Isolation, characterization, and evaluation of probiotic properties of Lactic Acid bacteria from different fermented yoghurt drinks available in Bangladesh

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Research Article

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Abstract

*Lactobacillus* spp. is the most commonly found probiotics strain in dairy products. In the present study, *Lactobacillus* spp. were isolated from four commercially available fermented yoghurt drinks, which were identified based on observing their morphological characteristics and through some biochemical tests such as, gram staining, MIU (Motility Indole Urease), catalase, citrate, TSI (Triplir Sugar Iron), gas from glucose, milk coagulation activities, starch hydrolysis and bile esculin test. It was observed that isolated *Lactobacillus* spp. growth rate decreased with the increasing concentration of inhibitory substances like NaCl (2,4,8%) and phenol (0.1-0.8%). Additionally, good growth were observed in the presence of 2% NaCl and 0.1% phenol. The isolated *Lactobacillus* spp. did show good survival abilities in acidic (pH 2) and alkaline (pH 8) conditions, while their maximum growth was observed at pH 8.0. Isolated *Lactobacilli* were able to coagulate skim milk and produce gas from glucose. All of the isolates showed negative results in hemolytic activity, indicating that lactic acid bacteria are safe for use by humans. All isolates were evaluated for their antimicrobial activity against *Staphylococcus aureus*, *Enterococcus* spp., *Bacillus* spp, *Escherichia coli*, *Salmonella typhi*, *Klebsiellas* spp, *Pseudomonas* spp, *Serratia* spp, *Candida albicans*, *Trichodermaressei*, *Aspergillus* spp. The majority of the isolates showed significant antimicrobial effectiveness against the targeted pathogens. Their susceptibility to selected eight antibiotics was determined in terms of zone of inhibition (mm). In conclusion, most of the results from the present experiments showed that, there were very few variations in probiotics properties of the isolated *Lactobacillus* spp. from different fermented yoghurt drinks.

Introduction

The World Health Organization (WHO) and the Food and Agriculture Organization (FAO) of the United State defined probiotics as “Live microorganisms which confer a beneficial effect on the host when consumed in adequate amounts” and are recommended safe for consumption (Joint, 2002). Nowadays, probiotics product with multistrains of probiotic mixtures are becoming increasingly popular because of their higher nutritional value, health benefits effect, and also for minimal adverse side effects (Reid, 2005). As probiotics belong to a part of functional food, they become top beneficiaries of the latest growing trend of functional foods (Lim et al., 2015). Probiotic strains can be either bacteria, molds, or yeast that are commercially added to various dairy or non-dairy products (Chapman et al., 2011). *Lactobacillus* spp and *Bifidobacterium* spp. are widely used in combination for most commercial probiotic products (Weese, 2003). *Lactobacillus* spp is an important part of human commensal microbiota and has been considered GRAS (generally recognized as safe). This group of bacteria is used as starter cultures for dairy fermented food products (yogurt, cheese, sauerkraut, pickles, beer, cider, kimchi, cocoa, kefir), animals feed as well as health products of many biotechnology industries (Mojgani et al., 2015).

Probiotics can act as gut-beneficial bacteria that create a physical barrier against unfriendly bacteria (Margo, 2007). Probiotics bacteria possess beneficial properties such as reduced intestinal pathogens, advantageous for the function of the epithelial cells and also capable of growing in stressful conditions of the stomach and intestine where acid and bile are the most detrimental factors (Anderson & Gilliland, 1999). Additionally, probiotics can help fix the bacterial imbalance brought on by antibiotic treatment. Antibiotics kill both beneficial and harmful bacteria, frequently causing gas, cramps, or diarrhea. Probiotics may be helpful in the treatment or prevention of a wide range of illnesses, including crohn's disease, ulcerative colitis, diarrhea, and irritable bowel syndrome (del Carmen et al., 2011).

The antibacterial activity of probiotics against a variety of pathogenic and unwanted microorganisms is one of the most crucial selection factors. According to Ouwehand and Vesterlund (2004), the antimicrobial activity of various probiotic bacteria is largely dependent on their capacity to produce compounds like lactic acid, propanoic acid, acetic acid, carbon dioxide, hydrogen peroxide, low molecular weight antimicrobial substances, and bacteriocins (Ouwehand & Vesterlund, 2004).

Additionally, evaluation of *Lactobacillus* spp safety attributes, such as its in vivo safety and absence of antibiotic resistance, is necessary before using it as a source of probiotics in the food and feed industries (BIoHAZ 2012). These analyses might result in enhancements to the probiotic product's quality and usefulness. Recently fermented dairy drinks such as acidophilus milk, yakult, kefir are popular worldwide due to their differences in taste and their favorable physiological effects.

In this study, we choose commercially available fermented yoghurt drinks for isolation, characterization, and evaluation of probiotics properties of *Lactobacillus* spp. These fermented yoghurt drinks were made using the *L. casei* Shirota strain. *L. casei* Shirota may populate and create antimicrobial compounds in the small intestine, is resistant to the stomach and duodenal acid, and an increase the activity and number of macrophages (Yerlikaya, 2014). Based on the above information, the current study was conducted to identify and characterize *Lactobacillus* spp. from commercially available fermented yoghurt drinks in Bangladesh as well as to evaluate their probiotic potential and anti-microbial efficacy against some common pathogens.

Materials And Methods

Sample collection & enrichment

Commercially available fermented yogurt drinks were collected from the local market of Dhaka, Bangladesh. Immediately after collection, the samples were stored aseptically in a low temperature (4°C) refrigerator to protect them from contamination and deterioration. Then 10g of each sample were separately enriched in 40ml MRS broth (De Man, Rogosa Sharpe Broth, Himedia) for 24 hours at 37°C.

Isolation of Lactic acid bacteria

From enriched MRS broth 1ml of sample was taken and poured on to the MRS agar (De Man, Rogosa Sharpe Agar, Himedia) and then incubated at 37°C for 24–48 hours (Kumar & Murugalatha, 2012). The presumptive *Lactobacillus* spp. were further cultured into MRS agar for morphological and biochemical identification.
Morphological and biochemical characterization of isolates

Morphological characterization including color, shape, margin, elevation, texture, and size, was determined by Bergey's manual (Bergey, 2001). Numerous biochemical tests including Gram staining, MIU (Motility, Indole, Urease), Catalase, Citrate, Triple Sugar Iron, Gas from glucose were performed. The results were evaluated using "Bergey's Manual of Determinative Bacteriology" (Holt et al., 1994).

Gas from glucose

Durham's tube (inverted and dipped) was placed in sterile test tubes containing 10 ml of glucose broth. After being inoculated with 1% Lactobacillus cultures, the test tubes were placed in an incubator at 37°C for 24–48 hours (Bhardwaj et al., 2012).

Milk coagulation test

Sterile test tube containing 10 ml of milk was sterilized at 121°C. Then 20µL of overnight fresh cultures was added into the test tube and incubated at 37°C for 24 hours. Milk coagulating is a good indicator that Lactic acid bacteria is present.

Starch hydrolysis test

Certain bacteria use starch, a branching polysaccharide, as a source of carbohydrates. After autoclaving at 121°C for 15 minutes, the starch-containing plates were incubated at 37°C for a duration of 24 to 48 hours. To check for hydrolysis, iodine was added to the plate and incubated for 5 minutes. Bacterial growth was surrounded by a clear zone if starch was hydrolyzed; otherwise, the agar remained blue-black with any clearing of the zone (Mac Faddin, 1976).

Bile esculin test

The isolates were tested for their capacity to hydrolyze the glycoside aesculetin into glucose and aesculetin. The development of a dark brown to black halo around the bacterial growth on the plates after incubation at 37°C for 24 to 48 hour showed that aesculin hydrolysis had occurred (Bhardwaj et al., 2012).

Screening for probiotic properties of lactic acid bacteria

pH Tolerance test

All isolates were examined under both alkaline and acidic conditions at pH values varying from 2 to 8. For this purpose, 15 ml MRS broth medium was inoculated with 20µL overnight grown bacterial cultures. After that, the test tubes were incubated at 37°C, and measurements of absorbance at 620 nm were made every four hours to assess the bacteria's capacity to survive at low pH levels.

Phenol Tolerance test

Four types of phenolic concentrations were used in our study (0.1%, 0.2%, 0.3% and 0.4%). For this purpose, 15 ml MRS broth medium was inoculated with 20µL overnight grown bacterial cultures. After that, the test tubes were incubated at 37°C, and measurements of absorbance at 620 nm were made every four hours to assess the bacteria's tolerance against phenol.

NaCl Tolerance test

The isolates' susceptibility to various NaCl concentrations (2, 4 and 8%) was examined. In MRS broth medium, NaCl concentrations of 2%, 4%, and 8% were employed for this purpose. 20µL of overnight-grown cultures in 15 ml of broth were used to inoculate the medium with various doses of NaCl. To assess whether the isolates tolerated NaCl, the test tubes were then incubated at 37°C and optical density at 620 nm was recorded every 4 hours.

Determination of optimum temperature

100 µl of overnight-grown tested strains were added to 10 ml sterilized MRS broth tubes as an inoculant, and the tubes were subsequently incubated for 24 hours at various temperatures 25°C, 30°C, 35°C, and 40°C. After incubation, the plate count method was used to record the bacterial growth (Reuben et al., 2019).

Hemolytic activity

Blood agar plates containing 5% sheep blood were incubated at 37°C for 48 hours. A distinct zone around bacterial colonies that indicated hemolytic activity was found (Mourad, n.d.).

Evaluation of antagonistic activity

To detect the antimicrobial activity of Lactobacillus isolates, Mueller Hinton agar (MHA) plates were consistently and extensively seeded with the test pathogenic organisms such as Staphylococcus aureus, Enterococcus spp, Bacillus spp, Escherichia coli, Salmonella typhi, Klebsiella spp, Pseudomonas spp, Serratia spp, Candida albicans, Trichoderma reessei, Aspergillus spp. In solid media, a well with a diameter of 8 mm was formed using sterile gel cutter. Then 100 µl of culture was added to the well, and the plates were kept at a low temperature (4°C) for 2–4 hours. After that, plates were incubated inverted for 18 to 24 hours at 37°C to determine the zone of inhibition (Bauer, 1966).

Test of isolates for antibiotic sensitivity

The sensitivity of Lactobacillus to different antibiotics was determined by the zone of inhibition. The assay was done using the method described by Kirby-Bauer disc diffusion method given by Bauer et al., (1966). Different antibiotics namely Vancomycin (30 µg), Erythromycin (15 µg), Gentamicin (10 µg), Tetracycline (30 µg), Cefoxitin (30 µg), Amikacin (30 µg), Ciprofloxacin (5 µg) and Kanamycin (30 µg) were used for this assay (Institute, 2017).
Growth curve analysis

To determine the growth curve, a sterile 500ml conical flask was filled with 250ml of autoclaved broth. After that, 5ml of an overnight-grown culture of isolates A, B, C, and D was added to the flask as an inoculum. A spectrophotometer was used to measure the optical density (OD) at intervals of 30 minutes until the reading became static. At the end of the experiment, a graph was generated using time in minutes on the X axis and optical density at 600 nm on the Y axis to produce a growth curve for the isolates.

Statistical Analysis

All experiments were carried out in triplicates and the results are presented as the mean of three independent observations.

Results and Observations

Morphology and growth on selective media

Four Lactobacillus spp. were isolated from the four types of fermented yoghurt drinks. Lactobacillus spp. produced creamy whitish colonies on an MRS agar plate (Figure 1) but E. coli showed no growth. The isolates were labeled as A, B, C, and D.

Biochemical Characterization

All of the isolates were characterized according to Bergey’s manual of systematic Bacteriology (Buchanan & Gibbons, 1984) (Bergey, 1994). Creamy whitish colonies were further identified for biochemical analysis. All isolates were gram positive, catalase negative, citrate positive, able to coagulate milk, produces no gas from glucose, showed positive results in starch hydrolysis test and negative in bile esculin test (Figure 2, Figure 3). Different morphological and biochemical characteristics are shown in Table 1.

Table 1: Morphological and biochemical characterization of the isolates

<table>
<thead>
<tr>
<th>Morphological and Biochemical Characteristics</th>
<th>Isolate A</th>
<th>Isolate B</th>
<th>Isolate C</th>
<th>Isolate D</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Morphological Characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell shape</td>
<td>Cocci</td>
<td>Cocci</td>
<td>Rod</td>
<td>Rod</td>
</tr>
<tr>
<td>Size</td>
<td>Small</td>
<td>Small</td>
<td>Small</td>
<td>Small</td>
</tr>
<tr>
<td>Form</td>
<td>Round</td>
<td>Round</td>
<td>Round</td>
<td>Round</td>
</tr>
<tr>
<td>Surface</td>
<td>Shiny</td>
<td>Shiny</td>
<td>Smooth</td>
<td>Smooth</td>
</tr>
<tr>
<td>Color</td>
<td>White creamy</td>
<td>White creamy</td>
<td>White creamy</td>
<td>White creamy</td>
</tr>
<tr>
<td>Opacity</td>
<td>Opaque</td>
<td>White creamy</td>
<td>White creamy</td>
<td>White creamy</td>
</tr>
<tr>
<td>Gram staining</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Biochemical Characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MIU</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Catalase test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Citrate test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>TSI test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gas from glucose</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Milk coagulation test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Starch hydrolysis test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Bile esculin test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+ indicate Positive growth, - indicate negative growth

Screening for probiotics properties

pH tolerance test

A pH range of 2 to 8 was used in this investigation to observe growth. Based on the results of the experiment, it was found that isolated Lactobacillus spp. from fermented yoghurt beverages can survive in both acidic pH (5 to 6) and basic pH environments (pH 7.5 to 8). Isolates tolerance activity at different pH are shown in Table 2.

Table 2: pH tolerance test
### Values are the mean±standard deviation of at least 3 determination

**Phenol tolerance test**

Four isolates showed a higher growth rate at the concentration of 0.1%. All of the isolates showed the lowest growth rate at 0.3% concentrations. Survival rate at 0.1 and 0.2% concentrations of isolate A and isolate B was higher than Isolate C and Isolate D (Figure 4). Isolate D showed higher growth at 0.4% phenolic concentration..

**NaCl tolerance test**

At 2% NaCl, the four isolates showed higher growth than the other two concentrations. Isolate A, B and D showed moderate growth at 4% NaCl concentration (Figure 5). At 8%, isolate C showed no growth as the absorbance was lower than the control. This bacterial species was sensitive to higher concentrations of NaCl. But the other three species showed a little growth at 8% NaCl concentrations.

**Determination of optimum temperature**

Isolate A was able to survive at temperature 25°C, 30°C, 35°C and 40°C and showed growth in MRS agar plate. Whereas isolate B, C, D showed growth in MRS agar plate at temperature 25°C, 30°C, 35°C but not in 40°C. Image of representative isolates were shown in figure 6, 7.

### Safety analysis

**Hemolytic activity**

When screened for hemolytic activity, every isolate showed a negative result (Figure 8), indicating that lactic acid bacteria are safe for use by humans.

**Antibiotic Sensitivity Test**

The disc diffusion method was used to analyze the antibiotic sensitivity pattern of some isolates in order to see the inhibitory effect of any antibiotics against them. Isolates A, B showed resistance against kanamycin (30 µg) whereas isolates C, D showed resistance against vancomycin (30 µg) (Table 3). Some images of antibiotic sensitivity patterns of isolates on MHA (Mueller Hinton Agar) were shown in Figure 9.

<table>
<thead>
<tr>
<th>Name of Antibiotic</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S=Sensitive, R=Resistant, I=Intermediate</td>
<td></td>
</tr>
</tbody>
</table>

### Table 3: Antisense of antibiotic sensitivity pattern

<table>
<thead>
<tr>
<th>Name of Antibiotic</th>
<th>Sensitive Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vancomycin (30µg)</td>
<td>S  S  R  R</td>
</tr>
<tr>
<td>Erythromycin (15 µg)</td>
<td>I  I  S  S</td>
</tr>
<tr>
<td>Gentamicin (10 µg)</td>
<td>I  I  S  S</td>
</tr>
<tr>
<td>Tetracycline (30 µg)</td>
<td>S  S  S  S</td>
</tr>
<tr>
<td>Cefoxitin (30 µg)</td>
<td>S  S  I  S</td>
</tr>
<tr>
<td>Amikacin (30 µg)</td>
<td>I  I  S  S</td>
</tr>
<tr>
<td>Ciprofloxacin (5 µg)</td>
<td>S  S  S  S</td>
</tr>
<tr>
<td>Kanamycin (30 µg)</td>
<td>R  R  S  S</td>
</tr>
</tbody>
</table>

### Evaluation of antagonistic activity

The isolates were tested for antimicrobial activity against some common pathogenic microorganisms. Isolates A, B, C, D showed maximum zone of inhibition against *E. coli* and *S. aureus* whereas all isolates showed resistance against *S. typhi* and *Pseudomonas* spp. Each isolates antimicrobial activity was shown in table 4.

<table>
<thead>
<tr>
<th>Name of Antibiotic</th>
<th>Sensitive Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S=Sensitive, R=Resistant, I=Intermediate</td>
<td></td>
</tr>
</tbody>
</table>

### Table 4: Antagonistic activity of isolates against common pathogenic microorganisms

<table>
<thead>
<tr>
<th>Name of Antibiotic</th>
<th>Sensitive Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vancomycin (30µg)</td>
<td>S  S  R  R</td>
</tr>
<tr>
<td>Erythromycin (15 µg)</td>
<td>I  I  S  S</td>
</tr>
<tr>
<td>Gentamicin (10 µg)</td>
<td>I  I  S  S</td>
</tr>
<tr>
<td>Tetracycline (30 µg)</td>
<td>S  S  S  S</td>
</tr>
<tr>
<td>Cefoxitin (30 µg)</td>
<td>S  S  I  S</td>
</tr>
<tr>
<td>Amikacin (30 µg)</td>
<td>I  I  S  S</td>
</tr>
<tr>
<td>Ciprofloxacin (5 µg)</td>
<td>S  S  S  S</td>
</tr>
<tr>
<td>Kanamycin (30 µg)</td>
<td>R  R  S  S</td>
</tr>
</tbody>
</table>
antibiotics were evaluated. Isolate C and D showed susceptibility towards all other antibiotic discs except Vancomycin (30 µg). Isolate A and B showed

Using the disk diffusion method on Muller Hinton agar medium, the patterns of susceptibility and resistance of isolated bacterial cultures to eight different

isolates have B-h

were obtained by Kumar et al. (Kumar & Murugalatha, 2012). In another study, the growth of the isolates ranging from pH 2–8. Based on the findings of the experiment, it was observed that the isolated Lactobacillus spp. from fermented yoghurt drinks can survive in both acidic pH (5 to 6) and basic pH (pH 7.5 to 8). According to another study, Lactobacillus spp. showed better growth at low pH level(Mannan et al., 2017). A research found that all of their isolates showed pH tolerance at pH 3.(Shirisha et al., 2021).Pundir R.K. et al. (2013) previously demonstrated that lactic acid bacteria isolated from fresh vegetables, fruits, and curds survived in pH ranges ranging from 3.5 to 7.0 (Pundir et al., 2013).

In addition, phenol an inhibitory substance is produced by the deamination of amino acids in the intestine(Suskovic et al., 1997). In the current investigation, phenol tolerance tests were conducted at concentrations of 0.1%, 0.2%, 0.3%, and 0.4%. Isolates A, B were able to survive at 0.1% and 0.2% of phenol, whereas isolates C, D cannot. According to Hoque's (2010) findings, the majority of Lactobacillus strains were able to endure 0.3% phenol. According to a related study of probiotics, they found that Lactobacillus spp. survived up to 0.4% of phenol(Akter et al., 2010). According to Mannan (2017), all of the isolates survived at concentrations of 0.1 and 0.2% phenol, however only half of the isolates survived at concentrations of 0.3 and 0.4%phenol (Mannan et al., 2017).

NaCl is an inhibitory substance that can prevent certain bacterial growth. The current findings show that Lactobacillus spp. isolated from fermented yoghurt drinks can tolerate 2%, 4% and 8% NaCl and grow well at 2% NaCl in case of isolates A, B. Another experiment by Elezete and Carlos revealed that Lactobacillus spp isolated from swine gastrointestinal tracts were tolerable to 4–8% NaCl(Diba et al., 2013). In another study, the growth of the isolates decreased with the increasing concentrations of NaCl(Ranjan et al., 2020). Lactobacillus spp. showed lower growth at higher NaCl concentration in another study isolated from yoghurt sample (Mannan et al., 2017). All of the isolates were able to survive at 25°C, 30°C, 35°C which indicate that they are mesophilic in nature. The findings of this research were remarkably comparable to the study conducted by Sakala et al. (Sakala et al., 2002).

Each isolates were tested for hemolytic activity showed negative results indicate that Lactic acid bacteria are safe for human consumption. Similar findings were obtained by Kumar et al. (Kumar & Murugalatha, 2012). According to another finding from (Tejero-Sarifiena et al., 2012) also found that none of their isolates have B-hemolytic properties.

Using the disk diffusion method on Muller Hinton agar medium, the patterns of susceptibility and resistance of isolated bacterial cultures to eight different antibiotics were evaluated. Isolate C and D showed susceptibility towards all other antibiotic discs except Vancomycin (30 µg). Isolate A and B showed

<table>
<thead>
<tr>
<th>Isolates name</th>
<th>Test organisms with zone of diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. aureus</td>
</tr>
<tr>
<td>Isolate A</td>
<td>++</td>
</tr>
<tr>
<td>Isolate B</td>
<td>++</td>
</tr>
<tr>
<td>Isolate C</td>
<td>++</td>
</tr>
<tr>
<td>Isolate D</td>
<td>++</td>
</tr>
</tbody>
</table>

Zone diameter: - indicates 0 mm; + indicates (1-5 mm); ++ indicates 6-12mm

Growth rate study

After a certain period, isolate A, isolate B and isolate C showed stationary growth rates but isolate D was different (Figure 10). It showed an increase and decrease from time to time. In the maximum case, the growth of the bacterial species was slowed down after four or five hours. Then their growth level was indifferent.

Discussion

Probiotic bacteria, which play an important role in the growth of gut microflora, were responsible for the production of probiotic foods and fermented milk products. It also aids the digestive system by increasing individuals immunity to pathogens in human health. These bacteria can be bred to produce a variety of pharmaceuticals and food products. They can also be utilized in the development of new functional foods(Shahriar et al., 2019). For this purpose commercially available fermented yoghurt drinks were isolated, identified and screened for their probiotic potential. The isolates were gram-positive, solitary, or rod-shaped in chain form. Gram stain results showed that the isolated bacteria could be identified as Lactobacillus spp. Similar findings were observed in some other studies on probiotics (De et al., 17)(Shirisha et al., 2021).

In this study, all isolates showed negative results in motility indole urease test. similar findings were obtained from probiotics milk samples (Hossain et al., 2016). In another research Lactobacillus spp. showed indole negative results (De et al., 2017). The catalase test or the decomposition of H₂O₂ to generate O₂ is one of the most useful diagnostic tests for detecting bacteria due to its simplicity. It is well known that Lactobacillus is catalase negative as similar results were found in many studies of yoghurt samples to the study of isolation and characterization of Enterococci from Yoghurts of Bangladesh by Tabrejee et al, (Tabrejee et al., 2018). The isolates were grown on Simmon's citrate agar slant test tube for the identification of acid or alkali-producing bacteria. From the Simmon's citrate test we observed that, all the samples showed negative results. Additionally, some probiotic-related research papers showed citrate negative results(Ranjan et al., 2020). The isolates had the ability to coagulate milk, produce no gas from glucose, showed positive starch hydrolysis results, and exhibited negative bile esculin results.

pH plays a key role in the development of bacterial growth. In this study growth was observed in various pH ranging from pH 2–8. Based on the findings of the experiment, it was observed that the isolated Lactobacillus spp. from fermented yoghurt drinks can survive in both acidic pH (5 to 6) and basic pH (pH 7.5 to 8). According to another study, Lactobacillus spp. showed better growth at low pH level(Mannan et al., 2017). A research found that all of their isolates showed pH tolerance at pH 3.(Shirisha et al., 2021).Pundir R.K. et al. (2013) previously demonstrated that lactic acid bacteria isolated from fresh vegetables, fruits, and curds survived in pH ranges ranging from 3.5 to 7.0 (Pundir et al., 2013).
resistance only towards Kanamycin among the eight antibiotics. De et al., used three antibiotics against Lactobacillus spp. and they showed sensitivity but in our study, we found some resistant isolates towards some antibiotics (De et al., 2017). According to another study Lactobacilli spp. have inherent resistance to streptomycin, kanamycin, gentamicin, vancomycin, bacitracin, metronidazole, and sulfamethoxazole (Lethycia et al., 2017).

All possible probiotic strains must have antagonistic activity, which is one of their most important characteristics. In this study, the isolates were evaluated against some common pathogenic microorganisms to see if they had any antagonistic effects on the growth of these bacteria. The isolates A, B, C, and D exhibited maximum antimicrobial activity against S. aureus and E. coli. In contrast, isolates had no inhibitory effect on S. typhi and other pseudomonas species. In another research Anas et al. (2008) showed that gram-positive organisms have higher degree of antagonistic activity than gram-negative bacteria (Anas et al., 2008). According to this study, the identified isolates can be utilized to produce functional dairy products.

**Conclusion**

In this work, Lactobacillus spp. from local fermented yoghurt drinks were separated and identified; they can live in extremely acidic and some what alkaline environments, with excessive salt concentrations, and low phenol contents. These results showed that the unique Lactobacillus strains can be used to thrive in the gastric environment of human’s tract. The isolates did not exhibit hemolytic activity, hence we regard it as safe, excellent probiotic potential and safety for humans. The antimicrobial activity of these potent isolates may contribute to efficacy, possibly through direct antimicrobial activity in vivo. Additional research is required to complete the molecular identification and detection of specific genes and proteins of interest that may aid in the development of pharmaceuticals medications which will be effective for both humans and animals.

**Abbreviations**

- **MIU:** Motility Indole Urease
- **TSI:** Triple Sugar Iron
- **pH:** Potential of hydrogen
- **GRAS:** Generally recognized as safe
- **MRS:** De Man, Rogosa Sharpe
- **MHA:** Mueller Hinton agar
- **OD:** Optical density

**Declarations**

**Authors Contributions**

Mala Khan contributed to the study conception and design. Material preparation, data collection and analysis were performed by Fatema Akter, Tabassum Jabin and Sahida Yeasmin. The first draft of the manuscript was written by Tabassum Jabin and Sahida Yeasmin and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

**Conflict of Interest**

The authors declare there is no conflict of interest.

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**Ethics approval**

Not applicable.

**Consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

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References


**Figures**

**Figure 1**

*Lactobacillus* spp. growth on MRS agar plate

**Figure 2**
Left side image indicate isolates showed no gas from glucose and right side image indicate isolates were able to coagulate milk.

Figure 3

Starch hydrolysis test (A indicates positive control, B indicates negative control and C indicates test isolates which were able to hydrolyze starch).

Figure 4

Phenol Tolerance Test
Figure 5

NaCl Tolerance Test

Figure 6

Images of optimum temperature determination of isolates.
Figure 7

Temperature activity of isolates in MRS agar plate.

Figure 8

Left side image indicates isolates' hemolytic activity in blood agar plate (no hemolysis). (B) Right side plate indicates isolates' activity in bile esculin agar plate.
Figure 9

Some images of antibiotic sensitivity pattern of isolates on MHA agar.

Figure 10

Growth rate study of different isolates