



## Original article

Probiotic characteristics of *Lactobacillus* strains isolated from cheese and their antibacterial properties against gastrointestinal tract pathogens

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## ABSTRACT

In the present study, four *Lactobacillus* strains from the cheese were analyzed for its probiotic potential against enteropathogenic bacteria. The probiotic properties of the selected strains were also analyzed and the selected bacterial strains showed high tolerance in bile salts and organic acid. The strain *L. plantarum* LP049 showed maximum survival rate ( $92 \pm 4.2\%$  and  $93.3 \pm 2\%$ ) after 3 h of treatment at 0.25% (w/v) bile salts and 0.25% (w/v) organic acid concentrations. The ability of the *Lactobacillus* strains to adhere to human epithelial cells (HT-29 cell lines) was evaluated and *L. plantarum* LP049 showed maximum adhesion property ( $19.2 \pm 1.1\%$ ) than other tested strains. The *Lactobacillus* strains produced lactic acid at various concentrations. Compared with other strains, maximum level of lactic acid (3.1 g/L), hydrogen peroxide (4.31 mM) and bacteriocin (31 AU/mg) was detected in LB049. The inhibitory activity of culture supernatant against various bacterial pathogens was observed. The zone of inhibition ranged between  $6 \pm 2$  mm and  $23 \pm 2$  mm. The cell free extract showed activity against, *Escherichia coli* (ATCC 10536), *Salmonella enteritidis* (ATCC 13076), *Shigella flexneri* (ATCC 29903), and *Enterococcus faecium* (ATCC 8459). Consequently, *L. plantarum* LP049 may be considered as a potential candidate for the production of novel bioactive metabolites for therapeutic and bio-protective applications.

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## 1. Introduction

Probiotics are live bacteria or other micro-organisms, that when ingested in suitable quantities confer numerous health benefits on the host organisms (Aarti et al., 2016; Al-Dhabi et al., 2020; Nagpal et al., 2012). Lactic acid bacteria (LAB) are Gram-positive, which are naturally available in the gastrointestinal tract of human and ruminants (Aarti et al., 2018). These LAB play significant role in maintaining the balance of microbial flora (Aarti et al., 2017; Kirtzalidou et al., 2011; Arasu et al., 2014a, 2016; George Kerry et al., 2018). Many of probiotic bacteria that effectively colonize

the gut of neonates have various origins and may be affected by many factors such as contact with hospital staff, parents, antibiotic treatment, feeding pattern, and type of delivery (Savino et al., 2011). In developing and under developing countries, diarrhea is one of the important diseases causing mortality and morbidity among infants (Lanata et al., 2013). The microorganisms such as, *Vibrio cholera*, *Campylobacter*, *Escherichia coli*, and Rotavirus cause diarrhea (Lanata et al., 2013). Probiotic bacteria effectively resist the growth and activities of various enteropathogenic microorganisms (Arasu et al., 2014b, 2014c; Fijan et al., 2018). Probiotics from the genus *Lactobacillus* are widely reported for their beneficial properties as adjunctive/preventive therapy for highly infectious diarrhea in the pediatric group (Arasu et al., 2014b; Delcaru et al., 2016). Some of the LAB produce bacteriocins and these substances are mainly considered as GRAS (generally recognized as safe) substances. In a study, Miao et al. (2014) characterized a novel bacteriocin F1 producing *Lactobacillus paracasei* strain FX-6 isolated from Tibetan kefir with 18 amino acids and the molecular weight was 2112.842 Da (Miao et al., 2016a, 2016b). Probiotics are highly effective in regulating adipocytes in both animal and human

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subjects (Kadooka et al., 2010). Also, probiotic food showed functional properties related to anti-obesity (Cui et al., 2015). The anti-obesity characteristics of fermented food may be attributed to various bioactive compounds and the LAB in fermented food (Park et al., 2012). *Lactobacillus* also enhanced immune organ indexes, SIgA secretion level and spleen lymphocyte transformation rate in experimental animal (Ren et al., 2015). It has been reported that the probiotic bacterium, *Lactobacillus acidophilus* could gently regulate the intestinal microbial flora of experimental mice with intestinal dysfunction and provide a protective response of immune system, which could be effectively replace synthetic antibiotics for broilers (Sun et al., 2015). Hence, probiotic bacteria have lot of potential in animal husbandry as an effective alternative to commercial antibiotics. However, it could be noted that the bacteria should have the ability to withstand various adverse climatic conditions such as resist to digestive juices, various proteases, bile salt and should tolerant to gastric acid (Erkkilä and Petäjä, 2000). The probiotic organisms should have potent colonization ability, so that rapid adhere to the intestinal mucosa is possible (Valan Arasu et al., 2013). Also, the selected probiotic organisms should not have any side effects and must be safe for host organisms (Saxami et al., 2012). LAB produce various antimicrobial agents. The synthesized metabolites showed an acidic pH in the gastrointestinal tract to facilitate the antagonistic properties against various acid sensitive bacterial and viral pathogens by affecting with the cell membrane. Probiotic bacteria showed activity against various Gram-negative and Gram-positive bacteria, fungi and yeast (Arasu et al., 2014b; Valan Arasu et al., 2014). *Lactobacilli* also showed protective activity in food-borne illness and in food preservation (Valan Arasu et al., 2015, 2013). In recent years an attempt has been made to analyze the health benefits of host organisms by improving the balance of gut microbial flora, reducing cholesterol, vitamin production providing immunomodulatory effects and regulating lactase activity and various metabolic processes. The bacteria from the genera, *Bifidobacterium* and *Lactobacillus* are widely reported probiotic culture, however the genera such as, *Saccharomyces*, *Enterococcus*, *Streptococcus* and *Lactococcus* are commonly used as probiotics (García-Ruiz et al., 2014; Ilavenil et al., 2015a; Valan Arasu et al., 2013b). The potential health benefits of probiotics on host health namely, production of anti-microbial metabolites and synthesis of B-group vitamins, anti-obesity effect (Kim et al., 2018), anti-diabetic effect, cholesterol-lowering effect and oxidative stress down-regulation (Yadav et al., 2018) have been reported previously. However, antibacterial potential and other characteristics vary from strains to strains. Hence, many studies are underway to evaluate probiotic characters of novel bacterial strain. The present study aimed to analyze the probiotic properties of *Lactobacillus* species isolated from the cheese to evaluate the antimicrobial property against various enteropathogenic bacteria. Fig. 1.

## 2. Materials and methods

### 2.1. Characterization of *Lactobacillus* isolates from cheese

This experiment was performed between July 2019 and May 2020. A total of 130 samples were collected from cheese. The collected samples were transferred to the laboratory within 4 h and cultured using MRS broth for 72 h at 37 °C. Then, the samples were cultured in MRS agar and incubated for 48 h at 37 °C. The isolated bacterial strains were tested based on their glucose-fermentation property, catalytic property, morphological characters and Gram's staining. The isolated organisms were incubated at various temperatures (30 °C, 35 °C and 40 °C) (Davoodabadi et al., 2015b; Kiliç and Karahan, 2010). The screened *Lactobacillus* species were

stored in MRS agar slants and the broth was suspended in glycerol (20%, v/v) and stored at – 80 °C. The identification of the tested isolates was performed by biochemical tests and 16S rDNA sequence analysis. Chromosomal DNA of the selected bacteria was extracted and purified as suggested earlier (Heilig et al., 2002). A forward primer (5'-AGAGTTTGATCCTGGCTCAG-3') and a reverse primer (5'-CCGTC AATTCCTTTGAGTTT-3') were used for the amplification of *Lactobacillus* 16S rRNA gene. The PCR reaction was carried out in an ppendorf thermal cycler PCR system (Germany) and the master mix was performed as suggested by the manufacturer (Ampliqon, Denmark). Millipore water has been used until otherwise stated. The amplified fragments were sequenced using Applied Biosystems, according to the instructions of the manufacturers. The sequences were compared and the submitted in GenBank data base

### 2.2. Characteristic feature of probiotics

#### 2.2.1. Acid and bile tolerance tests

The bile and acid tolerance of bacterial strains were analyzed as described earlier in literatures with little modifications (Donatien et al., 2012; Klingberg et al., 2005). Briefly, the acid resistance of the organisms were examined in MRS broth (Himedia, Mumbai, India) adjusted the pH to 2.5 using 1 N HCl. About 0.2 mL of 18 h culture of *Lactobacillus* isolates ( $10^6$  CFU/ml) was inoculated into 25 mL of MRS liquid medium and the pH was adjusted earlier. After 18 h incubation, the viable count of bacterial strain was performed using plating technique on MRS agar. The survival rate (%) of the bacterial strain was analyzed using plate count method on MRS agar, after incubation of 0 and 2 h. *Lactobacillus* isolates that grown in the acid medium were subjected to bile tolerance assay. *Lactobacillus* strains were inoculated in Erlenmeyer flask containing 50-mL ( $10^6$  CFU/ml) of 18 h grown culture was inoculated on 50 mL of MRS liquid medium with 0.3% (w/v) oxgall bile salt (Sigma, USA). To the control, oxgall bile salt was not added.

$$(N/NO) \times 100\%.$$

#### 2.2.2. *Lactobacillus* adhesion assay

The ability of the *Lactobacillus* strains to adhere to human epithelial cells was evaluated according to the method of Verdenelli et al. (2009). The percentage of adhesion of *Lactobacillus* strains was evaluated by comparison of adhered cells and total cells determined in bacterial suspension. Adhesion assay was performed in triplicates and an average value was considered for statistical analysis.

### 2.3. Antibacterial assay

The antibacterial activity of the selected *Lactobacillus* species against enteropathogenic bacterial strain was evaluated as described by Davoodabadi et al. (2015a) by well diffusion method. The pathogenic bacterial strains such as, *Escherichia coli* (ATCC 10536), *Salmonella enteritidis* (ATCC 13076), *Shigella flexneri* (ATCC 29903), and *Enterococcus faecium* (ATCC 8459) were inoculated in liquid medium (Nutrient broth) (Himedia, Mumbai, India) and cultured for overnight.

### 2.4. Antimicrobial agent characterization

In this study, the nature of the substances produced by *Lactobacillus* species responsible for antimicrobial activity was tested. The antimicrobial agents generally synthesized by *Lactobacillus* strains such as, bacteriocin, hydrogen peroxide and organic acid were determined as described previously by Shokryazdan et al. (2014). *Lactobacillus* strains were cultured in 50 mL MRS medium and centrifuged the sample for 10 min at  $5000 \times g$  at 4 °C. The cell free extract was used as the sample for all experiments. For the determination of bacteriocin, the culture supernatant (10 mL) was treated

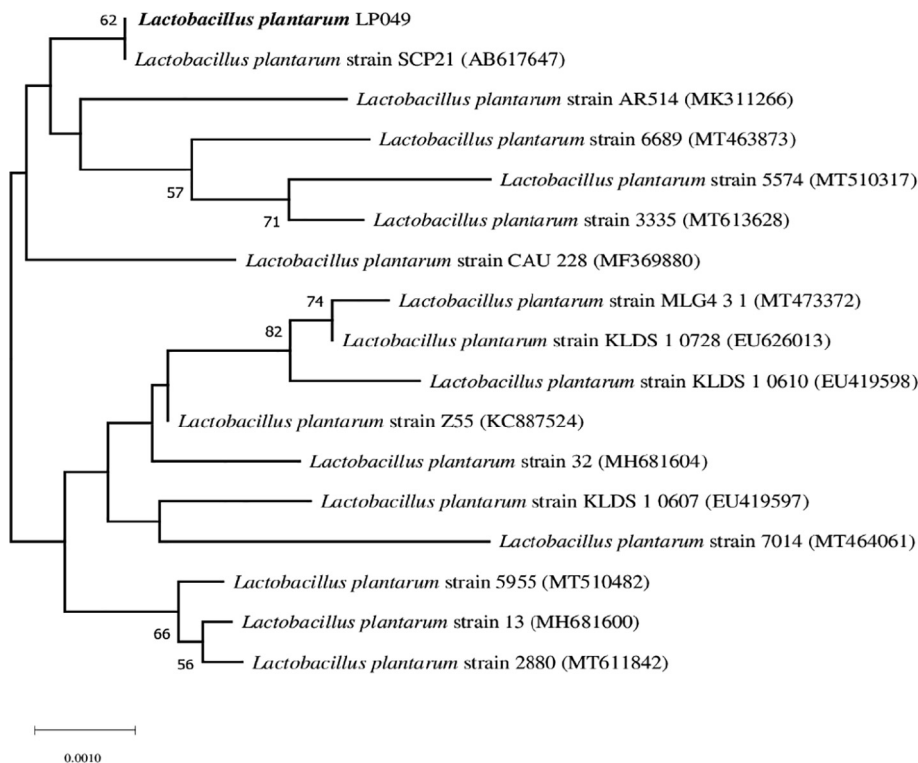


Fig. 1. Phylogenetic relation of *L. plantarum* LP049 with other relevant strains.

with 2 mg/mL trypsin (Sigma, USA). For the analysis of organic acids, the pH of the culture supernatant (10 mL) was adjusted to 6.5 using 1 N NaOH, and for the determination of hydrogen peroxide, the cell free sample (10 mL) was incubated with 1 mg/mL catalase. The cell free extract was sterilized using filtration (0.22 μm) and 50 μL sample was loaded into wells made in MRS agar plates, which was overlaid with soft nutrient agar (10 mL) (Himedia, Mumbai, India), and further inoculated with test pathogens.

### 2.5. Antimicrobial properties of *L. plantarum* strains

In this study, antibacterial potential of *Lactobacillus* species was evaluated by well diffusion method on MHA plates as described earlier by Valan Arasu et al. (2013a). The isolated Lactobacilli were cultured in MRS liquid medium at pH 6.5 and incubated for 24. After incubation period, antimicrobial properties were tested from the cell free supernatant. The cultures were centrifuged at 5000 × g for 10 min at 4 °C and the extract was sterilized using 0.22-μm hydrophilic Durapore PVDF membrane. The filtered sample was used for the determination antibacterial activity. To analyze the chemical nature of the inhibitory compound synthesized by *Lactobacillus* species, the filtered sample was subjected to various treatments as suggested by Herreros et al. (2005) with little modifications. The culture supernatant was heated for 5 min at 100 °C in the presence of 1 N NaOH and the pH value was attained to 6.5. To the supernatant pepsin was added in order to evaluate the role of antimicrobial peptides on antibacterial activity. Proteinase K and pepsin were added at the concentrations of 1 mg/mL.

## 3. Results and discussion

### 3.1. Identification and characterization of *Lactobacillus* species isolated from cheese

A total of 18 bacteria were characterized as *Lactobacillus* strains by various phenotypic tests and Gram's staining. The tested bacte-

rial strains were catalytic negative and all are Gram's positive bacterial strains. In this study, *Lactobacillus* species from five different species were identified. Among the species, five strains were characterized from *Lactobacillus plantarum* (Table 1). All nineteen bacterial strains were initially selected for acid tolerance, bile tolerance and adhesion assay. Later, potent strains were identified based on morphological characters and 16S rDNA gene sequencing. *Lactobacillus* species are probiotic organisms which help to balance the gastrointestinal microbial flora, destroy pathogenic microorganisms and produce various nutrients (Reid et al., 2003). Gastrointestinal diseases are mainly associated with maintaining microbial balance with the gastrointestinal tract (Dalcenserie et al., 2008). Davoodabadi et al. (2015a) reported that *Lactobacillus* inhibit in the gastrointestinal of human and play very critical role in the balance of microbial consortium in the intestine. The selection of probiotic *Lactobacillus* was mainly based on antibiotic activity against bacterial pathogens, survival ability in the gastrointestinal tract, antibiotic resistant, ability to adhere to the human intestinal cells and tolerance to bile salts and acidic pHs (Arasu and Al-Dhabi, 2017; Ilavenil et al., 2015b; Plessas et al., 2017)

### 3.2. Acid and bile tolerance ability of *Lactobacillus* species

The viability of the selected eighteen isolates was tested against acid (pH 2.5) and oxgall bile (0.25%) and the results were tabulated

Table 1  
*Lactobacillus* species isolated from the cheese and their numbers.

Bacterial strains	Strain number and number of strains
<i>L. plantarum</i>	Five strains, LP032, LP037, LP041, LP049 and LP058
<i>L. brevis</i>	Four strains, LB062, LB067, LB068 and LB0112
<i>L. fermentum</i> ,	Two strains, LF06 and LF089
<i>L. rhamnosus</i>	Three strains, LR013, LR019, LR087 and LR093
<i>L. furfuricola</i>	Three strains, LF038, LF073 and LF0162
<i>L. paracasei</i>	One strain, LP01

in Table 2. Among the *Lactobacillus* strains, *L. plantarum* LP049 showed potent tolerance against acid and bile salts (0.25%) and the survival rate was  $92 \pm 4.2\%$  and  $93.3 \pm 2$ , respectively. All isolated *Lactobacillus* strains were examined for bile and acid tolerance in the cell viabilities after 3 h of treatment showed variation in pH tolerance. At low pH, loss of bacterial population was registered because of loss of viable bacterial cells in the medium. It was previously reported that hydrochloric acid severely affect the biomolecules of cells, DNA, proteins and fatty acids (Sahadeva et al., 2011). At low pH, metabolism, viability of *Lactobacilli* and growth were significantly affected. Earlier studies also showed that exposure of *Lactobacilli* to gastric acid for 3 h caused marked reduction in the bacterial population (Mandal et al., 2006; Sahadeva et al., 2011). According to Sahadeva et al. (2011) and Prasad et al. (1998), the threshold limit to describe acid tolerance for gastrointestinal bacterial isolate was set between pH 2.0 and 3.0 for about 3 h incubation, as it generally stimulates microbial residency in the stomach of animals or humans. In a study, Liong and Shah (2005) described pH 3.0 as a standard value for the determination of pH tolerance test for any probiotic strains. The present finding revealed moderate inhibition at this pH indicated potential probiotic properties. The ability of tolerance on bile salt was evaluated in this study. *P. plantarum* LP049 showed maximum ( $93.3 \pm 2.8\%$ ) bile tolerant rate followed by *L. brevis* LB0112 ( $90 \pm 4.3\%$ ). Bile salt affect cellular homeostasis that cause the dissociation of lipid bilayer, affect integral protein of their cell membranes, leading in the leakage of intracellular content and finally death of cell (Mandal et al., 2006). There were various *Lactobacillus* strains with probiotic properties, thus they have potential applications in the prevention and treatment of various diseases. Their inhibitory

properties were mainly due to the production of deacetyl,  $H_2O_2$  and bacteriocins (Dave and Shah, 1998).

### 3.3. Adhesion assay

The selected *Lactobacillus* strains were subjected to HT-29 cell adhesion analysis. The strains showed varying adhesion properties and among all strains, *L. plantarum* LP049 showed maximum adhesion property ( $19.2 \pm 1.1\%$ ). The other bacterial strains, *L. brevis* LB062 and *L. brevis* LB068 showed  $18.3 \pm 1.5$  and  $17.2 \pm 1.1\%$  adhesion power, respectively (Fig. 2). The other tested *Lactobacillus* strains showed low and moderate adhesive properties. Probiotic bacteria have the ability to adhere intestinal epithelial cells and the selection of any bacterial strain based on adhesion properties. Adhesion properties of *Lactobacillus* strains have been described previously by Ren et al. (2014) and Davoodabadi et al. (2015b). The present finding is consistent with the finding of Halimi and Mirsalehian (2016). Also variation of adhesion level has also been reported previously by Shokryazdan et al. (2014). Bacteria from the genus *Lactobacillus* have adhesion abilities and this property is related to surface proteins. In bacteria various components from their cell surface involved in adherence to the epithelial cells, including, proteins, polysaccharides and (lipo) teichoic acids. Previously trypsin treated *Lactobacillus* strains showed least adhesive potentials indicated surface proteins have major role in bacterial adhesion (Glenting et al., 2013; Jensen et al., 2014). In some experiments revealed adhesive properties after trypsin treatment, indicated the role of non-proteinaceous compounds and non-surface proteins in the adhesion of *Lactobacillus* species (Polak-Berecka et al., 2014; Yadav et al., 2018). Also moonlighting proteins reported in *Lactobacillus* species have various functions due to multiple proteolytic fragments, splice variants and gene fusions. These proteins have been reported in various *Lactobacillus* species (Kinoshita et al., 2016). The surface proteins of *Lactobacilli* have the ability to adhere with the host cell. In a study Glenting et al. (2013) observed removal of various surface proteins from *Lactobacilli* after trypsin treatment and these confirmed the use of adhesive proteins on adhesion.

### 3.4. Characterization of antimicrobial agents

The *Lactobacillus* strains produced lactic acid at various concentrations. Compared with other strains, maximum level of lactic acid (3.1 g/L) was detected with strain LB049. The other strains, LB068, LB062 and LF06 showed 2.92 g/L, 2.65 g/L and 1.08 g/L, respectively (Fig. 3). *Lactobacilli* such as, *L. plantarum* produced organic acids reduce the pH of the environment (Ođdak et al., 2017). Li et al. (2016) reported that most of the *L. plantarum* produce various organic acids and these were useful to preserve fermented food. The antibacterial mechanism of organic acids varied widely. The previous findings showed that various kinds of *Lactobacilli* produce various types of organic acids, and some of the *Lactobacilli* produce more quantities of acidic acid than lactic acid (Rowland et al., 2010). This antimicrobial phenomenon clearly indicated that antimicrobial property of the selected bacterial strain is mainly due to the secretion of organic acids. *Lactobacilli* showed antibacterial activity mainly because of the production of lactic acid. However, other acids such as, citric acid and tartaric acid were also reported previously (Zalán et al., 2010). Hydrogen peroxide production improved along with the growth of the organisms. Hydrogen peroxide production was maximum and detected 4.31 mM in medium inoculated with the probiotic strain LB049. The other strains, LB062, LB068, and LF06 showed 3.87 mM,

**Table 2**  
Tolerance of isolated *Lactobacillus* strains at pH 3.0.

<i>Lactobacillus</i> strains	Acid resistant survival rate (%)	Oxgall bile tolerance rate (%)
<i>L. plantarum</i> LP032	$89 \pm 2.5$	$75 \pm 1.3$
<i>L. plantarum</i> LP037	$62 \pm 1.3$	$82 \pm 1.1$
<i>L. plantarum</i> LP041	$43 \pm 0.7$	$59 \pm 3.4$
<i>L. plantarum</i> LP058	$87 \pm 3.8$	$65 \pm 1.4$
<i>P. plantarum</i> LP049	$92 \pm 4.2$	$93.3 \pm 2.8$
<i>L. brevis</i> LB062	$90 \pm 1.38$	$85 \pm 1.6$
<i>L. brevis</i> LB067	$52 \pm 2.3$	$49 \pm 1.3$
<i>L. brevis</i> LB068	$35 \pm 1.8$	$53 \pm 1.8$
<i>L. brevis</i> LB0112	$86 \pm 4.2$	$90 \pm 4.3$
<i>L. fermentum</i> LF06	$48 \pm 3.3$	$59 \pm 3.2$
<i>L. fermentum</i> LF089	$59 \pm 2.2$	$62.3 \pm 2.7$
<i>L. rhamnosus</i> LR013	$57 \pm 1.1$	$59.5 \pm 2.2$
<i>L. rhamnosus</i> LR019	$40 \pm 2.0$	$45.5 \pm 1.9$
<i>L. rhamnosus</i> LR087	$67 \pm 3.2$	$67.5 \pm 2.8$
<i>L. rhamnosus</i> LR093	$84 \pm 0.0$	$58 \pm 2.7$
<i>L. furfuricola</i> LF038	$29 \pm 4.3$	$33 \pm 0.08$
<i>L. furfuricola</i> LF073	$19 \pm 1.2$	$13.3 \pm 0.0$
<i>L. furfuricola</i> LF0162	$4 \pm 2.5$	$5.1 \pm 1.03$
<i>L. paracasei</i> LP01	$59 \pm 2.8$	$60.2 \pm 1.05$



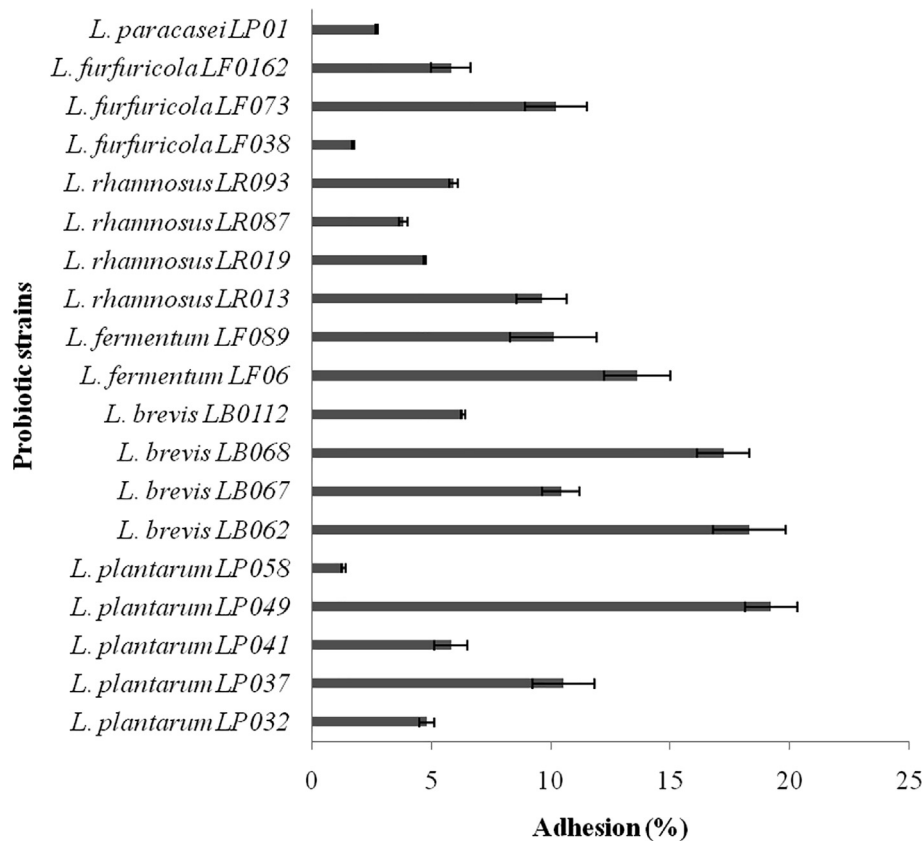


Fig. 2. Lactobacilli strains and their adhesion property.

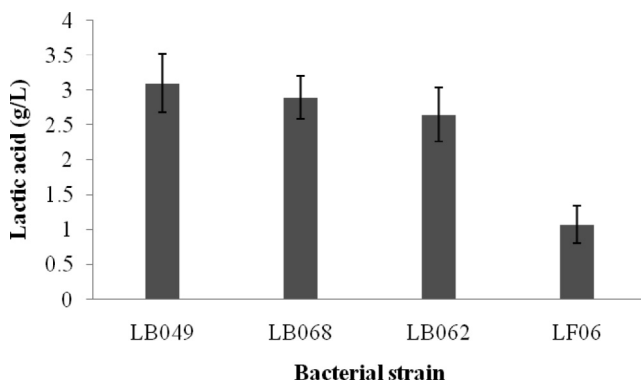


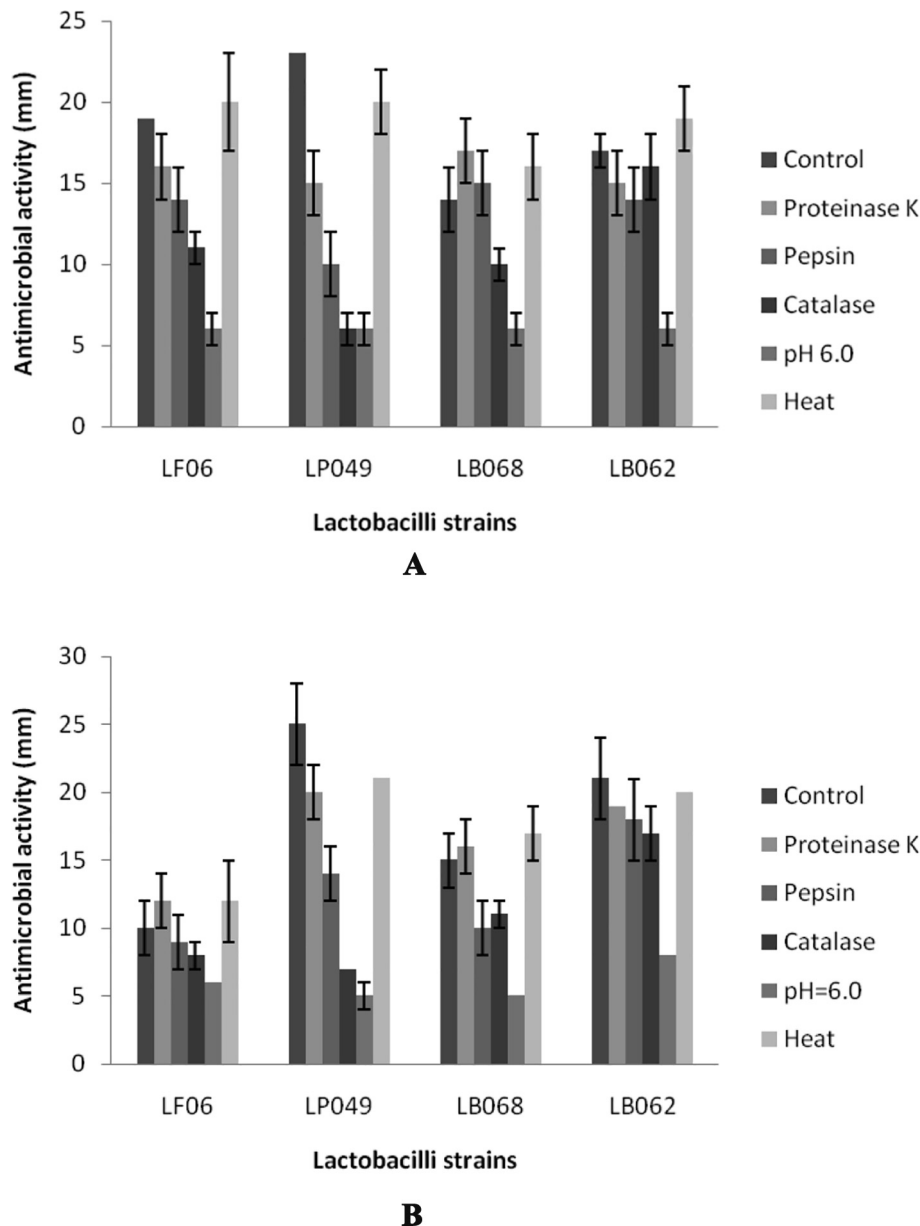
Fig. 3. Lactic acid production by the bacterial strain isolated from the cheese.

2.14 mM and 0.98 mM, H<sub>2</sub>O<sub>2</sub> respectively. Hydrogen peroxide production and antimicrobial activity were positively correlated. In *Lactobacillus crispatus* cell free extract total H<sub>2</sub>O<sub>2</sub> level reached maximum (3.29 mM) under optimized agitation condition. This H<sub>2</sub>O<sub>2</sub> producing property by *Lactobacillus* has been described previously by various researchers. Yap and Gilliland (2000) reported hydrogen peroxide producing ability by the strain, *Lactobacillus delbrueckii* subsp. lactis. Martín et al. (2005) characterized hydrogen peroxide-producing strain, *Lactobacillus coryniformis* which was isolated from milk cheese. All selected Lactobacilli strains showed bacteriocin in the cell free extract. The specific activity of bacteriocin was 31 AU/mg, 29.3 AU/mg, 11.8 AU/mg, and 3.9 AU/mg, respectively for the *Lactobacillus* strains, LB049, LB062,

LB068, and LF06. Bacteriocins producing Lactobacilli has been identified by various researchers. In a study, Ghanbari et al. (2013) isolated bacteriocins producing lactobacilli strains from Sturgeon fish. In another study, Mohammadi et al. (2018) characterized bacteriocin from the cell free extract of *Lactobacillus* spp. isolated from mother’s milk.

### 3.5. Antibacterial activity of *Lactobacillus* sp.

The inhibitory activity of culture supernatant was observed and the zone of diameter ranged between 6 ± 2 mm and 23 ± 2 mm. Four bacterial pathogens were selected to determine antimicrobial potential (Fig. 4a-d). The crude sample showed activity against, *Escherichia coli* (ATCC 10536), *Salmonella enteritidis* (ATCC 13076), *Shigella flexneri* (ATCC 29903), and *Enterococcus faecium* (ATCC 8459). Antibacterial activity varied among the selected bacterial species. As described in the Fig. 4, pepsin and catalase treated samples did not vary much than control experiments. At increased pH of the sample, (pH 6.0) antibacterial activity suddenly decreased than other treatments. Lactobacilli are Gram-positive, nonspore forming, fermenting various sugars into organic acids. This low pH value generally affected the growth of other group of bacteria. In some studies, it was reported the production of H<sub>2</sub>O<sub>2</sub> during metabolic process critically inhibit other pathogenic bacterial growth (Charlier et al., 2009; Ilavenil et al., 2016). Some lactobacilli produce bacteriocin-like substances or bacteriocins to affect the growth of pathogens (Zhao et al., 2016). These kinds of lactobacilli have the ability to affect the growth of types of pathogenic bacteria and widely used for the preservation of food. Zhao et al. (2016) isolated antimicrobial compounds produced by *L. plantarum* JLA-9



**Fig. 4.** Antibacterial activity of fermentation broth of four *Lactobacillus* strains after subjected to various treatments. Control sample: Filtered, unheated sample; Proteinase K: Samples treated with proteinase K at 1 mg/mL concentration; Pepsin: Samples treated with Pepsin at 1 mg/mL concentration; Catalase: Sample treated with catalase at 1 mg/mL concentration; pH: Samples adjusted the pH to 6.0; Heat: Sample treated at 100 °C for 5 min. A: *Escherichia coli* (ATCC 10536), B: *Salmonella enteritidis* (ATCC 13076), C: *Shigella flexneri* (ATCC 29903), and D: *Enterococcus faecium* (ATCC 8459).

which showed potential activity against *Bacillus* spp. have been used in various commercial applications. Some of the *Lactobacillus* species produce phenyllactic acid, a natural food preservative (Valerio et al., 2004; Vijayakumar et al., 2015).

#### 4. Conclusions

The present results revealed that, the selected strains were highly tolerant to various in vitro gastrointestinal conditions and increased survival rate. The *Lactobacillus* strains possessed a good antibacterial activity against various gastrointestinal bacterial pathogens. These bacterial strains also showed high quantities of hydrogen peroxide, lactic acid and bacteriocins. Therefore, all *Lactobacilli*, especially, *L. plantarum* LP049 could be used as a novel

probiotic strain for feed, food and various technological applications.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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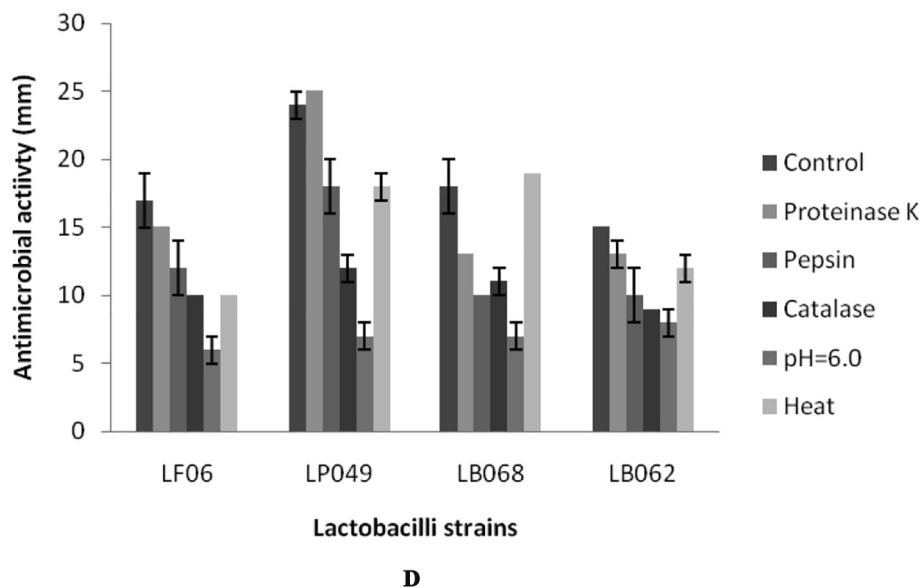
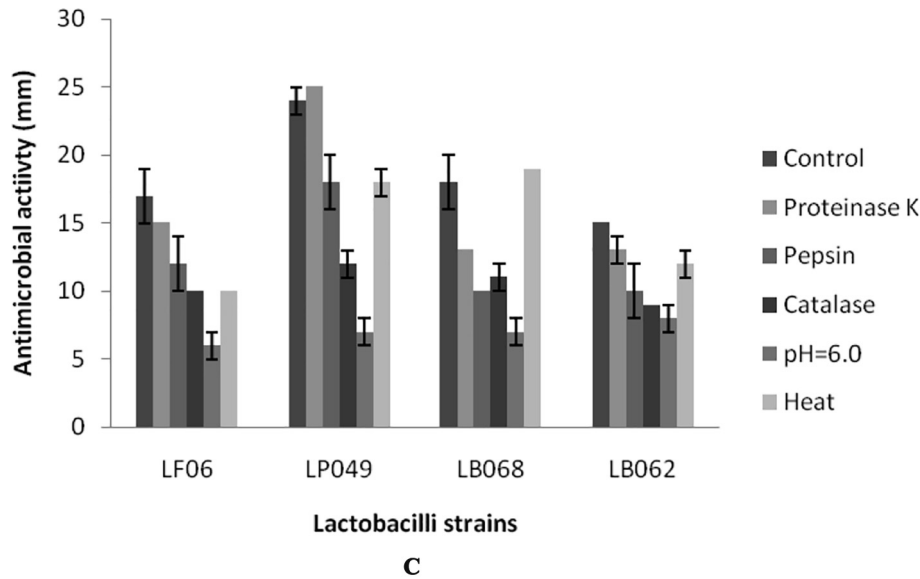


Fig. 4 (continued)

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