INTRODUCTION

*Streptococcus thermophilus* is a common LAB with intrinsic functional characteristics, such as technological, organoleptic, and nutritional properties, which are considered important in the food and dairy industry (Quigley et al., 2013). This microorganism is not only able to produce lactic acid by a fermentation process but also with EPS that can act as a bio-stabilizer in yogurt, contributing to texture, firmness, and viscosity of the final product (De Vuyst et al., 2003). In order to reduce syneresis and enhance the consistency of yogurt, hydrocolloids or synthetic stabilizers have been used in the past. These stabilizers were mostly chemically modified and declared banned in some countries. However, consumer demand for cost-effective, free from synthetic additives, natural, cholesterol-lowering, and diabetic-friendly products has increased. These problems can be overcome, and consumer demands can be fulfilled, by using EPSs as a viable alternative.
2 | MATERIALS AND METHODS

Dahi samples \((n = 101)\) were collected from the local markets of Rawalpindi and Islamabad. These samples were collected under aseptic conditions and taken immediately to the laboratory for analysis.

2.1 | Isolation and identification of \(S.\ \text{thermophilus}\)

The selective medium \(M17\) \((CM0817)\) Oxoid England was used to recover isolates of \(S.\ \text{thermophilus}\) from Dahi samples. \(M17\) agar medium \((\text{composed of 3.725g M17 broth, 1.5% Technological agar and 10% lactose})\) was prepared in 100ml distilled water according to the instructions of the manufacturer; \(pH\) was adjusted to 6.9 with 6N \(\text{NaOH}\), mixed on a magnetic stirrer and sterilized in a digital autoclave at 121°C for 15 min. (Hirayama, Japan). The agar was then poured into sterilized Petri dishes and allowed to solidify. Inoculation of collected samples was performed by the streaking method, on \(M17\) agar plates, and incubated at 37°C for 24–48 h. The obtained isolates were tested through Gram staining and only Gram \((\text{+})\) colonies were further screened based on their morphological and biochemical properties, according to Buchanan and Gibbons \((1974)\) and as mentioned in Bergey’s manual. The selected colonies were then further analyzed and identified using the API kit method. For this purpose, API \((\text{Analytical Profile Index})\) 50CHL \((\text{API System, BioMerieux, France})\) was used according to the instructions given by its producer. Finally, \(S.\ \text{thermophilus}\) was confirmed at the molecular level with polymerase chain reaction \((\text{PCR})\) by amplifying the 16S rRNA region of these isolates using specific primers \(5’\ \text{ACGCTGAAGAGCTTG} 3’\) and \(3’\ \text{GCAATTGCCCTTTCAATA} 5’\) according to the standard method of Tab asco et al. \((2007)\) with some modifications.

2.2 | Exopolysaccharide production of strains

The identified and characterized \(S.\ \text{thermophilus}\) strains were further evaluated for having exopolysaccharide production ability. Initially, the production of EPSs on skim milk \((\text{Oxoid})\) plus nutrient agar \((\text{Oxoid})\) medium plates after incubation \((37°C\ \text{for 24 h})\) were visually observed by checking ropiness, mucidness, or capsulation of the strains using a sterile wire loop \((\text{or toothpick, like in Zivkovic et al., 2015})\) \((\text{Ali et al., 2019; Muigei et al., 2013; Paulo et al., 2012})\). Finally, mucoid or ropy \((\text{EPS producing})\) colonies of \(S.\ \text{thermophilus}\) were selected and further assessed for their EPS production.

In order to estimate EPS production, strains were inoculated \((2%)\) in sterilized fermentation medium and incubated at 42°C for 24 h. 100ml of fermentation medium was prepared by adding 7ml of 11% skim milk \((\text{LP0031})\) Oxoid England, 3.0g nutrient agar \((\text{Oxoid, England})\), and 1.0g tryptone \((\text{Oxoid, England})\) in distilled water to make the volume up to 100ml, mixed on magnetic stirrer, and autoclaved at 121°C for 15 min.

2.2.1 | EPS isolation

Exopolysaccharides were isolated from the fermented medium according to the method described by Rimada and Abraham \((2003)\), with slight modifications. The fermented sample \((100ml)\) was taken and heated to boiling point \((100°C)\) in a water bath for about 15 min. in order to remove proteins \((\text{to inactivate enzymes})\) and
polysaccharides attached to the cell walls. After cooling to room temperature, the sample was centrifuged at 8,000 rpm for 10 min. to remove the cells. 17ml of 85% trichloro-acetic acid (TCA) was then added to the sample (100 ml), cooled to 4°C, and centrifuged again at 8,000 rpm for 10 min. EPS concentration in the supernatant was increased by precipitation with cold ethanol (-20°C), with a 1:3 concentration and stored overnight at 4°C. The final precipitate, obtained by centrifugation at 8000 rpm for 10 min., was dissolved in distilled water (100ml) and stored at 4°C. The collected pellets of EPS were again suspended and filtered through a dialysis tube (molecular weight cut-off 8–14 KDa, Beijing Solarbio Science & Technology Co., Ltd., China). The dialysis was performed against water for 48h., with water removal after every 8th hour. For further quantity determination, the solutions were prepared according to the method of Xu et al. (2010).

2.2.2 EPS quantification

EPS quantification was carried out according to the method of Dubois (Dubois et al., 1956) based on the phenol-sulfuric acid method, with slight modifications. Firstly, 5% of phenol red solution was prepared in the distilled water then 2ml of sample (EPS solution) and 1ml of phenol solution were mixed in the test tube. 5ml of concentrated sulfuric acid was added to the mixture and left for 10 min. Then, the mixture was shaken by vortex and incubated at 30°C for 10 min. (until development of a yellow-orange color). The control was prepared by adding 400µl phenol solution in 400µl of distilled water. Afterward, the absorbance of samples was measured by spectrophotometer (UV-9200) at 490nm, and readings were compared with the control to measure total carbohydrate content. The amount of EPSs in each sample was interpreted by using glucose standard calibration line and expressed as mg/L glucose equivalent. Calibration line is prepared by glucose solution (1mg/ml) as standard, using 6 different proportions as defined by Feldmane et al. (2013) and Muigei et al. (2013).

2.2.3 Evaluation of technological properties of EPS-producing strains

The strains identified as EPS producers were used to ferment milk in order to determine technological properties, such as titratable acidity, curdling time, flavor, body, and texture of the curd. Sensory attributes (flavor, body, and texture) were determined through sensory evaluation method and titratable acidity by titration method. All the experiments were conducted in triplicates.

2.3 Antibacterial activity of S. thermophilus strains

For measuring antibacterial activity, pathogenic strains E. coli ATCC25922, S. aureus ATCC6538, P. aeruginosa ATCC25923, and L. monocytogenes ATCC 19;115 were obtained from the Department of Pathology, Pakistan Institute of Medical Science (PIMS) (Mahmood et al., 2013). The stocks of all the strains were maintained in 20% (v/v) glycerol and stored at −80°C.

For this purpose, the paper disc method was used, as described by Soomro and Masud (2012), with slight modifications. Sterilized paper discs of 6-mm diameter made of Whatman filter paper no. 1, were kept on nutrient agar plates having a target pathogenic strain, whereas discs carried an adsorbed aliquot (20µl) of cell-free supernatant. pH of the nutrient agar medium was adjusted to 7.2. To obtain a cell-free supernatant, freshly overnight-grown culture was attained in broth medium, and its pH was adjusted to 5.5 with 1 M NaOH. It was then centrifuged at 13,000 rpm for 10 min and the supernatant (cell-free) was collected to send through a syringe filter (0.2μm) to remove bacterial cells. For comparison with the control, Ampicillin disc (10µg) was used as a reference antibiotic. The concentration of the overnight-grown culture of indicator strains was adjusted according to 0.5 McFarland turbidity standard. The plates were then kept in an incubator for 24 h at 37°C. Resulting clear inhibition zones, formed around paper discs, were then measured for evaluation of antibacterial activity. Inhibition zones or spectra round discs were computed in diameter (mm).

2.4 Bile salt resistance and acid tolerance test

Bile salt tolerance and acid tolerance of isolates was conducted by the methods of Hassanzadazar et al. (2012) and Singhal et al. (2010), with slight modifications. For the acid tolerance test, M17 broth medium with adjusted pH values (2 and 3) was used to create in vitro acidic conditions of the gastrointestinal tract. pH 2 and 3 were adjusted with 1N HCl while pH 6.9 was adjusted to serve as a control. Overnight-grown culture of S. thermophilus strains (1%) were then inoculated to M17 broth and incubated at 37°C for 5h. Percentage acid tolerance was found by measuring optical density (O.D.) at 600 nm using following formula:

$$\text{Survival} \% = \frac{\text{O.D. (pH2 or 3)} - \text{O.D. (pH7)}}{\text{O.D. (pH7)}} \times 100$$

For bile salt tolerance, a fresh overnight-grown culture of S. thermophilus strains (1%) was used for inoculation in M17 broth medium, supplemented with 0.3% and 1.5% bile salts (w/V), while M17 broth without bile salt (0%) supplementation was used as the control. Samples were incubated at 37°C for 6 h. and optical density (O.D.) was measured at 600 nm to determine the bile tolerance percentage of strains using the following formula:

$$\text{Survival} \% = \frac{\text{O.D. (0% bile)} - \text{O.D. (0.3% or 1.5% bile)}}{\text{O.D. (0% bile)}} \times 100$$

2.5 Auto-aggregation assay

Auto-aggregation assay was performed in line with the method as outlined by Kaushik et al. (2009), with slight modifications. For this purpose, cell pellets from fresh growth of isolates were obtained
by centrifugation (8000 rpm for 10 min.). The cell pellet was then washed and resuspended in 0.01 M phosphate buffer saline (PBS). Initial cell concentration (initial absorbance) was adjusted according to 0.5 McFarland standard at 600 nm and then incubated at 37°C for 2h. After 2 h, the suspension was centrifuged to obtain the cell pellet and mixed with the respective broth of equal volume removed. The supernatant was used to measure its absorbance (final absorbance) while the broth was used as the control. The following formula was used for calculating the percentage of auto-aggregation capability.

\[
\text{Auto-aggregation(\%)} = \frac{\text{Abs. (initial)} - \text{Abs. (final)}}{\text{Abs. (initial)}} \times 100
\]

### 2.6 Bacterial adherence to hydrocarbons (BATH) test

The method used, with some modifications, for determining percentage of bacterial adherence or hydrophobicity of S. thermophilus strains, was described by Kaushik et al. (2009). For this purpose, three different hydrocarbons (xylene, n-hexadecane, and dichloromethane) were selected for measuring selected strains adherence percentage to these hydrocarbons. Briefly, fresh overnight-grown culture was centrifuged (at 8000 rpm for 10 min.) to obtain the cell pellet. The cell pellet was then washed and resuspended in 2.5 ml of 0.01 M phosphate urea magnesium (PUM) buffer. Initial absorbance of cell suspension was set to 0.7 at 600 nm and then 1 ml of any tested hydrocarbon (xylene, n-hexadecane, or dichloromethane) was added to the cell suspension. This suspension was then incubated at 37°C for 10 min. and vortexed (2 min.) to mix the two phases and again incubated at 37°C for 1 hr. After the incubation period, phases were separated and the aqueous phase was collected carefully to measure its absorbance at 600 nm, using the following formula:

\[
\text{Hydrophobicity(\%)} = \frac{\text{Abs. (initial)} - \text{Abs. (final)}}{\text{Abs. (initial)}} \times 100
\]

### 2.7 Antibiotic susceptibility assay

The disc diffusion method (paper disc method) was used to determine the S. thermophilus strains’ susceptibility to antibiotics, as defined by Pisano et al. (2014), with some modifications. Antibiotics (10), which are currently used for the treatment of infections in the Allied hospitals of Pakistan, including Erythromycin (15 µg), Amoxicillin (10 µg), Vancomycin (30 µg), Kanamycin (30 µg), Teicoplanin (30 µg), Tetracycline (30 µg), Ciprofloxacin (5 µg), Streptomycin (10 µg), Ampicillin (10 µg), and Gentamicin (10 µg), were selected for this test.

In this method, a bacterial lawn was prepared on agar plates with the concentration adjusted according to 0.5 McFarland standard and antibiotic discs were kept on it. These plates were then incubated at 37°C for 24 h and after 24 h clear zones or zones of inhibition (ZoI) were measured in diameters (mm) and compared with the interpretative zone diameters (CLSI M100–S21, 2011). The results were indicated as susceptible, moderately susceptible, or resistant.

### 2.8 Statistical study of data

The resulting data were statistically examined using Statistical package (SPSS 16.0 version). For this purpose, completely randomized design (CRD) was used and for graphical representation of the data Microsoft Excel was used. ANOVA (two way) followed by Tukey’s test was also applied for statistical differences with a level of significance = 0.05 (Han et al., 2016).

### 3 RESULT AND DISCUSSION

#### 3.1 Isolation and identification of S. thermophilus

One hundred and one samples of indigenous Dahi were collected from different areas of Islamabad and Rawalpindi to isolate S. thermophilus strains. On the basis of Gram staining, catalase test, and acid production, 76 isolates of lactic acid bacteria (LAB) were recovered and further characterized by morphological studies. Out of 76 strains, 44 were identified as bacilli Gram positive catalase negative, and 32 as cocci Gram positive and catalase negative. All selected isolates resulted negative for motility and spore formation ability as also reported by Sharma (2014).

In order to screen out S. thermophilus from 32 isolates of cocci, isolated Gram-positive and catalase-negative cocci were further differentiated on the basis of their growth at different temperatures and NaCl concentrations as well as their carbon dioxide gas production from glucose and confirmed through analytical profile index (API) test. Each experiment was conducted in triplicates and only promising isolates were further propagated for selection. The isolates which grew at 45°C but could not grow at even 2% NaCl concentration and did not produce carbon dioxide gas from glucose (homo-fermentative), were selected. Sharma (2014) also used similar criteria of homofermentative bacteria for the screening of LAB isolates. Further biochemical testing through API confirmed that out of 32 LAB cocci, 20 isolates were S. thermophilus, three isolates were S. cremoris, and five were L. lactis, while only four isolates were identified as Leuconostoc spp. All 20 biochemically identified strains were subjected to PCR amplification of 16S rRNA regions. The specific primers used according to the sequence 5’ ACGCTGAAGAGGAGCTTG 3’ and 3’ GCAATTGCCCTTCTAAAATA 5’ published in NCBI gene bank and consequently isolates which gave PCR product of 200-230bp were finally selected. These findings are similar to the results of PCR products of S. thermophilus previously described by Tab asco et al., (2007) and Mahmood et al., (2013). The PCR results confirmed the 10 selected strains as S. thermophilus (Ali et al., 2019; Kullen et al., 2000; Suhartatik et al., 2014).
3.2 | Exopolysaccharide production of selected strains of S. thermophilus

3.2.1 | Screening of ropy and mucoid strains to assess EPS-producing ability

The 10 strains identified as S. thermophilus were tested for EPS production ability. For this purpose, initially their ropiness and mucoid nature was assessed through a visual observation method, that is, ropiness test. The strains which formed long roppy like structures when picked with a sterile inoculation wire loop, were considered as roppy strains. According to Gomez (2006) and Zivkovic et al. (2015), this phenotypic character can be associated with the production of exopolysaccharides on solid medium, however, exopolysaccharides can be capsular polysaccharides (CPS) or roppy polysaccharides (RPS). Capsulation was determined through staining with crystal violet and subsequently rinsing with 20% copper sulphate solution. The results obtained are shown in Table 1 and it can be seen that all ten selected strains showed positive mucoid colony growth on M17 and skim milk agar medium plates. However, two isolates (RIRT and RIR1L) did not show any roppy polymer production through visual observation while all others were positive for ropiness, including two isolates (RIY and RIH4) which were highly roppy. With regard to the capsulation-forming ability, all the strains resulted positive to the capsulation test, except for the RIRT isolate. Capsule staining showed that some strains (RIH4) formed large and thick capsules while some formed relatively small or no capsules (Figure 1 and Figure 2). The disparity among different strains for production of roppy or capsular polysaccharides is due to different cultures and some strains even produced both roppy and capsular polysaccharides. Mozzi et al. (2009) also reported that roppy or capsular exopolysaccharide production is varied from culture to culture and hence some strains have the ability to form only one type of polymer, while others form both capsular and roppy polysaccharides.

Table 1: Assessment and quantification of EPS production by S. thermophilus strains isolated from local Dahi

| Strain code | Type of EPS | EPS in skim milk media (mg/L) ± S.D. |
|-------------|-------------|-------------------------------------|
| RIY         | +           | 133.0 ± 0.06                        |
| RIK         | +           | 93.17 ± 0.28                        |
| RIM         | +           | 27.43 ± 0.40                        |
| RIRT2       | +           | 95.77 ± 0.22                        |
| RIH4        | +           | 103.83 ± 0.76                       |
| RIRT        | -           | 19.67 ± 0.57                        |
| RHQ3        | +           | 24.67 ± 0.57                        |
| RIR1L       | +           | 78.33 ± 0.57                        |
| RIH1        | +           | 37.67 ± 1.52                        |
| RH3         | +           | 41.01 ± 1.00                        |

Note: All values are means of three replications and means carrying different letters are significantly different at alpha 0.05 (p < .05). Indications used are for highly roppy (+ +), less roppy (+), and nonroppy (−) strains.

Mostefaoui et al. (2014) also used a similar mucoid and ropiness test for screening of EPS-producing isolates after incubation for 48 h at 42°C. According to Ruas-Madiedo and de Los Reyes-Gavilan (2005), Welman et al. (2003), and Ricciardi et al. (1997) mucoidy of strains was assessed through appearance and visual observation of colonies’ growth and confirmed through an ethanol precipitation technique. According to Behare et al. (2010), the strains forming roppy polysaccharides were considered to be better than strains forming capsular EPS and, due to this, can be used in dairy industry as a biothickener.

3.2.2 | Exopolysaccharide isolation and quantification

EPSs produced by the tested strains (ropy or capsular) were further isolated and then quantified by the trichloroacetic acid method followed by precipitation through the cold ethanol method. A similar method was used by Han et al. (2016) for isolation and measuring the concentration of these polysaccharides. The results obtained regarding EPS concentration are summarized in Table 1. It can be observed that different strains produced different amounts of extracellular polymers with a significant difference (p < .05) among all the tested strains. The selected strains were able to produce EPS in skim milk medium, from 19.67 to 133.0 mg/l. Stingele et al. (1996) that reported the presence in S. thermophilus SF16 of epsM and epsA genes responsible of exopolysaccharides synthesis. Maximum EPS production was observed in RIY in skim milk medium (133.0 ± 0.06) followed by RIH4 (103.83 ± 0.76) while minimum EPS production was observed in RIRT (19.67 ± 0.57). This variation in the results of EPS production might be attributed to the reason that exopolysaccharides production is dependent on the strains, which might be associated with the gene encoding on chromosome for EPS formation. In the literature it was reported that the total yield of EPS produced by the lactic acid bacteria (LAB) depends on the composition of the medium and conditions in which the organisms grow (i.e., medium, temperature, and incubation time) (Cerning et al., 1990). Gamar et al. (1997) also reported that EPS production and yield were influenced by the carbon source and concentration. Consequently, those strains which produced maximum quantity of EPS have a potential to replace the usage of chemical stabilizers in the dairy industry.

3.2.3 | Technological screening—a comparison of EPS-producing strains

Technological properties including acidity, curdling time, body and texture of curd, and other sensory features are summarized in Table 2. As shown, EPS production greatly affects sensory evaluation, body, and texture of the curd. In detail, four strains (RIY, RIH4, RIK, and
S. thermophilus, which is identified as a probiotic bacteria (Bhowmik et al., 2009; Mahmood et al., 2013). In addition to the primary role of their milk acidification, these bacterial strains of S. thermophilus produce secondary metabolites such as antibacterial peptides and possess other probiotic features.

EPS-producing strains were firstly investigated to ascertain their possible antimicrobial activity against food pathogens before determining other probiotic properties. Four pathogenic strains were used for this purpose (as shown in Table 3), namely L. monocytogenes ATCC 19115, E. coli ATCC25922, S. aureus ATCC6538, and P. aeruginosa ATCC25923 as also previously used by Mahmood et al. (2013). Therefore, determination of the antibacterial activity of S. thermophilus strains against these indicator strains would be a novel character. It is revealed from the results that all the ten tested strains gave variable results and showed a wide range of antimicrobial activity against different pathogenic/indicator strains, having more or less zone of inhibition against one pathogen or more. These differences in the inhibitory activities of tested strains against different indicator strains may be due to their genotype or environmental factors.

The results of antibacterial activity of cell-free supernatants, from S. thermophilus strains, are presented in Table 3. The maximum zone of inhibition (16mm) was observed against L. monocytogenes ATCC 19115 using RIK and RIRT2 supernatants, while S. aureus ATCC6538 was observed to be most sensitive on a maximum number of isolates, with a maximum zone of inhibition of 8 mm by RIM and RIRT2 supernatants. Mahmood et al. (2013) also reported maximum antibacterial activity against L. monocytogenes ATCC 19115 and S. aureus ATCC6538, by four S. thermophilus strains (S02FT, S03FT, S05FT, S06FT). Similar results were reported by Khalil (2009) who found that the crude extract of S. thermophilus CHCC3534 produced broad-spectrum bacteriocin that is effective against S. typhimurium and S. aureus. Fontaine and Hols (2008) studied thermophilin 9, produced by S. thermophilus LMD-9, that was active against other S. thermophilus and L. monocytogenes strains due to the presence of three mutative operons (bacSt operons) and the blpGSt gene, which encodes a putative modification protein to the inhibitory activity of LMD-9.

E. coli ATCC25922 was found to be less sensitive to cell-free supernatants from all the tested strains, with three strains (RIY, RIH4, and RIH3) showing no antibacterial activity or no detectable zone of inhibition. However, only a lower antibacterial activity was found by cell-free supernatants of the remaining seven strains with two (RIK and RIR1L) having a zone of inhibition up to 4mm and five (RIRT, RIRT2, RIM, RIHQ3, and RIH1) having visible zones of inhibition (<1mm). Mahmood et al. (2013) also reported weak antibacterial activity (≤4mm Zoi) against E. coli ATCC25922 from cell-free supernatants of 3 tested strains out of 11.

A mixed response was observed against P. aeruginosa ATCC 25923 by cell-free supernatants of the tested strains. The maximum zone of inhibition (12mm) was observed using RIK and RIR1L supernatant, while minimum zone of inhibition (<1mm) was observed using RIRT, RIRT2, and RIH3 supernatants. The supernatants of two strains (RIHQ3 and RIH1) showed no activity against P. aeruginosa ATCC 25923, while others showed medium (in between) zones of inhibition (≤4mm Zoi) with the production of extracellular polymers was found. Thus, the relationship between acidity and curdling time but no relationship with the production of extracellular polymers was found. Thus, the four strains RIY, RIH4, RIK, and RIRT2, showing comparatively better results, can be used as potential candidates for yogurt making.

### 3.3 Probiotic potential of S. thermophilus strains

#### 3.3.1 Antibacterial activity of S. thermophilus strains

Our traditional fermented dairy product, Dahi, can be used as a source of probiotics because the microbial isolates included strains of S. thermophilus. Figure 1 and 2 show the capsule formation by S. thermophilus strains detected using staining method.
inhibition. Mahmood et al. (2013) stated that supernatants of only 3 strains out of 11 tested showed an inhibitory effect (≤8mm ZoI) against *P. aeruginosa* ATCC 25923.

### 3.3.2 Acid and bile tolerance

**Acid tolerance**

If a minimum amount of 10⁶ log CFU (Nagpal et al., 2012) bacterial culture tolerates pH up to 2–3, it can be a potential candidate for a probiotic, as the initial pH of the stomach is 1.5 and it reaches up to pH 3–4 as food enters, which can remain for 4–5 h (Slavin, 2013).

Low pH tolerance or acid tolerance of *S. thermophilus* strains was measured in vitro at two pH levels (pH 2 and pH 3). Only six strains out of ten selected were found to be tolerant to acid at both pH levels (2 and 3). The maximum tolerance under acidic conditions was observed in RIY with a 69% survival after 5h of incubation at pH 3 and 25% at pH 2, followed by RIH4, showing 65% survival at pH 3 for 5h and 20% at pH 2 (Figure 3). RIH4 is further followed by RIK with 62% survival at pH 3 and 19% survival at pH 2. Strain RIRT2 has 58% survival at pH 3 and 16% at pH 2 while strains RIRT and RIR1L showed almost similar survival rates at both pH levels with 52% survival at pH 3 and 15% at pH 2. Control strain gave survival percentages of 10% at pH2 and 47% at pH 3 as compared to other selected strains. Consequently, it can be said that pH 2 was more harmful for *S. thermophilus* than pH 3, however, the viability of cells declined during incubation. All six strains which remained viable at pH 3 had a survival rate of more than 50% and hence can be probable candidates as a probiotic culture (Liong & Shah, 2005).

Several studies have determined that *S. thermophilus* strains were unable to grow at low pH levels (Haller et al., 2001; Khalil, 2009; Mahmood et al., 2013; Maurad & Meriem, 2008; Tuncer and Tuncer (2014) reported that pH 1 was more lethal to *S. thermophilus* ST8.01 than pH 3, but during incubation at pH 3 viability of cells still declined and the percentage of inhibition was found to be more than 99.99%, whereas at pH 5 it was 95.43% and viability was retained. Another study by Mahmood et al. (2013) also reported that *S. thermophilus* strain S02FT was not capable to grow at pH 3.5 but maintained its viability at lower pH values. Our results are also correlated with the study of Khalil (2009) who found that *S. thermophilus* CHCC3534

### Table 2: Technological properties of EPS-producing strains

| Cell-free supernatant strain code | Acidity (% Lactic acid) | Curdling time (hrs) | Flavor, body, and texture |
|----------------------------------|-------------------------|---------------------|--------------------------|
| RIY                              | 0.83 ± 0.01             | 5.0                 | Good body and texture, pleasant flavor |
| RIK                              | 0.68 ± 0.02             | 7.0                 | Good body and texture, acidic flavor |
| RIM                              | 0.63 ± 0.01             | 8.5                 | Good body and texture, mild flavor |
| RIRT2                            | 0.71 ± 0.04             | 6.5                 | Poor body and texture, pleasant flavor |
| RIH4                             | 0.78 ± 0.01             | 5.5                 | Good body and texture, pleasant flavor |
| RIRT                             | 0.60 ± 0.03             | 9.0                 | Good body and texture, acidic flavor |
| RIHQ3                            | 0.64 ± 0.02             | 8.5                 | Good mouth feel but acidic flavor |
| RIR1L                            | 0.67 ± 0.02             | 7.0                 | Good body and texture, pleasant flavor |
| RIH1                             | 0.66 ± 0.04             | 8.0                 | Good mouth feel but acidic flavor |
| RIH3                             | 0.65 ± 0.01             | 7.5                 | Good texture, pleasant flavor |

Note: Zone of inhibition (−) = no activity, (+) = visible inhibition, 1–4 mm (++), 5–12 mm (+++), ≥13mm (++++) (Mahmood et al., 2013).

### Table 3: Antibacterial activity of cell-free supernatants from *S. thermophilus* strains against different food pathogens

| Cell-free supernatant strain code | Indicator strain (Pathogen) |
|----------------------------------|-----------------------------|
|                                  | *E. coli* ATCC25922 | *L. monocytogenes* ATCC19115 | *P. aeruginosa* ATCC25923 | *S. aureus* ATCC6538 |
| RIY                              | − | +++ | ++ | + |
| RIK                              | ++ | ++++ | +++ | ++ | + |
| RIHQ3                            | + | +++ | ++ | +++ |
| RIM                              | + | +++ | ++ | +++ |
| RIRT                             | ++ | ++ | + | + |
| RIRT2                            | ++ | ++++ | + | +++ |
| RIH1                             | + | +++ | − | ++ |
| RIH3                             | − | +++ | + | ++ |
| RIH4                             | − | +++ | ++ | + |
| RIR1L                            | ++ | +++ | +++ | ++ |

Note: Zone of inhibition (−) = no activity, (+) = visible inhibition, 1–4 mm (++), 5–12 mm (+++), ≥13mm (++++) (Mahmood et al., 2013).
was resistant to pH greater than 2 but nonresistant to 1.5. Some studies related to other probiotic bacteria also gave similar findings to our results. Maurad and Meriem (2008) reported that L. plantarum strains survived up to 6h after incubation at pH 2. According to Aswathy et al. (2008), LAB including Streptococcus growth increased at pH 5 and facilitated in the production of fermented vegetable and milk products.

**Bile tolerance**

Bile tolerance is one of the most essential criteria for a strain to be used as a probiotic culture (Hassanzadazar et al., 2012; Soleimanian-Zad et al., 2009; Vizoso-Pinto et al., 2006). Bile resistance and the ability of LAB to inhabit the intestinal tract appear to be correlated (Soomro & Masud, 2012). According to Aswathy et al. (2008), probiotic strains which are intended to be used for humans must have resistance to bile salts at 0.3% concentration.

Bile tolerance of 10 selected EPS-producing *S. thermophilus* strains was measured in vitro at two bile salt concentrations (0.3% and 1.5%). Seven strains out of ten were found to be tolerant at both concentrations. Maximum bile tolerance or survival percentage at 0.3% bile concentration was observed for RIY (about 85% survival), followed by RIH4 (80% survival) and RIRT2. RIR1L, RIK with >70% survival, while RIRT RIHQ3 had >60% survival. At 1.5% bile concentration maximum tolerance was observed for RIY (70% survival) followed by RIH4 (69% survival), RIRT2 (52% survival), RIR1L (51% survival), RIK (52% survival), and RIRT (49% survival) while least survival (21%) was observed for RIHQ3 (Figure 4). The control strain gave 64% survivability at 0.3% bile salt concentration and 30% at 1.5% as compared to other selected strains. Hence, all the seven strains (RIRT2, RIR1L, RIK, RIRT, RIHQ3, RIY, RIH4), which survived at 0.3% bile salt, fulfilled the criteria for being probiotic strains as reported by Boke et al. (2010). It is also described by Brashears et al. (1998) that 2% bile salt concentration is equal to that of the alimentary canal. Similar to our findings, Tuncer and Tuncer, (2014) found in their study that *S. thermophilus* strain ST8.01 was able to survive after incubation (24 h) at three different concentrations (0.3, 0.5, and 1%) of bile salt (w/v) and highest inhibition (38.34%) was observed at 1% bile salt concentration. Similarly, several other studies reported the bile tolerance of *S. thermophilus* strains even at 2% concentrations (Arias & Murray, 2009; Iyer et al., 2010). Mahmood et al. (2013) studied growth of *S. thermophilus* strain S02FT at three different concentrations (1, 2, and 3%) of bile salt and found that S02FT was able to survive at 2% concentration but unable to grow at 3%. However, Vinderola and Reinheimer (2003) reported much lower bile tolerance of *S. thermophilus* and stated that mostly strains were unable to survive at 0.5% bile concentration. From the mentioned literature, it can be stated that there is different growth at different bile salt concentrations, and this is probably due to a strain’s specificity.

### 3.3.3 Cell aggregation

Auto-aggregation ability of probiotics is a prerequisite for their adherence with the epithelium cells of the intestine (Aslim et al., 2007; Collado et al., 2008). It can be seen from Figure 5 that the cellular aggregation percentage was variable for all the six selected strains. Maximum auto-aggregation was found for RIRT (98.8 ± 0.6) followed by RIY (97.8 ± 0.4), RIRT2 (61.2 ± 1.0), and RIH4 (53.6 ± 0.6), respectively, while the minimum was observed for RIR1L (12.0 ± 0.5) and RIK (8.8 ± 0.6). These variations can be probably due to the auto-aggregation ability of the single strain as also observed by other researchers (Kos et al., 2003; Todorov et al., 2009; Tuncer & Tuncer, 2014; Vlkova et al., 2008) reporting that physico-chemical properties of cell surfaces such as hydrophobicity might have affected the auto-aggregation abilities. The results in Table 4 show that high EPS-producing strains exhibited more aggregation. Aslim et al. (2007) and Darilmaz and Beyatlı (2012) also reported that high EPS-producing strains exhibited significant aggregation. However, RIRT strain is less EPS producing but showed high auto-aggregation ability which might be due to the strain specificity.

Hence, the strains with aggregation percentages of 97.8 ± 0.4, 61.2 ± 1.0, and 53.6 ± 0.6 (Figure 5) are very interesting because
their aggregating ability is higher than the reported aggregation of other S. thermophilus strains, except RIRT which is a high aggregating but low EPS-producing strain. Tuncer and Tuncer (2014) reported 62.4% auto-aggregation of S. thermophilus ST8.01 strain. Some previous studies conducted also reported somewhat comparable auto-aggregation percentages of LAB (Canzi et al., 2005; Koll et al., 2010; Rahman et al., 2008). Miljkovic et al. (2015) determined the important role of AggLb cell surface protein of strain L. paracasei subsp. paracasei BGNJ1-64 for cell aggregation and collagen binding.

### 3.3.4 | Bacterial adherence to hydrocarbons (BATH)

The ability of bacteria to adhere to different hydrocarbons is the measure of the bacterial hydrophobicity to assess adherence of bacterial strains to the intestinal lining. Previously, in vitro analysis of bacterial adhesion to hydrocarbons using n-hexadecane and xylene was carried out by Schillinger et al. (2005) and Kaushik et al. (2009), while using dichloromethane as a source of hydrocarbons was conducted by Jose et al. (2015).

In the present study, three hydrocarbons were used, namely n-hexadecane, xylene, and dichloromethane (DCM) for testing the adherence percentage of selected strains of S. thermophilus, as shown in Figure 6. Among the three different hydrocarbons used, there is significant difference (p < 0.05) between n-hexadecane and the other two hydrocarbons (xylene and dichloromethane), whereas between xylene and dichloromethane there is a nonsignificant difference (p > 0.05). In contrast to this study, Kaushik et al. (2009) reported that no significant difference was found in the adherence property of bacterial strains by using different hydrocarbons, while n-hexadecane showed a lower mean value (47.1%) compared to xylene (49.9%) and DCM (49.8%). This decrease might be due to its toxic or destructive action on microbial cells. It can also be observed that among the adherence percentages of all the strains except RIRT (58.3%) and RIY (57.7%) there is a significant difference (p < 0.05). The maximum mean value was observed as 62.2 μg for RIRT2 followed by RIRT (58.3%), RIY (57.7%), and RIH4 (56.8%), respectively, whereas the minimum adherence percentage was observed as 11.2% for RIK. These differences might be due to the reason that cell surface hydrophobicity or adhesion of bacteria is basically a strain-specific property which depends on the origin of the strain, its surface-adhering or mucus-adhering property (Grajek et al., 2016), or variation in cell surface protein expression levels along with the influence of the environment on the expression of certain proteins (De Vries et al., 2006).

Previously, Tuncer and Tuncer, (2014) reported S. thermophilus ST8.01 adherence percentage to xylene as 67.23 ± 7.16% and similar results of higher adhering capacity percentage were observed in a study by Iyer et al. (2010). Although small differences exist for adherence percentage, the present study values are still higher than many other findings (Figure 6). According to the criterion as described by Tyfa et al. (2015) all the selected strains fall under the category of strongly hydrophobic except RIK, while RIR1L showed moderate hydrophobic behavior for hydrocarbon n-hexadecane and strong hydrophobic behavior for xylene and DCM. It might be due to expression of surface proteins of cells or their preference for hydrocarbons (Draksler et al., 2004).
3.3.5 | Antibiotic susceptibility

A key requirement for probiotic strains is that they should not carry transmissible antibiotic resistance genes. Ingestion of bacteria carrying such genes is undesirable, as horizontal gene transfer to recipient bacteria in the gut could lead to the development of new antibiotic-resistant pathogens (Guglielmetti et al., 2009; Saarela et al., 2000; Salminen et al., 1998). For this, the assessment of \( S. \) thermophilus strains’ susceptibility to clinically important antibiotics becomes important (Tuncer & Tuncer, 2014). The six selected strains were tested against 10 antibiotics by agar diffusion method as presented in Table 4. These strains were grouped as susceptible (S:20mm or >), resistant (R:0-10mm), or intermediate (I:10-19mm) to a particular antibiotic. It can be seen that the strains exhibited different behaviors to variable antibiotics as well as their concentrations. All the strains were found susceptible to Vancomycin (25-40mm Zol), Tetracycline (20-40mm Zol), and Erythromycin (20-40mm Zol) as well as to Teicoplanin (20-30mm Zol) with the exception of RIK strain which showed an intermediate effect (14mm Zol). The strains RIRT, RIRT2, RIH4, and RIK exhibited intermediate response (12-15mm Zol), while RIY was susceptible (22mm Zol) and RIR1L was resistant (0mm Zol) against Gentamicin. Four strains RIRT, RIH4, RIY, and RIK showed intermediate responses (14-18mm Zol) to Amoxicillin except RIRT2 and RIR1L which showed susceptibility (30-40mm Zol). However, all strains were found susceptible (20-35mm Zol) to Ampicillin except RIRT2 and RIR1L which showed intermediate responses (12-15mm Zol).

The strains RIRT, RIR1L, and RIK were found to be resistant (0-10mm Zol) to Ciprofloxacin, Kanamycin, and Streptomycin while other strains showed intermediate (12-18mm Zol) to low susceptibility (20-25mm Zol). Similar results were reported by Katla et al. (2001), Temmerman et al. (2003), Aslim and Beyatli, (2004), Tosi et al. (2007), and Mahmood et al. (2013). These differences in the degree of inhibition with various antibiotics were possibly due to the difference in environment of strain isolation, as this is not the intrinsic feature of strains, or might be due to their different actions on the cell components such as the cell wall, protein and DNA synthesis, DNA gyrase, and RNA polymerase (Neu, 1992).

Briefly, RIY showed susceptibility to the maximum number (against eight) of antibiotics, while RIR1L showed resistance to the minimum number (against three) of antibiotics. All other strains showed mixed response to different antibiotics. Mahmood et al. (2013) and Tosi et al. (2007) also found behavioral variations in the susceptibility patterns of \( S. \) thermophilus strains for different antibiotics. These behavioral changes in strains toward different antibiotics are due to the continuous exposure of strain culture to antibiotic-resistant environments (Arias & Murray, 2009; Mathur & Singh, 2005).

Many previous studies also testified the susceptibility of \( S. \) thermophilus to several antibiotics, including E, chloramphenicol, CIP, TE, cephalothin, quinupristin, and reported medium susceptibility to highly resistant to CN, K, and S (Aslim & Beyatli, 2004; Katla et al., 2001; Temmerman et al., 2003; Tosi et al., 2007).

4 | CONCLUSION

Today, the selection of probiotic, natural, EPS-producing strains is gaining importance throughout the world for replacing artificial stabilizers. The present in vitro findings reflected that these three novel EPS-producing strains of \( S. \) thermophilus (RIRT2, RIH4, and RIY), isolated from indigenous Dahi samples fulfill the basic criteria for the selection of probiotics with additional health benefits. Thus, these strains have a potential to be used as a source of bio-stabilizer starter culture for the different probiotic fermented milk products.

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CONFLICT OF INTEREST
The authors declare that they do not have any conflict of interest.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are openly available in [repository name e.g "figshare"] at http://doi.org/ [doi], reference number [reference number].

ORCID
Bai Kumari Sharma Khanal https://orcid.org/0000-0001-8550-4352

REFERENCES
Ali, K., Mehmoon, M. H., Iqbal, M. A., Masud, T., Qazalbash, M., Saleem, S., Ahmed, S., Tariq, M. R., Safdar, W., Nasir, M. A., Saeed, M. T., Muhammad, A., & Sheas, M. N. (2019). Isolation and characterization of exopolysaccharide-producing strains of Lactobacillus bulgaricus from curd. Food Science and Nutrition, 7, 1207–1213.

Arias, C. A., & Murray, B. E. (2009). Antibiotic-resistant bugs in the 21st century – A clinical super-challenge. The New England Journal of Medicine, 360, 439–443. https://doi.org/10.1056/nejmp080465

Aslim, B., & Beyatli, Y. (2004). Antibiotic resistance and plasmid DNA contents of Streptococcus thermophilus strains isolated from Turkish yogurts. Turkish Journal of Veterinary and Animal Sciences, 28, 257–263.

Aslim, B., Oral, D., & Beyatli, Y. (2007). Factors influencing autoaggregation and aggregation of Lactobacillus delbrueckii subsp. bulgaricus isolated from handmade yogurt. Journal of Food Protection, 70, 223–227. https://doi.org/10.4315/0362-028X.JFP-70.1.223

Aswathy, R. G., Ismail, B., John, R. P., & Nampoothiri, K. M. (2008). Evaluation of the probiotic characteristics of newly isolated lactic acid bacteria. Applied Biochemistry Biotechnology, 151, 244–255. https://doi.org/10.1007/s12100-008-8183-6

Behare, P., Singh, R., Tomar, S., Nagpal, R., Kumar, M., & Mohania, D. (2010). Effect of exopolysaccharide-producing strains of Streptococcus thermophilus on technological attributes of fat-free lassi. Journal of Dairy Science, 93, 2874–2879. https://doi.org/10.3168/jds.2009-2300

Bhowmik, D., Dubey, J., & Mehra, S. (2009). Probiotic efficiency of S. platensis stimulating growth of lactic acid bacteria. World Journal of Dairy Science, 4, 160–163.

Boke, H., Aslim, B., & Alp, G. (2010). The role of resistance to bile salts and acid tolerance of exopolysaccharides (EPSs) produced by yoghurt starter bacteria. Archives of Biological Sciences, Belgrade, 62(2), 323–328. https://doi.org/10.2298/ABBS1002329L

Brashears, M. M., Gilliland, S. E., & Buck, L. M. (1998). Bile salt deconjugation and cholesterol removal from media by Lactobacillus casei. Journal of Dairy Science, 81, 2103–2110. https://doi.org/10.3168/jds.S0022-0302(98)75785-6

Buchanan, R. E., & Gibbons, N. E. (1974). Bergey’s manual of determinative bacteriology, 8th edn (1268 pp). Williams and Wilkins.

Canzi, E., Guglielmetti, S., Mora, D., Tamagnini, I., & Parini, C. (2005). Conditions affecting cell surface properties of human intestinal Bifidobacteria. Antonie Leeuwenhoek, 88, 207–219. https://doi.org/10.1007/s10482-005-6501-3

Cerning, J., Bouillanne, C., Landon, M., & Desmazeaud, M. (1990). Comparison of extracellular polysaccharide production by thermophilic lactic acid bacteria. Sei Al Ments, 10, 443–451.

CLSI M100-S21 (2011). Performance standards for antimicrobial susceptibility testing 21st informational supplement. Clinical and Laboratory Standards Institute.

Collado, M. C., Merluquo, J., & Salminen, S. (2008). Adhesion and aggregation properties of probiotic and pathogen strains. European Food Research Technology, 226, 1065–1073. https://doi.org/10.1007/s00217-007-0632-x

Darilmaz, D. O., & Beyatli, Y. (2012). Investigating hydrophobicity and the effect of exopolysaccharide on aggregation properties of dairy Propionibacteria isolated from Turkish homemade cheeses. Journal of Food Protection, 75(2), 359–365. https://doi.org/10.4315/0362-028X.JFP-11-225

De Souza, B. M. S., Borgonovi, T. F., Casarotti, S. N., Todorov, S. D., & Penna, A. L. B. (2019). Lactobacillus casei and Lactobacillus fermentum strains isolated from mozzarella cheese: Probiotic potential, safety, acidifying kinetic parameters and viability under gastrointestinal tract conditions. Probiotics and Antimicrobial Proteins, 11(2), 382–396. https://doi.org/10.1007/s12602-018-9406-y

De Vries, M. C., Vaughan, E. E., Kleerebezem, M., & de Vos, W. M. (2006). Lactobacillus plantarum-Survival, functional and potential probiotic properties in the human intestinal tract. International Dairy Journal, 16(9), 1018–1028. https://doi.org/10.1016/j.idairyj.2005.09.003

De Vuyst, L., Zamfir, M., Mozzi, F., Adriany, T., Marshall, V., Degeest, B., & Vanengelgem, F. (2003). Exopolysaccharides-producing Streptococcus thermophilus strains as functional starter cultures in the production of fermented milks. International Dairy Journal, 13, 707–717. https://doi.org/10.1016/S0959-6946(03)00105-5

Draksler, D., Gonzales, S., & Oliver, G. (2004). Preliminary assays for the development of a probiotic for goats. Reproduction Nutrition Development, 44, 397–405. https://doi.org/10.1016/rind.20040406

Dubois, M., Gilles, K. A., Hamilton, J. K., Peters, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. Analytical Chemistry, 28, 350–356. https://doi.org/10.1021/ac60111a017

FAO, WHO (2006). Probiotics in food: Health and nutritional properties and guidelines for evaluation. FAO, WHO.

Farid, W., Masud, T., Sohail, A., Ahmad, N., Naqi, S. M. S., Khan, S., Ali, A., Khaliﬁ, S. A., Hussain, A., Ali, S., Saghir, M., Siddeeg, A., & Manzoor, M. F. (2021). Gastrointestinal transit tolerance, surface hydrophobicity, and functional attributes of Lactobacillus acidophilus strains isolated from Indigenous Dahi. Food Science and Nutrition, 9(9), 5092–5102.

Feldmane, M., Semjonovs, P., & Ciprovica, I. (2013). Potential of exopolysaccharides in yoghurt production. International Journal of Nutrition and Food Engineering, 7(8), 767–770.

Fontaine, L., & Hols, P. (2008). The Inhibitory Spectrum of Thermophilin 9 from Streptococcus thermophilus LMD-9 Depends on the Production of multiple peptides and the Activity of BlpGSt, a Thiol-Disulﬁde Oxidase. Applied and Environmental Microbiology, 74(4), 1102–1110.

Gamar, L., Blondeau, K., & Simonet, J. M. (1997). Physiological approach to extracellular polysaccharide production by Lactobacillus rhamnosus strain C83. Journal of Applied Microbiology, 83, 281–287.

Gomez, I. (2006). Characterization of the exopolysaccharides produced by shalopholic microorganisms belonging to the genera Halomonas, Alteromonas, Idiomarina, Palleronia and Salipiger. 20 f. Thesis (Doctoral)-University of Granada, grenade, Spain.

Grajek, K., Sip, A., Foksowicz-Flaczyk, J., Dobrowolska, A., & Wita, A. (2019). Transfer of plasmid-mediated resistance to extracellular polysaccharide production by Lactobacillus casei strain C83. Probiotics and Antimicrobial Proteins, 11(2), 382–396. https://doi.org/10.1007/s12602-018-9406-y

Guglielmetti, E., Guglielmetti, S., Majone, C., Morelli, L., & Wright, A. V. (2009). Transfer of plasmid-mediated resistance to...
tetracycline in pathogenic bacteria from fish and aquaculture environments. *FEMS Microbiology Letters*, 293(1), 28–34. https://doi.org/10.1111/j.1574-6968.2009.01512.x

Haller, D., Colbus, H., Ganzel, M. G., Scherenbacher, P., Bode, C., & Hammes, W. P. (2001). Metabolic and functional properties of lactic acid bacteria in the gastro-intestinal ecosystem: A comparative in vitro study between bacteria of intestinal and fermented food origin. *Systematic Applied Microbiology*, 24, 218–226. https://doi.org/10.1078/0723-2020-00023

Han, X., Yang, Z., Jing, X., Yu, P., Zhang, Y., Yi, H., & Zhang, L. (2016). Improvement of the texture of yogurt by use of exopolysaccharide producing lactic acid bacteria. *BioMed Research International*, 2016, 1–6. https://doi.org/10.1155/2016/7945675

Hassanzadazad, H., Ehsani, A., Mardani, K., & Hesari, J. (2012). Investigation of antibacterial, acid and bile tolerance properties of *Lactobacilli* isolated from Koozeh cheese. *Veterinary Research Forum*, 3(3), 181–185.

Iyer, R., Tomar, S. K., Kapila, S., Mani, J., & Singh, R. (2010). Probiotic properties of lactic acid bacteria isolated from Algerian raw camel milk. *Veterinary Research Forum*, 32, 103–110. https://doi.org/10.1016/j.j.foodres.2009.09.011

Jose, N. M., Bunt, C. R., & Hussain, M. A. (2015). Comparison of microbiological and probiotic characteristics of *Lactobacillus* isolates from dairy food products and animal rumen contents. *Microorganisms*, 3(2), 198–212. https://doi.org/10.3390/microorganisms3020198

Katia, A. K., Kruse, H., Jhonsen, G., & Herikstad, H. (2001). Antimicrobial susceptibility of starter culture bacteria used in Norwegian dairy products. *International Journal of Food Microbiology*, 67, 147–152. https://doi.org/10.1016/S0168-1605(00)00522-5

Kaushik, J. K., Kumar, A., Duary, R. K., Mohanty, A. K., Grover, S., & Batish, V. K. (2009). Functional and probiotic attributes of an indigenous isolate of *Lactobacillus plantarum*. *PLoS One*, 4(12), 8099. https://doi.org/10.1371/journal.pone.0008099

Khalli, R. (2009). Evidence for probiotic potential of a capsular-producing *Streptococcus thermophilus* CHCC 3534 strain. *Polish Journal of Microbiology*, 58(1), 49–55.

Koll, P., Mandar, R., Smidt, I., Hutt, P., Trussalu, K., Mikelsaar, R. H., Shchepetova, J., Krogh-Andersen, K., Marcotte, H., Hammarstrom, L., & Mikelsaar, M. (2010). Screening and evaluation of human indigenous isolate of *Lactobacillus acidophilus* LbCHCC 3534 strain. *Veterinary Research Forum*, 3(3), 28–34. https://doi.org/10.1016/j.jfoodmicro.2005.03.008

Maurad, K., & Meriem, K. (2008). Probiotic characteristics of *Lactobacillus plantarum* strains from traditional butter made from camel milk in arid regions (Sahara) of Algeria. *Grasas Y Aceites*, 59, 210–224.

Mlijkovic, M., Strahanic, I., Tolinacki, M., Zivkovic, M., Kojic, S., Golic, N., & Kojic, M. (2015). AggLb is the largest cell-aggregation factor from *Lactobacillus paracasei* subsp. *paracasei* BGNJ1-64, functions in collagen adhesion, and pathogen exclusion in vitro. *PLoS One*, 10, e0126387. https://doi.org/10.1371/journal.pone.0126387

Monteagudo-Mera, A., Rastall, R. A., Gibson, G. R., Charalampopoulos, D., & Chatzifragkou, A. (2019). Adhesion mechanisms mediated by probiotics and prebiotics and their potential impact on human health. *Applied Microbiology and Biotechnology*, 103(16), 6463–6472. https://doi.org/10.1007/s00253-019-09978-7

Mosteafoui, A., Hakem, A., Yabirib, B., Boutaiba, S., & Badis, A. (2014). Screening for exopolysaccharide producing strains of thermophilic lactic acid bacteria isolated from Algerian raw camel milk. *African Journal of Microbiology Research*, 8(22), 2208–2214. https://doi.org/10.5987/AJMR2014.6759

Mozzí, F., Gerbino, E., de Valdez, G. F., & Torino, M. I. (2009). Functionality of exopolysaccharides produced by lactic acid bacteria in in vitro gastric system. *Journal of Applied Microbiology*, 107, 56–64.

Muigai, S. C., Shitandi, A., Muliro, P., & Bitonga, O. R. (2013). Production of exopolysaccharides in the Kenyan fermented milk, Mursik. *International Journal of Science and Research*, 2(12), 79–89.

Nagpal, R., Kumar, A., Behare, P. V., Jain, S., & Yadav, H. (2012). Probiotics, their health benefits and applications for developing healthier foods: A review. *FEMS Microbiology Letters*, 334(1), 1–15. https://doi.org/10.1111/j.1574-6968.2012.02593.x

Neu, H. C. (1992). The crisis in antibiotic resistance. *Science*, 257, 1064–1078. https://doi.org/10.1126/science.257.5073.1064

Paulo, E. M., Vasconcelos, M. P., Oliveira, I. S., Affe, H. M., Nascimento, R., de Melo, I. S., Roque, M. R., & de Assis, S. A. (2012). An alternative method for screening lactic acid bacteria for the production of exopolysaccharides with rapid confirmation. *Food Science and Technology (Campinas)*, 32(4), 710–714. https://doi.org/10.1590/S0101-20612012005000094

Pisano, M. B., Viale, S., Conti, S., Fadda, M. E., Deplano, M., Melis, M. P., Deiana, M., & Cosentino, S. (2014). Preliminary evaluation of probiotic properties of *Lactobacillus* strains isolated from Sardinian dairy products. *Biomed Research International*, 2014, 1–9.

Quigley, L., O’Sullivan, O., Stanton, C., Tom, P., Beresford, R., Ross, P., Gerald, F., Paul, F., & Cotter, D. (2013). The complex microbiota of raw milk. *FEMS Microbiology Reviews*, 37(5), 664–698. https://doi.org/10.1111/1574-6976.12030

Rahman, M. M., Kim, W. S., Kumura, H., & Shimazaki, K. (2008). Auto-aggregation and surface hydrophobicity of *Bifidobacteria*. *World Journal of Microbial Biotechnology*, 24, 1593–1598. https://doi.org/10.1007/s11274-007-9650-x

Ricciardi, A., Parente, E., & Clementi, F. (1997). Exopolysaccharide production in a whey based medium by *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* in pure culture and in association. *Annual Microbiology Enzymes*, 47, 213–222.

Rimada, P. S., & Abraham, A. G. (2003). Comparative study of different methodologies to determine the exopolysaccharide produced by kefir grains in milk and whey. *Le Lait*, 83, 79–87.

Rusas-Madiedo, P., & de Los Reys-Gavilan, C. G. (2005). Methods for the screening, isolation and characterization of exopolysaccharides produced by lactic acid bacteria. *Journal of Dairy Science*, 88, 843–856.
