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# Probiotic Properties of Lactic Acid Bacteria Isolated from Traditional Yoghurt (Dahi) in Sindh Pakistan

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**Abstract.** The probiotic properties of *Lactobacillus* isolates of traditional yoghurt were investigated during the study. For that seven species of *Lactobacillus* were identified phenotypically. Out of 84 isolates, 23 *L. acidophilus*, 21 *L. casei*, 11 *L. helveticus*, 09 each *L. delbrueckii* sub sp. *bulgaricus* and *L. delbrueckii* sub sp. *lactis*, 03 *L. viridescense* and 02 *L. plantarum* were identified. All of these identified species were screened for antibacterial activity against foodborne pathogens, acid and bile tolerance and antibiotic resistance pattern. The results indicated that *L. casei* S66, *L. delbrueckii* sub sp. *bulgaricus* S65, *L. acidophilus* S26 and *L. plantarum* S19 produced antimicrobial substances against the indicator organisms and showed tolerance against acidic pH of 2.5 and survival against the bile salt concentration of 0.1 and 0.2. The *L. acidophilus* S26 survived the 0.3% bile salt concentration and was resistant to antibiotics ciprofloxacin, kanamycin, penicillin and vancomycin.

**Keywords:** lactic acid bacteria, bile tolerance, antibiotic resistance, probiotic culture, yoghurt

## Introduction

Dahi similar to yoghurt is a popular traditional fermented milk product of Pakistan produced from buffalo milk using an indigenous non-descriptive starter culture containing unknown mixture of lactic acid bacteria (LAB). It is believed that dahi assists in digestion and cures intestinal disorders including diarrhea and dysentery.

Historically, incorporation of LAB in food fermentation is to prevent spoilage. However, the development of antibiotic resistance and occurrence of different pathogens resulted in research on LAB to combat bacterial infections. The LAB are gram +ve, non-respiring, non-sporing rods or cocci. They produce lactic acid as their main end-product during fermentation. These have a 'GRAS' (generally recognized as safe) status therefore are considered as a safe food grade microorganisms (Metchnikoff, 1908).

Probiotics are group of viable bacteria that exert health related benefits on host (Saarela *et al*., 2000). These organisms can be incorporated into yoghurt or to other fermented milk products because of their apparent health benefits. The functional foods containing probiotics became popular due to the development of new products with incorporation of these cultures not only in dairy products but also in beverages, cereals and even in meat products (Erkkilä *et al*., 2001).

Some probiotics including *Bifidobacterium* and *Lactobacillus* are normal occupants of complex ecosystem of gastrointestinal tract (Mitsuoka, 1992). These probiotic organisms may prevent the gastro-intestinal infections by host cells adherence, reduce pathogenic bacteria by producing bacteriocins and ultimately reinforces the host's natural defense mechanism and present an antagonistic environment for pathogens (Rashmi and Gayathri, 2014).

However, the main criteria for selection of probiotics are based on acid and bile tolerance, gastrointestinal tract survival, antagonism against pathogens, ability of the organism to adhere to gut epithelium and colonize temporarily with good technological properties (Tuomola *et al*., 2001). The evaluation of antibiotic resistance patterns among probiotic *Lactobacilli* isolates to check the safety in the human health is suggested by Danielsen and Wind (2003). The *Lactobacilli* are present naturally or added intentionally in yoghurt, cheeses and other fermented milks to enhance the technological properties to produce health benefits to the consumer (Vernoux *et al*., 2003) and yoghurt is the best known food containing probiotics (Oskar *et al*., 2004).

Thus, knowing the importance of the probiotic cultures this research was planned to isolate and identify

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*Lactobacillus* species and to explore the probiotic potential of these isolates from traditional yoghurt (dahi) samples collected from the local market.

## Materials and Methods

**Isolation and identification of *Lactobacillus* species.** Thirty samples of traditional yoghurt were collected randomly from different shops of Hyderabad and Tandojam, in sterilized glass bottles were brought to the Food Microbiology Laboratory, Institute of Food Sciences and Technology, Sindh Agriculture University Tandojam under refrigeration to achieve the objectives of the study.

The *Lactobacillus* spp. were isolated using streak plate method and were identified morphologically and by means of cultural and biochemical characteristics according to the method by Collins and Lyne (1984).

**Bacterial isolates and growth conditions.** The seven species of *Lactobacillus* were identified phenotypically, all these were preserved in MRS broth (Oxoid, Basingstoke, UK) with 80% glycerol and stored at -40ºC. The isolates were anaerobically grown in MRS broth (Oxoid, Basingstoke, UK) at 37ºC. All indicator bacteria namely *Escherichia coli*, *Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteous mirabilis* and *Staphylococcus aureus* were obtained from Research and Diagnostic Laboratory, Liaquat University of Medical and Health Sciences, Jamshoro and preserved in nutrient agar slants (Oxoid, Basingstoke, UK) at 4 °C and were transferred to fresh broth 24 h before start of the experiment.

**Detection of antimicrobial activity.** For the detection of antimicrobial activity sterile filter paper discs were used as described by Ohmomo *et al*. (2000).

**Acid tolerance.** The acid tolerance of *Lactobacillus* isolates was observed at pH 2.5 for 3 h as described by Charteris *et al*. (1998).

**Bile tolerance.** The *Lactobacilli* were incubated with 0.1, 0.2 and 0.3% (w/v) oxgall (Difco) for total viable count determination at 37 °C.

**Antibiotic susceptibility.** The antibiotic susceptibility was determined by agar disk diffusion method as described by Bauer *et al*. (1966). Antibiotic discs of ciprofloxacin, erythromycin, kanamycin, penicillin, tetracycline and vancomycin (Oxoid, Basingstoke, UK) were used.

## Results and Discussion

**Isolation and identification of *Lactobacilli*.** The results presented in Fig. 1 showed that out of 84 *Lactobacillus* isolates, 27% were *L. acidophilus*, 25% *L. casei*, 13% *L. helveticus*, 11% *L. delbrueckii* sub sp. *bulgaricus,* 11% *L. delbrueckii lactis*, 4% *L. viridescence*, 2% *L. plantarum*, while 7% of the isolates could not be identified.

A total of 84 isolates from traditional made yoghurt samples were identified phenotypically, it was observed that the most of isolates were gram +ve and catalyst -ve. When incubated with 2% NaCl concentration growth was observed in all the *Lactobacillus* isolates. Moreover, there was variation in growth pattern at 4% NaCl concentration, whereas at 6.5% NaCl concentration growth was not observed (Table 1).

In the sugar fermentation profile of the isolates 12 sugars (glucose, fructose, galactose, lactose, sucrose, maltose, arbinose, raffinose, rhamnose, xylose, sorbitol and manitol) were used for identification of *Lactobacillus* spp. When these sugar utilization patterns were compared

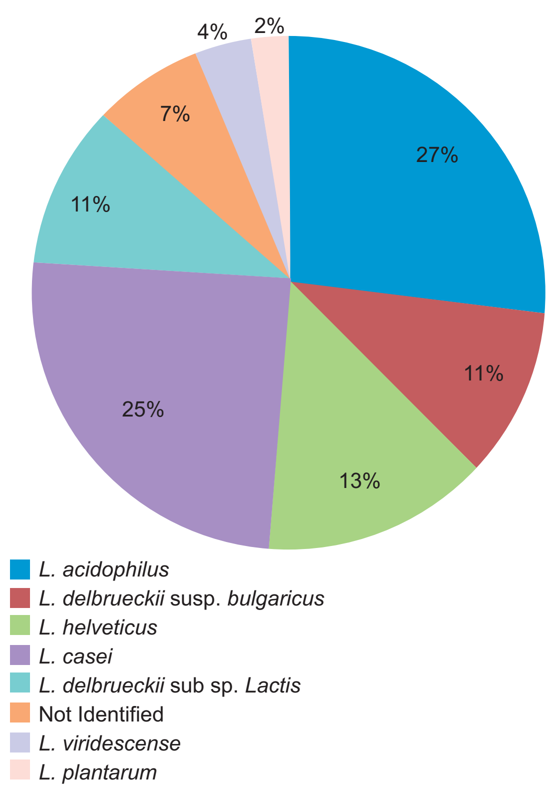


**Fig. 1.** Prevalence of *Lactobacillus* spp. identified from traditional yoghurt (dahi).

**Table 1.** Characteristics of the isolated *Lactobacillus* spp. from dahi

| lsolate no.(s) | Colonial morphology | | | Gram staining | Catalyst | Cell shape and arrange-ment | Growth at different temperatures | | | Growth at different NaCl concentrations | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Colour | Shape | Margin | | | | 37°C | 10°C | 42°C | 2 | 4 | 6.5 |
| 1-3, 10, 13, 20, 21, 23, 26, 27, 32, 38, 46, 56, 59, 60, 61, 71, 72, 73, 75, 83, 84 | White | Circular; large; rough and irregular | Undulate | + | – | Rods with rounded ends single/short chains | + | + | – | + | – | – |
| 5, 9, 12, 14, 15, 17, 29, 30, 41, 44, 45, 49, 51, 52, 57, 58, 62, 66, 78, 79, 80 | White | Circular | Entire | + | – | Rods in chains | + | – | + | + | + | – |
| 35,37,65 ,67, 68 ,69, 76, 82 | White | Irregular convex | Entire | + | – | Rods | + | – | + | + | – | – |
| 8, 31, 33, 48, 53, 55, 70, 77, 81 | White | Circular and large | Undulate | + | – | Rods | + | – | + | + | – | – |
| 4, 7, 11, 24, 25, 34, 36, 39, 40, 47, 54, 74 | White | Circular and compact | Entire | + | – | Rods in chains | + | – | + | + | – | – |
| 18, 16, 42, 43, 63, 64 | White | Circular and large | Entire | + | – | Rods | + | + | + | + | + | – |
| 19,28 | Creamy white | Pin point; circular; smooth; compact and convex | Entire | + | – | Rods | + | + | – | + | + | – |
| 6, 22,50 | Creamy white | Circular | Entire | + | – | Rods | + | + | + | + | – | – |

with those given for *Lactobacillus* species in the Bergey's manual of determinative bacteriology, the isolates were identified as *L. acidophilus*, *L. casei*, *L. delbruckeii bulgaricus*, *L. plantarum*, *L. acidophilus*, *L. helveticus*, *L. viridescense* and *L. delbruckeii lactis* (Table 2).

**Detection of antimicrobial activity.** The results presented in Table 3 showed that among the *L. acidophilus* eight strains showed antimicrobial activity and *L. acidophilus* S26 showed maximum zone of inhibition (16 mm) against all of the indicator strains except *Proteous mirabilis*. Whereas, five strains of *L. delbrueckii* sub sp. *bulgaricus* showed various zones of inhibition against *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* followed by *L. casei* S49, S66 which produced similar antimicrobial activity agains the tested pathogens. However, none of these strains inhibited growth of *Proteous mirabilis.* Both strains of *L. plantarum* S18 and S28 showed zone of inhibition (6-10 mm) against all five indicator stains. The results further revealed that three species namely *L. helveticus*, *L. viridescense*, *L. delbrueckii* sub sp. *lactis* showed no inhibition against any of the indicator strains.

**Acid tolerance.** The isolates exhibiting antimicrobial activity were screened for acid tolerance by observing growth at pH 2.5 after incubating at 0 h to 3 h as shown in Table 4. *L. acidophilus*, *L. casei*, *L. delbrueckii* sub sp. *bulgaricus* and *L. plantarum* strains were used for acid tolerance after detecting their activity against pathogens. The viable cell number was counted in terms

**Table 2.** Sugar fermentation pattern of *Lactobacillus* isolates from dahi

| Isolate(s) | A | F | G | Gu | L | M | Mn | Rf | Rh | Sr | Su | X | Identified *Lactobacilli* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1-3, 10, 13<br>20, 21, 23<br>26, 27, 32<br>38, 46, 56<br>59, 60, 61<br>71, 72, 73<br>75, 83, 84 | – | + | w | + | + | – | + | + | + | – | + | + | *L. acidophilus* |
| 5, 9, 12, 14<br>15, 17, 29<br>30, 41, 44<br>45, 49, 51<br>52, 57, 58<br>62, 66, 78<br>79, 80 | – | + | + | + | + | + | + | – | – | + | + | – | *L. casei* |
| 35, 37, 65<br>67, 68, 69<br>76, 82 | – | + | – | + | + | + | – | – | – | – | + | – | *L. delbrueckii* sub sp.<br>*bulgaricus* |
| ~~8, 31, 33,~~<br>48, 53, 55, 70<br>77, 81 | ~~–~~ | ~~–~~ | ~~+~~ | ~~+~~ | ~~+~~ | ~~+~~ | ~~–~~ | ~~+~~ | ~~–~~ | ~~–~~ | ~~v~~ | ~~–~~ | ~~*L. delbrueckii* sub sp.~~<br>*lactis.* |
| 4, 7, 11, 24<br>25, 34, 36<br>39, 40, 47<br>54, 74 | – | + | + | + | + | – | – | – | – | – | + | – | *L. helveticus* |
| 19, 28 | v | + | + | + | + | + | + | + | – | + | + | v | *L. plantarum* |
| 6, 22, 50 | – | – | – | – | – | + | – | – | – | – | – | – | *L. viridescense* |

A = Arabinose; F = Frustose; GU = Glucose; G = Galactose; L = Lactose; M = Maltose; Mn = Mannitol; Rf = Raffinose; Rh = Rhamnose; Sr = Sorbitol; Su = Sucrose; X = Xylose; (–) = not able to ferment sugar; (+) = able to ferment sugar; v = variable fermentation; w = weak fermentation.

of $\log_{10}$ cfu/mL. Among all the isolates *L. acidophilus* (S1, S26, S27, S41, S71, S72 and S73), three isolates of *L. delbrueckii* sub sp. *bulgaricus* (S35, S65 and S68), three isolates of *L. casei* (S 29, S41, and S66) and two isolate of *L. plantarum* (S19 and S28) showed tolerance towards acid as they were able to resist acidic pH values of 2.5 even after 3 h of incubation. The results revealed that the viability of these isolates was reduced at pH 2.5. However, *L. acidophilus* S26 and S72, *L. casei* S41 and the *L. plantarum* S19 appeared to be more acid tolerant as compared to other tested strains.

**Bile tolerance.** Survival of *Lactobacillus* spp. were screened for their resistance to different bile concentrations (0.1, 0.2 and 0.3%). Table 5 exhibits that all the strains have variable range of resistance to bile salt concentrations at different levels during 3 h of incubation. While, the more resistant strain in this study appeared to be *L. acidophilus* S26 which showed higher growth in 0.3% bile after 3 h of incubation as compared to other tested strains. Whereas, *L. acidophilus* S1, S41, *L. casei* S66, *L. plantarum* S19 and S28 showed survival to 0.1, 0.2 and 0.3% bile concentrations after 3 h. The growth appeared reciprocal to bile salt concentrations, and with an increase in bile concentration, the growth decreased in all the tested *Lactobacillus* spp. isolates.

**Antibiotic susceptibility.** The *Lactobacillus* isolates were further screened for susceptibility pattern against selected antibiotics. For this purpose six different and frequently used antibiotics were selected i.e. ciprofloxacin, erythromycin, kanamycin, penicillin, tetracycline and vancomycin.

The results presented in Table 6 revealed that all *Lacobacillus* spp. were resistant to ciprofloxacin and kanamycin were susceptible to tetracycline except *L.*

**Table 3.** Screening of antimicrobial activity from identified isolates of *Lactobacillus* spp. against pathogens.

| Isolate no(s). | *Escherichia coli* | *Staphylococcus aureus* | *Pseudomonas aeruginosa* | *Klebsiella pneumoniae* | *Proteous mirabilis* |
|---|---|---|---|---|---|
| *L. acidophilus* | | | | | |
| S1 | ++++ | +++ | ++++ | +++ | – |
| S2 | ++ | +++ | ++ | +++ | – |
| S3 | – | – | – | – | – |
| S10 | – | – | – | – | – |
| S13 | – | – | – | – | – |
| S20 | – | – | – | – | – |
| S21 | ++ | +++ | ++ | +++ | – |
| S23 | – | – | – | – | – |
| S26 | ++++ | ++++ | ++++ | +++ | – |
| S27 | +++ | ++ | +++ | ++ | – |
| S32 | – | – | – | – | – |
| S38 | – | – | – | – | – |
| S42 | | | | | |
| S46 | – | – | – | – | – |
| S56 | – | – | – | – | – |
| S59 | – | – | – | – | – |
| S60 | – | – | – | – | – |
| S61 | – | – | – | – | – |
| S71 | +++ | ++ | ++++ | +++ | – |
| S72 | +++ | ++ | ++++ | +++ | – |
| S73 | +++ | +++ | – | +++ | – |
| S75 | +++ | +++ | ++++ | +++ | – |
| S83 | – | – | – | – | – |
| S84 | – | – | – | – | – |
| *L. delbrueckii* sub sp. *bulgaricus* | | | | | |
| S35 | +++ | +++ | ++ | ++ | – |
| S37 | +++ | ++ | ++ | ++ | – |
| S65 | ++++ | +++ | +++ | +++ | – |
| S67 | – | – | – | – | – |
| S68 | ++++ | ++ | ++ | ++ | – |
| S69 | ++++ | +++ | ++ | +++ | – |
| S74 | – | – | – | – | – |
| S76 | – | – | – | – | – |
| S82 | – | – | – | – | – |
| *L. helveticus* | | | | | |
| S4 | – | – | – | – | – |
| S7 | – | – | – | – | – |
| S11 | – | – | – | – | – |
| S24 | – | – | – | – | – |
| S25 | – | – | – | – | – |
| S34 | – | – | – | – | – |
| S36 | – | – | – | – | – |
| S39 | – | – | – | – | – |
| S40 | – | – | – | – | – |
| S47 | – | – | – | – | – |
| S54 | – | – | – | – | – |
| *L. casei* | | | | | |
| S5 | – | – | – | – | – |
| S9 | – | – | – | – | – |

*continued on next page.....*

| Isolate no(s). | *Escherichia coli* | *Staphylococcus aureus* | *Pseudomonas aeruginosa* | *Klebsiella pneumoniae* | *Proteous mirabilis* |
|---|---|---|---|---|---|
| S12 | +++ | ++ | ++ | – | – |
| S14 | – | – | – | – | – |
| S15 | – | – | – | – | – |
| S17 | – | – | – | – | – |
| S28 | – | – | – | – | – |
| S29 | ++ | ++ | – | – | – |
| S30 | – | – | – | – | – |
| S41 | ++ | ++ | – | – | – |
| S44 | – | – | – | – | – |
| S45 | – | – | – | – | – |
| S49 | +++ | +++ | ++++ | +++ | – |
| S51 | ++ | ++ | +++ | ++ | – |
| S52 | – | – | – | – | – |
| S57 | – | – | – | – | – |
| S58 | – | – | – | – | – |
| S62 | – | – | – | – | – |
| S66 | +++ | +++ | ++++ | +++ | – |
| S78 | – | – | – | – | – |
| S79 | – | – | – | – | – |
| *L. plantarum* | | | | | |
| S19 | ++ | ++ | +++ | ++ | ++ |
| S28 | ++ | ++ | ++ | ++ | ++ |
| *L. delbrueckii* subs. *lactis* | | | | | |
| S8 | – | – | – | – | – |
| S31 | – | – | – | – | – |
| S33 | – | – | – | – | – |
| S48 | – | – | – | – | – |
| S53 | – | – | – | – | – |
| S55 | – | – | – | – | – |
| S70 | – | – | – | – | – |
| S77 | – | – | – | – | – |
| S81 | – | – | – | – | – |
| *L. viridescense* | | | | | |
| S6 | – | – | – | – | – |
| S22 | – | – | – | – | – |
| S50 | – | – | – | – | – |

**Note:** (–) = No inhibition; (+) = zone inhibition from 1 to 5 mm; (++) = inhibition zone inhibition from 6 to 10 mm; (+++) = zone inhibition from 11 to 15 mm; and (++++) = zone of inhibition more than 16 mm.

*plantarum*, whereas S71, S72, S73, S75 were erythromycin resistant. All isolates of *L. acidophilus* and *L. plantarum* showed resistance to penicillin, while, all isolates of the *L. delbrueckii* sub sp. *bulgaricus* remained susceptible to penicillin. The *L. casei* isolates were also resistant to penicillin, whereas S41 and S49 strains were susceptible to penicillin.

Among the isolates of *L. acidophilus* only three strains S21, S27 and S41 were susceptible to vancomycin and S1, S2, S26, S71, S72, S73 were found resistant to vancomycin. On the otherhand *L. delbrueckii* sub sp. *bulgaricus* S35 and S37 were susceptible to vancomycin and S65, S68, S69 showed vancomycin resistance. Only *L. casei* S49 was susceptible to erythromycin, penicillin, tetracycline and vancomycin. However, *L. plantarum* S19 and S28 were resistant to all the antibiotics except erythromycin.

The present study was carried out with the objective of identification of potential probiotic species of lactic acid bacteria from traditional dahi (curd). The results

**Table 4.** Survival of *Lactobacillus* spp. isolates at pH 2.5 after incubating at 0 to 3 h. The values are viable cell count $\log_{10}$ (CFU/mL)

| Isolate no(s). | 0h | 3h |
|---|---|---|
| *L. acidophilus* | | |
| S1 | 7.80±0.17 | 4.53 ± 0.15 |
| S26 | 7.83 ± 0.05 | 5.86 ± 0.05 |
| S27 | 7.56 ± 0.15 | 2.03 ± 0.15 |
| S41 | 8.16 ± 0.20 | 3.06 ± 0.25 |
| S71 | 7.63 ± 0.15 | 4.53 ± 0.15 |
| S72 | 7.83±0.05 | 5.56 ± 0.15 |
| S73 | 7.76 ± 0.05 | 5.23 ± 0.15 |
| *L. delbrueckii* sub sp. *bulgaricus* | | |
| S35 | 6.70 ± 0.20 | 2.16 ± 0.11 |
| S65 | 7.66 ± 0.20 | 3.56 ± 0.30 |
| S68 | 7.30 ± 0.10 | 3.12 ± 0.15 |
| *L. casei* | | |
| S29 | 7.20 ± 0.10 | 3.36 ± 0.15 |
| S41 | 7.63 ± 0.15 | 5.36+0.17 |
| S66 | 7.63 ± 0.15 | 3.63 ± 0.11 |
| *L. plantarum* | | |
| S19 | 7.96 ± 0.11 | 5.00 ± 0.10 |
| S28 | 7.83 ± 0.11 | 4.80 ± 0.20 |

Each value in the table represents the mean value ± standard deviation (SD) from three replications.

revealed the presence of a wide variety of *Lactobacillus* spp. in this traditional fermented milk product.

All the species identified on the basis of morphological and biochemical characteristics were rod shaped, convex, rough, smooth, shiny, irregular, circular, gram positive, facultative anaerobic, non-spore forming and catalase negative which indicated that they belong to the *Lactobacillus* group of bacteria (Holt *et al*., 1994), *Lactobacilli* are able to survive highly acidic environment of pH 4 to 5 or even lower, due to these properties *Lactobacilli* are responsible for final stages of fermentation in the products. These results are further supported by (Tzanetakis and Litopoulou-Tzanetaki, 1992). That low pH conditions favor the growth of *Lactobacilli*. NaCl is an inhibitory substance which may inhibit growth of certain types of bacteria and probiotic organisms have to withstand high salt concentration in human gut. The *Lactobacillus* spp. isolated from dahi were able to tolerate 2-4% NaCl. The presence of *L. acidophilus* (27%) in the market dahi samples is beneficial and could be used in combination with *L. delbrueckii* sub sp. *bulgaricus* in the preparation of yoghurt as probiotic culture; these results are in accord with the findings of Raquib *et al*. (2003), who were of the view that these species could

**Table 5.** Survival of *Lactobacillus* isolates under different bile concentrations. The values are viable cell count $\log_{10}$ (CFU/mL)

| Isolate no(s). | 0.1% | | 0.2% | | 0.3% | |
|---|---|---|---|---|---|---|
| | 0h | 3h | 0h | 3h | 0h | 3h |
| *L. acidophilus* | | | | | | |
| S1 | 7.74 ± 0.11 | 3.71 ± 0.34 | 7.76±0.11 | 3.00±0.15 | 7.68±0.11 | 2.20±0.20 |
| S27 | 7.67 ± 0.27 | 5.54±0.25 | 7.48±0.07 | 0 | 7.65±0.06 | 0 |
| S41 | 8.17±0.16 | 3.7±0.5 1 | 7.66±0.02 | 2.95±0.16 | 7.78±0.20 | 2.89±0.11 |
| S26 | 7.88±0.10 | 7.20±0.06 | 7.65±0.05 | 7.28±0.01 | 7.80±0.35 | 6.68±0.04 |
| S71 | 7.69 ± 0.10 | 3.99± 0.19 | 6.90±0.40 | 2.80±0.10 | 7.76±0.50 | 0 |
| S72 | 7.78 ±0.20 | 2.80±0.10 | 7.68±0.04 | 3.9±0.5 | 7.68±0.04 | 0 |
| S73 | 7.65±0.05 | 2.80±0.10 | 7.00±0.26 | 0 | 7.65±0.05 | 0 |
| *L. delbrueckii* sub sp. *bulgaricus* | | | | | | |
| S35 | 7.39±0.07 | 3.58±0.06 | 7.48±0.14 | 0 | 7.36±0.10 | 0 |
| S65 | 6.85± 0.08 | 2.54 ± 0.07 | 6.95±0.14 | 6.36±0.15 | 7.26±0.15 | 0 |
| S68 | 7.10 ± 0.10 | 2.58±0.09 | 7.00±0.34 | 0 | 6.90±0.20 | 0 |
| *L. casei* | | | | | | |
| S29 | 7.54±0.12 | 7.04±0.14 | 7.62±0.20 | 0 | 7.72±0.09 | 0 |
| S41 | 7.48±0.14 | 4.50±0.40 | 6.21±0.10 | 0 | 7.20±0.10 | 0 |
| S66 | 7.62±0.26 | 5.00±0.20 | 7.75±0.19 | 4.08±0.24 | 7.50±0.32 | 3.1±0.9 |
| *L. plantarum* | | | | | | |
| S19 | 8.20±0.10 | 6.1±0.5 | 7.58±0.31 | 6.7±0.3 | 7.50±0.32 | 3.2±0.3 |
| S28 | 7.20±0.10 | 4.1±0.3 | 7.98±0.01 | 4.5±0.40 | 7.00±0.34 | 3.3±0.5 |

Each value in the table represents the mean value ± standard deviation (SD) from three replications.

**Table 6.** Antibiotic susceptibility pattern of *Lactobacillus* spp. isolates

| Isolate(s) | Antibiotics used (µg/ disc) | | | | | |
|---|---|---|---|---|---|---|
| | CIP 5 | E 15 | K 30 | P 10 | TE 30 | V 30 |
| *L. acidophilus* | | | | | | |
| S1 | R | S | R | R | S | R |
| S2 | R | S | R | R | S | R |
| S21 | R | S | R | R | S | S |
| S26 | R | S | R | R | S | R |
| S27 | R | S | R | R | S | S |
| S41 | R | S | R | R | S | S |
| S71 | R | R | R | R | S | R |
| S72 | R | R | R | R | S | R |
| S73 | R | R | R | R | S | R |
| S75 | R | R | R | R | S | R |
| *L. delbrueckii* sub sp. *bulgaricus* | | | | | | |
| S35 | R | S | R | S | S | S |
| S37 | R | S | S | S | S | |
| S65 | R | S | R | S | S | R |
| S68 | R | S | R | S | S | R |
| S69 | R | S | R | S | S | R |
| *L. casei* | | | | | | |
| S12 | R | S | R | R | S | R |
| S29 | R | S | R | R | S | R |
| S41 | R | S | R | S | S | R |
| S49 | R | S | R | S | S | S |
| S51 | R | S | R | R | S | R |
| S66 | R | S | R | R | S | R |
| *L. plantarum* | | | | | | |
| S19 | R | S | R | R | R | R |
| S28 | R | S | R | R | R | R |

R = Resistant; S = Susceptible; CIP 5 = Ciprofloxacin; E 15 = Erythromycin; K 30 = Kanamycin; P 10 = Penicillin; TE 30 = Tetracycline; V = Vancomycin

be successfully used as mixed culture for youghurt production with improved organoleptic characteristics and enhanced therapeutic benefits.

Antimicrobial activity is one of the most important selection criteria for probiotics. As a potential probiotic, antimicrobial activity is one important property to avoid gastrointestinal infection (Kanmani *et al*., 2013). The lactic acid bacteria produce organic acids, hydrogen peroxide, diacetyl, carbon dioxide, low molecular weight antimicrobial compounds and bacteriocins with antimicrobial effect against foodborne pathogens (Amenu, 2013).

For the screening of the antimicrobial activity of *Lactobacillus* isolates five different pathogens *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumonia* and *Proteous mirabilis* were used as indicator strains. Among the tested strains *L. acidophilus*, *L. plantarum*, *L. casei and L. delbrueckii* sub sp. *bulgaricus* inhibited growth of the indicator strains, however, *L. acidophilus* showed maximum inhibition among the test strains. Similar results were recorded by (Fernandez *et al*., 2003) that two human *Lactobacillus* strains exhibited strong inhibition against the food borne pathogens. Moreover, Jacobsen *et al*. (1999) also observed that from 47 *Lactobacillus* isolates only 20 showed inhibition in growth of gram-positive as well as negative pathogenic bacteria. Similar findings were recorded by Savadogo *et al.* (2004) that all the *Lactobacillus* isolates inhibited the growth of indicator organisms though they vary in zone of inhibition.

Moreover, probiotics including *Lactobacillus* spp. are known to be inhibitory to the growth of a wide range of intestinal pathogens in human also. In addition to the favorable effects against disease caused by an imbalance of the gut microflora, several experimental observations have showed a potential protective effect of probiotic bacteria against the development of colon tumors (Dunne *et al*., 2001).

The effects of pH on the *Lactobacillus* isolates showed that there was a decrease in the number of viable cells after 3 h of incubation at pH value of 2.5. The viable counts of *L. acidophilus* S26, S72, S73, *L. casei* S41 and *L. plantarum* S19 strains were higher than the other strains. In most *in vitro* assays, pH 3.0 has been chosen to evaluate resistance to gastric transit due to substantial decrease in the viability of strains at pH 2.0 or lower (Vinderola and Reinheimer, 2003). Our results also showed that acid tolerance is strain-specific, as described by Zanoni *et al.* (2008). It is a trait of individual strains and strongly affected by experimental conditions. Thus, a direct comparison with other studies is difficult to be established. Nevertheless, similar studies often demonstrated that lactobacilli had increased sensitivity at pH values below 3.0 (Azat *et al*., 2016; Pitino *et al*., 2012; Ortu *et al*., 2007; Maragkoudakis *et al*., 2006).

Variable tolerances to 0.3% oxgall were observed among the strains tested. Most of the strains showed resistance to bile, though; the growth was delayed in all the strains. The presence of bile salts in the small intestine is another biological barrier for probiotic bacteria survival and colonization. The results of present findings are in accordance with the findings of Azat *et al*. (2016) and Maragkoudakis *et al.* (2006). They also screend six LAB strains isolated from cheese for bile tolerance.

However, some lactobacillus strains can grow in higher bile salt concentration (Ortu *et al*., 2007; Vinderola and Reinheimer, 2003). Similarly, infant feces isolates of *L. acidophilus*, *L. rhamnosus*, *L. gasseri* and *L. reuteri* tolerated low pH and bile salt (Xanthopoulos *et al*., 2000). Moreover, Magdoub *et al*. (2015), also indicated that survival (log cfu/mL) of all tested strains were affected gradually with increase in the bile salts concentrations.

The antibiotics may cause intestinal disorders by disrupting the balance of healthy gut microflora of the host. The administration of antibiotic-resistant strains can help retain the normal bacterial ratio in the intestines or its fast restoration if administered after antibiotic treatment (Hummel *et al*., 2007). The *L. acidophilus* strains showed resistance against an inhibitor of the cell wall synthesis such as penicillin. However, majority of the isolates showed sensitivity to the cell wall and protein synthesis inhibitors such as tetracycline. Similar observations were recorded by Beyatli *et al*. (2007). These probiotic bacteria may have negative impact on human health due to these resistant traits. Furthermore, the transfer of antibiotic-resistance genes and plasmids the conjugative transposons in the *Lactobacillus* species have also been reported (Ito *et al*., 2009). It has also been observed that plasmid borne resistance is present among probiotic cultures against certain antibiotics that include tetracycline, kanamycin, erythromycin, gentamycin, streptomycin and chloramphenicol (Hummel *et al*., 2007; Temmerman *et al*., 2002). Thus, there exists risk related to potential transfer of antibiotic resistance from probiotic strains to other bacteria either commensally residing in intestine or pathogens which is undesirable and detrimental.

## Conclusion

The results concluded that isolated *Lactobacillus acidophilus* from traditional yoghurt showed potentially important properties and it is valuable for use as starter and protective culture.

**Conflict of Interest.** The authors declare no conflict of interest

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