability in *Bifidobacteria* and *Clostridia*. *Folia Microbiol.* (2008) 53:263–69. doi: 10.1007/s12223-008-0040-z
32. Keller MK, Hasslöf P, Stecksén Blinks C, Twetman S. Co-aggregation and growth inhibition of probiotic *Lactobacilli* and clinical isolates of mutans *Streptococci*: an *in vitro* study. *Acta Odontol Scandin.* (2011) 69:263–68. doi: 10.3109/00016357.2011.554863
33. Arqués JL, Rodríguez E, Langa S, Landete JM, Medina M. Antimicrobial activity of lactic acid bacteria in dairy products and gut: effect on pathogens. *BioMed Res Int.* (2015) 2015:584183. doi: 10.1155/2015/584183
34. Hudzicki J. Kirby-Bauer disk diffusion susceptibility test protocol. *Am Soc Microbiol.* (2009) 1–23.
35. Serata M, Kiwaki M, Iino T. Functional analysis of a novel hydrogen peroxide resistance gene in *Lactobacillus casei* strain Shirota. *Microbiology.* (2016) 162:1885–94. doi: 10.1099/mic.0.000379
36. Wu YY, Liu FJ, Li LH, Yang XQ, Deng JC, Chen SJ. Isolation and identification of nitrite-degrading lactic acid bacteria from salted fish. In: *Advanced Materials Research*. Kapellweg: Trans Tech Publications (2012) 393:828–34. doi: 10.4028/www.scientific.net/AMR.393-395.828
37. Yadav R, Puniya AK, Shukla P. Probiotic properties of *Lactobacillus plantarum* RYPRI from an indigenous fermented beverage Raabadi. *Front Microbiol.* (2016) 7:1683. doi: 10.3389/fmicb.2016.01683
38. Shuhadha MF, Panagoda GJ, Madhujith T, Jayawardana IA. Evaluation of probiotic attributes of *Lactobacillus* sp. isolated from cow and buffalo

- curd samples collected from Kandy. *Ceyl Medic J.* (2017) 62:159–66. doi: 10.4038/cmj.v62i3.8519
39. Padmavathi T, Bhargavi R, Priyanka PR, Niranjan NR, Pavitra PV. Screening of potential probiotic lactic acid bacteria and production of amylase and its partial purification. *J Gen Eng Biotechnol.* (2018) 16:357–62. doi: 10.1016/j.jgeb.2018.03.005
 40. Paul J. Blotting of coomassie blue-stained proteins from page gels to transparencies. *InWest Blott.* (2015) 1312:415–17. doi: 10.1007/978-1-4939-2694-7_41
 41. Ramasamy K, Rahman NZ, Chin SC, Alitheen NJ, Abdullah N, Wan HY. Probiotic potential of lactic acid bacteria from fermented Malaysian food or milk products. *Int J Food Sci Technol.* (2012) 47:2175–83. doi: 10.1111/j.1365-2621.2012.03085.x
 42. Syakila RN, Lim SM, Agatonovic KS, Lim FT, Ramasamy K. *In vitro* assessment of *pediococci* and *Lactobacilli* induced cholesterol lowering effect using digitally enhanced high performance thin layer chromatography and confocal microscopy. *Anal Bioanal Chem.* (2019) 411:1181–92. doi: 10.1007/s00216-018-1544-2
 43. Gilliland SE, Nelson CR, Maxwell C. Assimilation of cholesterol by *Lactobacillus acidophilus*. *Appl Env Microbiol.* (1985) 49:377–81. doi: 10.1128/AEM.49.2.377-381.1985
 44. Abrahamsson TR, Sinkiewicz G, Jakobsson T, Fredrikson M, Björkstén B. Probiotic *Lactobacilli* in breast milk and infant stool in relation to oral intake during the first year of life. *J Pediatric Gastroenterol Nutr.* (2009) 49:349–54. doi: 10.1097/MPG.0b013e31818f091b
 45. Ardeshir A, Narayan NR, Mendez-Lagares G, Lu D, Rauch M, Huang Y, et al. Breast-fed and bottle-fed infant rhesus macaques develop distinct gut microbiotas and immune systems. *Sci Transl Med.* (2014) 6:252ra120. doi: 10.1126/scitranslmed.3008791
 46. Pundir RK, Rana S, Kashyap N, Kaur A. Probiotic potential of lactic acid bacteria isolated from food samples: an *in vitro* study. *J Appl Pharm Sci.* (2013) 3:85–93. doi: 10.7324/JAPS.2013.30317
 47. Chou LS, Weimer B. Isolation and characterization of acid and bile tolerant isolates from strains of *Lactobacillus acidophilus*. *J Dair Sci.* (1999) 82:23–31. doi: 10.3168/jds.S0022-0302(99)75204-5
 48. Maragkoudakis PA, Miaris C, Rojcz P, Manalis N, Magkanari F, Kalantzopoulos G, et al. Production of traditional Greek yoghurt using *Lactobacillus* strains with probiotic potential as starter adjuncts. *Int Dair J.* (2006) 16:52–60. doi: 10.1016/j.idairyj.2004.12.013
 49. Begley M, Gahan CG, Hill C. The interaction between bacteria and bile. *FEMS Microbiol Rev.* (2005) 29:625–51. doi: 10.1016/j.femsre.2004.09.003
 50. Azuma Y, Ito K, Sato M. Simulated digestive juice tolerance and inhibitory effect on harmful intestinal bacteria of *Lactobacillus gasseri* NY0509 and *Lactobacillus casei* NY1301. *J Japan Soc Food Sci Technol.* (2001) 48:656–63. doi: 10.3136/nskkk.48.656
 51. Gut AM, Vasiljevic T, Yeager T, Donkor ON. Characterization of yeasts isolated from traditional kefir grains for potential probiotic properties. *J Funct Foods.* (2019) 58:56–66. doi: 10.1016/j.jff.2019.04.046
 52. Darsanaki RK, Rokhi ML, Aliabadi MA, Issazadeh K. Antimicrobial activities of *Lactobacillus* strains isolated from fresh vegetables. *Middle East J Sci Res.* (2012) 11:1216–19. doi: 10.5829/idosi.mejsr.2012.11.09.64152
 53. Savadogo A, Ouattara CA, Bassole IH, Traore AS. Antimicrobial activities of lactic acid bacteria strains isolated from Burkina Faso fermented milk. *Pak J Nutr.* (2004) 3:174–79. doi: 10.3923/pjn.2004.174.179
 54. Lee NK, Han KJ, Son SH, Eom SJ, Lee SK, Paik HD. Multifunctional effect of probiotic *Lactococcus lactis* KC24 isolated from kimchi. *LWT Food Sci Technol.* (2015) 64:1036–41. doi: 10.1016/j.lwt.2015.07.019
 55. Soubra L, Sarkis D, Hilan C, Verger P. Dietary exposure of children and teenagers to benzoates, sulphites, butylhydroxyanisole (BHA) and butylhydroxytoluene (BHT) in Beirut (Lebanon). *Regul Toxicol Pharmacol.* (2007) 47:68–77. doi: 10.1016/j.yrtph.2006.07.005
 56. Maragkoudakis PA, Zoumpopoulou G, Miaris C, Kalantzopoulos G, Pot B, Tsakalidou E. Probiotic potential of *Lactobacillus* strains isolated from dairy products. *Int Dairy J.* (2006) 16:189–99. doi: 10.1016/j.idairyj.2005.02.009
 57. Ren D, Li C, Qin Y, Yin R, Du S, Ye F, et al. *In vitro* evaluation of the probiotic and functional potential of *Lactobacillus* strains isolated from fermented food and human intestine. *Anaerobe.* (2014) 30:1–10. doi: 10.1016/j.anaerobe.2014.07.004
 58. Oh YJ, Jung DS. Evaluation of probiotic properties of *Lactobacillus* and *Pediococcus* strains isolated from Omegisool, a traditionally fermented millet alcoholic beverage in Korea. *LWT Food Sci Technol.* (2015) 63:437–44. doi: 10.1016/j.lwt.2015.03.005
 59. Wang J, Wang J, Yang K, Liu M, Zhang J, Wei X, et al. Screening for potential probiotic from spontaneously fermented non-dairy foods based on *in vitro* probiotic and safety properties. *Ann Microbiol.* (2018) 68:803–13. doi: 10.1007/s13213-018-1386-3
 60. Ghazi F, Henni DE, Benmecherrine Z, Kihal M. Phenotypic and whole cell protein analysis by SDS-PAGE for identification of dominant lactic acid bacteria isolated from Algerian raw milk. *World J Dairy Food Sci.* (2009) 4:78–87.
 61. Huang Y, Wang X, Wang J, Wu F, Sui Y, Yang L, et al. *Lactobacillus plantarum* strains as potential probiotic cultures with cholesterol lowering activity. *J Dairy Sci.* (2013) 96:2746–53. doi: 10.3168/jds.2012-6123

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2021 Afrin, Akter, Begum and Hossain. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.