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Research Article
Characterization and Confirmation of *Lactobacillus* spp. from Selective Regional Yoghurts for Probiotic and Interference with Pathogenic Bacterial Growth

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Abstract
A probiotic is a viable microbial dietary supplement that is beneficial for the host through its effects in the intestinal tract. Probiotics are widely used to prepare fermented dairy products such as yoghurt and it is an important source of probiotic Lactobacilli. In this study, the samples were collected from different superstores of Chittagong and Bogra city of Bangladesh. Pure culture of specific probiotic isolates from each sample was performed and identified on the basis of their colonies morphologies and some biochemical tests such as catalase, oxidase and IMViC (indole, methyl- red, voges proskavar, citrate utilization) test. Their identification as *Lactobacillus* spp., was confirmed through PCR reaction using genus specific primer. The isolated *Lactobacillus* spp., were resistant to inhibitory substances like NaCl (1-9%) and bile acid (0.05-0.3%). In addition, the satisfactory growth of isolated *Lactobacillus* spp., was observed in alkaline condition. The isolated *Lactobacillus* spp., showed positive result in different carbohydrate source such as glucose, xylose, galactose, etc and were able to produce organic acid in skim milk which was determined by titrimetic method. The *Lactobacillus* spp., was examined for their antimicrobial activities against some test pathogens by modified agar overlay method and the high inhibition zones showed their potential antibacterial effects. The yoghurt is suggested as a source of potential probiotic strains and there were variations in probiotic properties of the isolated *Lactobacillus* spp., obtained from selective regional yoghurt samples in this study.

Key words: *Lactobacillus* spp., probiotic, bile salt tolerance, genus specific PCR, antibacterial activity

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Yoghurt is a dairy product prepared by fermentation of milk with *Lactobacillus* spp. Now a days, Therapeutic, prophylactic and nutritional properties of yogurt are widely accepted (Boor, 2001). When administered in a sufficient amount, probiotics give health benefit to the host and increase microbial balance (Fuller, 1989; Guarner *et al*., 2005). So, probiotics are living, non-pathogenic, friendly microorganisms which play beneficial roles in the micro flora compartment of the host (Schrezenmeir and de Vrese, 2001). Lactic Acid Bacteria (LAB) are the most important probiotic group of microorganisms especially *Lactobacillus* sp., *Bifidobacterium* sp. and *Enterococcous* sp. (Klein *et al*., 1998). Dietary and therapeutic qualities of milk product are determined by probiotic microorganism (Boor, 2001).

Yoghurt is a potential source of probiotic *Lactobacillus* spp. Yoghurt is also known as the richest probiotic contaminating food. The nutritional value of milk product is increase by probiotic microorganisms and metabolites produced as result of fermentation. Regular intake of yogurt reduces the excessive fat form the liver and enhance by secretion. It is also necessary for those who suffer from heart disease, atherosclerosis, hypertension and inflammation. Gastric juice which is secreted by the action of yogurt leads to a high digestive capability. The beneficial effect of probiotic microorganisms especially *Lactobacillus* sp., which exists in milk products which evidence reported furthermore, many researchers studied the effects of probiotic microorganisms against pathogenic organisms using different method (Mercenier *et al*., 2003).

Lactobacilli are members of the lactic acid bacteria whose primary fermentation end product is lactic acid. They are commercially important bacteria with a wide variety of application both in food and non food industries due to their "Generally Recognized As Safe" (GRAS) status. Lactobacilli have been extensively studied for their molecular biology in order to improve their specific beneficial characteristics (Pouwels and Leer, 1993).

The mode of action of probiotics is based on the ability of probiotic bacteria to bind pathogens in intestinal epithelial tissue. Anti-pathogenic action of probiotics consists in production of lactic acid which decreases the pH, interacts with the toxins produced by pathogens with the production of hydrogen peroxide and synthesis bacteriocin (Corcionivoschi *et al*., 2010).

An effective probiotic product requires proper identification and characterization of a bacterial species used. The selection of probiotic organisms that can be helpful therapeutically and nutritionally would be based on specific properties (Fuller, 1989; Quewand and Vesterlund, 2004).

The aim of this research was to collect yoghurt from various regions of Bangladesh and identification of lactobacilli by bacteriological as well as genus specific PCR and analysis of their probiotic properties following the interference with pathogenic bacterial growth.

MATERIALS AND METHODS

Sample collection and isolation of Lactic Acid Bacteria (LAB): Two types of yoghurt samples were collected from different superstores of Chittagong and Bogra city in Bangladesh which were sour (Sample M1, M2 and M3) and sweet (sample S2) in taste. Then samples were stored immediately after collection in low temperature (4°C) refrigerator aseptically to protect form contamination and deterioration. The *Lactobacillus* spp. was isolated from yogurt samples by appropriate dilutions with 0.9% salt solution. The MRS (Man, Rogosa and Sharpe) broth and MRS (Man, Rogosa and Sharpe) agar media were used for the growth of the organism. The pH of the media was adjusted to 6.5. The plates were aerobically incubated at 37°C for 48 h. Finally, the single colony of *Lactobacillus* was isolated by observing their colony morphology and some biochemical tests such as gram staining, catalase and oxidase test. Well isolated colonies were picked up and transferred to MRS broth for enrichment of *Lactobacillus* at 37°C.

Identification of Lactic Acid Bacteria (LAB) by bacteriological analysis: Identification was carried out according to the methods described in Bergey's manual of systemic bacteriology. Without the use of anaerobic conditions all strains grew well on MRS agar at 37°C for 48 h for selective outgrowth of lactobacilli. From appropriate dilutions one representative colony was picked and tentatively identified as lactobacilli after Gram stain reaction, colony appearance, cell morphology, catalase test, oxidase test, indole test, methyl red test, voges-proskauer test, citrate utilization test and carbohydrate fermentation patterns as delineated by Bergey's manual (Hensyl, 1994).

DNA extraction from isolates: DNA was extracted from 4 samples isolated from yogurt according to the classical heat-thaw method (Salehi *et al*., 2005). Pure bacterial culture from MRS agar slant was subcultured in MRS broth medium from which 1.5 mL broth culture was taken in eppendorf tube and centrifuged at 10,000 rpm for 5 min. After that the

supernatant was discarded and the pellet was collected. About 200 µL of autoclaved deionized water was added to the pellet and dissolved by finger shaking. The cap of the eppendorf tube was pierced by sterile needle and then the tube was boiled in water bath at 100°C for 10 min. Just after boiling, the eppendorf tube was kept in ice for 10 min and then centrifuged at 10,000 rpm for 10 min. Then 100-150 µL supernatant containing bacterial chromosomal DNA was collected.

Genus specific PCR amplification: To determine the affiliation to genus level of all 4 isolates, PCR was carried out with *Lactobacillus* genus-specific primer set LbLMA1-rev (5'-CTCAA AACTAAACAAAGTTTC-3') and R16-1 (5'-CTTGTACACACCG CCCGTCA-3') designed by Dubernet *et al.* (2002). The PCR analysis was done based on 16-23S ribosomal RNA intergenic spacer region as described before (Dubernet *et al*., 2002).

The reaction mixture (20 µL) contained 1 µL (100 ng μL^{-1}) of each primer added with 10 µL 10× PCR Master Mix, 6 µL of PCR water and 2 µL of template. The run condition was initial denaturation at 95°C for 5 min, followed by 30 cycles consisting of denaturation at 95°C for 30 sec, annealing a 55°C for 30 sec, extension at 72°C for 30 sec and a 7 min final extension step at 72°C. The products were stored at 4°C until analysis. The amplified products were subjected to electrophoresis in 1% agarose gels in TAE buffer (40 mM Tris acetate, 1 mM EDTA, pH 8.2). Gels were stained with ethidium bromide (5 µg mL^{-1}) and visualized under UV transilluminator (Biometra GmBH, Germany).

Analysis of probiotic characteristics: The probiotic characteristics were determined by analyzing the following tests.

NaCl tolerance test: For the determination of NaCl tolerance, isolated Lactobacilli were grown in MRS broth containing nine test tubes that were adjusted with different concentrations of NaCl (1-9%). After autoclaving for 15 min in 15 Ibs pressure at 121°C, each test tube was inoculated with 10 µL over night culture of *Lactobacillus* and incubated anaerobically at 37°C for 24 h. After 24 h incubation, the bacterial growth was measured using a spectrophotometer at 560 nm (Graciela and Maruia, 2001).

Bile salt tolerance test: Growth rate of bacterial cultures was determined in MRS broth containing different levels (0.05, 0.1, 0.15, 0.3 and 0.5%) of bile salts. Freshly prepared cultures were inoculated (1%) into medium and incubated at 37°C for 24 h under anaerobic condition. Then optical density of each sample was measured using a spectrophotometer at 560 nm (Graciela and Maruia, 2001).

Determination of optimal pH for growth: For the determination of optimal pH for growth, 100 µL of fresh *Lactobacillus* overnight culture was inoculated into MRS broth containing test tubes with varying pH ranging from 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0. To determine the effect of pH on growth acetate buffer (pH -4, 4.5, 5, 5.5, 6, 6.5), Tris-HCl buffer (pH-7) and borate buffer (pH-8) were used. The inoculated broth was incubated in anaerobic condition for 24 h at 37°C. After incubation, growth of the bacteria was measured using a spectrophotometer at 560 nm against uninoculated control broth.

Quantification of organic acid and determination pH value: According to Hoque *et al*. (2010), quantification of organic acids produced by the isolates and determination of their pH values were performed. The MRS broth supplemented with 10% skim milk inoculated with 1% (v/v) or 100 µL overnight culture of the isolates and incubated in anaerobic condition at 37°C for 72 h. Fermented samples were collected in every 24, 48 and 72 h and liquids of coagulated milk were separated by filtration. After filtration, the pH of the separated liquid was recorded using a digital electrode pH meter and quantification of organic acid was done through titration with 0.1 N NaOH using phenophthalien as pH indicator (Hoque *et al*., 2010).

Screening of interference with pathogenic bacteria: The antibacterial activities of the isolated *Lactobacillus* spp., against some pathogenic bacteria were determined by modified agar overlay method (Aween *et al*., 2012). Eight different human pathogens, *Shigella dysenteriae*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Bacillus megaterium*, *Staphylococcus aureus*, *Vibrio cholerae*, *Escherichia coli* and *Shigella sonnei* were used in this study as test pathogen. Antibacterial activity was further characterized by determining whether bacteriostatic or bactericidal. The test was performed by swabbing of the growth inhibition zone. The swab was streaked onto nutrient agar plate and incubated aerobically at 37°C for 72 h. The presence of growth in nutrient agar plate was interpreted as an inhibitory activity i.e., bacteriostatic while no growth was interpreted as bactericidal.

RESULTS AND DISCUSSION

Identification of bacteria through bacteriological and biochemical tests: The four isolates were grown in Man, Rogosa and Sharpe (MRS) medium at pH 6.5. All the isolates were produced small, irregular and round shape with shiny whitish cream or brownish colored which were morphologically similar to *Lactobacillus* spp.

Then all isolates were examined under bright field microscope to observe their microscopic features. These isolates were found gram positive, short and medium rod shaped non-spore forming bacterium (Fig. 1a-d) which indicate them to be member of *Lactobacillus* spp. (Thamaraj and Shah, 2003).

In addition, some biochemical tests such as catalase test, oxidase test, indole test, Methyl Red (MR) test, Voges Proskauer (VP) test, citrate utilization test and carbohydrate fermentation patterns were performed as delineated by Bergey's manual systematic bacteriology (Hensyl, 1994).

The isolates were found catalase and oxidase negative and in IMViC (indole, methyl-red, voges proskauar, citrate utilization) tests all isolates were also found negative, thereby these might confirm the isolates were *Lactobacillus* spp. (Dhanasekaran *et al*., 2010).

In this study, all the four isolates were able to ferment 11 different carbohydrates, i.e., glucose, sucrose, fructose, lactose, xylose, ribose, galactose, maltose, mannitol, rhamnose and dextrose indicating that they are able to grow in variety of habitats utilizing different type of carbohydrates. The summarized results of all bacteriological and biochemical tests are presented in Table 1. All these results are found relevant to the findings of Chowdhury *et al*. (2012).

Molecular identification through genus specific PCR: In this study, we used a genus specific primer prepared by analyzing similarities between the nucleotide sequences of the spacer region between the 16 and 23S ribosomal RNA genes of *Lactobacillus* (Dubernet *et al*., 2002). The specificity of this genus specific primer combined with a universal primer was tested against 23 strains of *Lactobacillus* of varied origin. The PCR products were ran through gel electrophoresis in 1% agarose and visualized by UV transilluminator. An expected sharp band of 200 bp amplicon was found for each sample (M1, M2, M3 and S2) which is corresponding to the 16-23S rRNA intergenic spacer region of *Lactobacillus* spp. (Dubernet *et al*., 2002). Thus all 4 isolates were confirmed at genus level as *Lactobacillus* spp. The negative control without any template did not give any band, which is suggesting that all the 4 PCR products were correspondence of the template DNA (Fig. 2).

Analysis of probiotic characteristics: Probiotic bacteria produce a variety of substances with antibacterial properties including organic acid, H_2O_2, bacteriocins that affect bacterial metabolism or toxin production (Rafter, 2003; Rolfe, 2000;

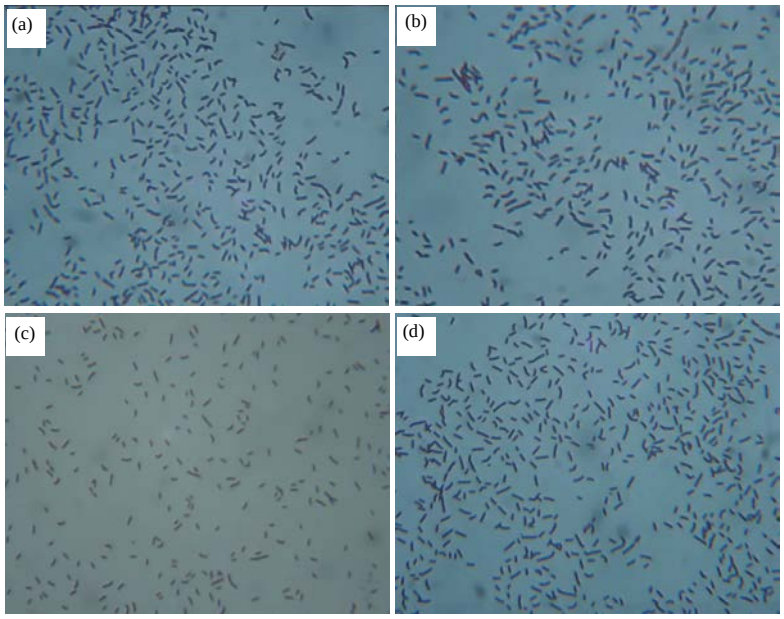

Fig. 1(a-d): Microscopic view (40X) of *Lactobacillus* after gram staining. Gram positive bacteria stained with violet colour

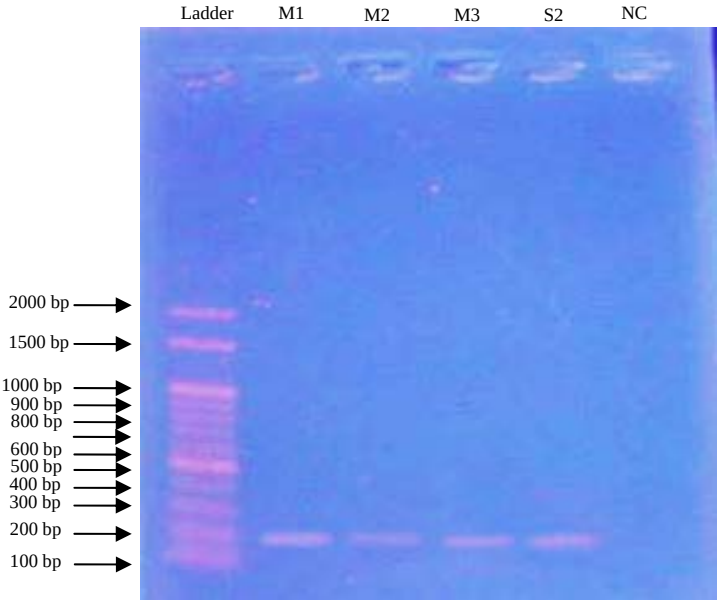

Fig. 2: Gel visualization on UV transilluminator after performing PCR in 1% agarose gel. M1, M2, M3 and S2 lane indicate the PCR products of isolate M1, M2, M3 and S2 consecutively, which are showing sharp bands besides the 200 bp of ladder sequence. Left well was loaded with ladder while the right most well was loaded with the PCR product from NC (negative control) (showing no band)

Table 1: Summarized result of bacteriological and biochemical analysis of isolates M1, M2, M3 and S2

Isolates	M_1	M_2	M_3	S_4
Colonies morphology	White irregular and round	Small cream color and shiny	Small circular and brownish	Brownish color and shiny
Macroscopic view	Short and rod shaped	Medium and rod shaped	Short rod shaped	Short and rod shaped
Gram staining	+	+	+	+
Catalase test	-	-	-	-
Oxidase test	-	-	-	-
Indole test	-	-	-	-
MR test	-	-	-	-
VP test	-	-	-	-
Citrate test	-	-	-	-
Glucose	+	+	+	+
D(-) fructose	+	+	+	+
Sucrose	+	+	+	+
Galactose	+	+	+	+
Dextrose	+	+	+	+
Lactose	+	+	+	+
Maltose	+	+	+	+
D(-)Ribose	+	+	+	+
D(+)xylose	+	+	+	+
L(+)rhamnose	+	+	+	+
D(-)mannitol	+	+	+	+

+: Positive result (Gram positive in case of Gram staining and capability in case of sugar fermentation), -: Negative result (Gram negative in case of Gram staining and inability in case of sugar fermentation)

Vandenbergh, 1993). In order to do this, they must able to withstand with the unfavorable condition in the gut like NaCl and bile salt.

NaCl tolerance test: The isolate *Lactobacillus* spp., from yogurts was able to tolerate 1-9% NaCl. To determine the NaCl tolerance of the isolates, optical density was measured at 560 nm and the data was plotted. Isolate M1, M2, M3 and S2 grew well in 1% NaCl concentration. Maximum growth (OD) of isolates M1, M2, M3 and S2 were found 1.420, 2.143, 1.662 and 2.207, respectively in 1% NaCl (Fig. 3). High salt tolerance is a desirable property for organism to be used as probiotics.

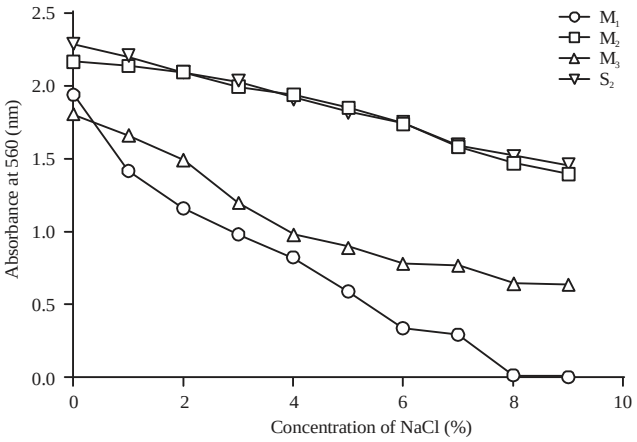

Fig. 3: NaCl tolerance test of identified isolates M1, M2, M3 and S2

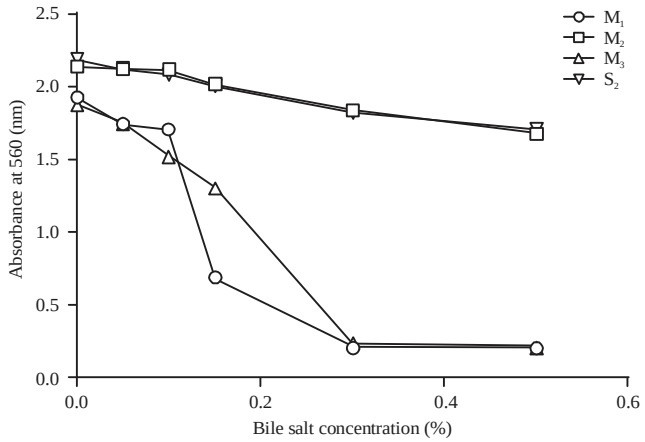

Fig. 4: Bile salt tolerance test of identified isolates M1, M2, M3 and S2

It is known that NaCl is an inhibitory substance which may inhibit growth of certain types of bacteria. In this study, the results showed that *Lactobacillus* spp., isolated from yogurts were able to tolerate 1-9% of NaCl and optimal growth was observed at 1-5% NaCl (Hoque *et al*., 2010).

Bile salt tolerance test: Isolated *Lactobacillus* spp., was able to survive in 0.05, 0.1, 0.15 and 0.3% bile acid. The isolated *Lactobacillus* spp., was also able to multiply in above mentioned concentrations of bile acid. Optical density was measured at 560 nm and the data was plotted. All isolates grow well in 0.05% bile salt concentration. Maximum growths (OD) of isolates M1, M2, M3 and S2 were found 1.741, 2.213, 1.758 and 2.125, respectively. The growth rate was decreased with the increasing level of bile salt concentration (Fig. 4).

In this experiment 0.05-0.3% of bile concentration were used which may found in the human intestinal tract and maximum concentration of bile that is present in healthy man is 0.3% (Graciela and Maruia, 2001). It is reported that, before selecting a probiotic bacteriam for human consumption it must be tolerable to 0.3% bile concentration (Gilliland *et al*., 1984). Based on the result, it is suggested that, these strains may be potential for use as probiotic organism because all of the isolates were resistant and able to grow in 0.3% bile salt concentration.

Determination of optimal pH for growth: The isolated *Lactobacillus* spp., from yogurts was able to grow up pH ranges from 4.0-8.0. Optical density was measured at 560 nm and the data was plotted. Maximum growth (OD) of isolates M1, M3 and S2 were found 2.201, 2.0619 and 2.237, respectively at pH 6.0 whereas maximum growth of isolates M2 was 2.259 at pH 6.5 (Fig. 5). The isolates were able to grow at pH between 4.0 and 8.0 but the optimum growth was observed at pH between 5.0 and 6.5 when grown in MRS broth at 37°C. From this study it can be concluded that the growth rate of *Lactobacillus* spp., decreases at certain stage when pH concentration increases (Chowdhury *et al*., 2012).

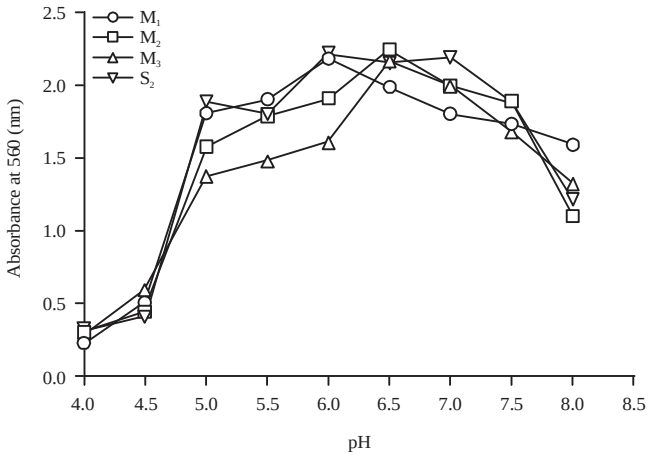

Fig. 5: Effect of pH on growth of identified isolates M1, M2, M3 and S2

Table 2: Quantification of organic acid and determination of pH value

Sources of bacteria	Incubation time (h)	Incubation temperature (°C)	Organic acid (%)	pH
***Lactobacillus* spp.**				
Isolate M1	24	37	1.213	4.01
	48	37	1.641	3.84
	72	37	1.827	3.69
Isolate M2	24	37	1.397	4.00
	48	37	1.820	3.79
	72	37	2.042	3.68
Isolate M3	24	37	1.450	3.99
	48	37	2.001	3.71
	72	37	2.119	3.65
Isolate S2	24	37	1.372	3.99
	48	37	1.565	3.72
	72	37	1.900	3.64

Quantification of organic acid and determination pH value: For the quantification of organic acid, titration method was used.

$$\text{Quantification of organic acid} = V \times N \times D$$

where, V is volume of NaOH, N is strength of NaOH and D is dilution factor. The result of organic acid production is presented in Table 2.

This study indicates that organic acid production was increased with the incubation time but at the same time pH of the media decreased with the increasing acid production. From the result (Table 2) highest acidity (1.9%) and lowest pH 3.64 was observed after 72 h incubation at 37°C for probiotic *Lactobacillus* isolated form Bogra yoghurt. Other probiotic bacteria isolated form yoghurt of different supermarkets of Chittagong showed the highest acidity 1.83, 2.11 and 2.11% and lowest pH 3.9, 3.68 and 3.65, respectively after 72 h incubation. There is a minor variation in organic acid production by lactobacilli due to their regional differences, indicating that these isolates are slightly climate and environment dependent (Hoque *et al*., 2010).

Table 3: Screening of antibacterial activity against eight pathogenic bacteria after 72 h of four isolates

	Inhibition zone (mm)			
Test pathogens	Isolate M1	Isolate M2	Isolate M3	Isolate S2
Shigella dysenteriae	42.66±1.23	30.03±1.20	27.76±1.14	39.23±1.23
Bacillus cereus	51.20±1.22	27.43±1.00	34.53±1.10	34.43±1.20
Pseudomonas aeruginosa	26.70±1.00	36.46±1.30	42.90±1.20	35.13±1.20
Bacillus megaterium	44.03±1.33	20.10±1.00	28.80±1.06	24.50±1.22
Staphylococcus aureus	27.73±1.30	20.60±1.33	17.83±1.10	21.63±1.10
Vibrio cholerae	28.40±1.20	26.20±0.00	26.70±1.14	29.93±1.20
Escherichia coli	46.70±1.10	38.43±1.00	36.50±1.00	43.80±1.20
Shigella sonnei	23.46±1.00	25.30±1.22	20.10±1.10	26.23±1.40

Screening of interference with pathogenic bacteria: Probiotics including *Lactobacillus*, *Bifidobacterium* and *Streptococcus* spp., are known to be inhibitory to the growth of a wide range of intestinal pathogens in human. In addition to the favorable effects against disease caused by an imbalance of the gut microflora several experimental observations of bacteria against the development of colon tumors are reported by Dunne *et al*. (2001) and Wollowski *et al*. (2001).

In this study, the selected 4 isolates were examined according to their antibacterial activity against different pathogenic bacteria such as *Shigella dysenteriae*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Bacillus megaterium*, *Staphylococcus aureus*, *Vibrio cholerae*, *Escherichia coli* and *Shigella sonnei* associated with food borne diseases. The comparison of their inhibition (in mm) against 8 tests pathogens is shown in Table 3.

The experimental results showed that the highest inhibitory activity of isolate M1 was demonstrated against *Bacillus cereus* (51.20±1.22 mm) and lowest zone of inhibition was (23.46±1.00 mm) against *Shigella sonnei* after 72 h incubation. The highest diameter of inhibition zone of isolate M2 was showed against *E. coli* (38.43±1.00 mm) and lowest zone (20.10±1.00 mm) against *Bacillus megaterium* after 72 h of incubation. Similarly, the highest diameter of inhibition zone of isolate M3 was showed against *P. aeruginosa* (42.90±1.20 mm) and lowest zone (17.83±1.10 mm) against *S. aureus*. And finally, in isolate S2, highest zone was observed against *E. coli* (43.80±1.20 mm) and lowest zone (21.63±1.10 mm) against *S. aureus* after 72 h incubation. The results related to *Bacillus cereus*, *Pseudomonas aeruginosa*, *Bacillus megaterium*, *Staphylococcus aureus*, *Vibrio cholerae* and *Escherichia coli* were found relevant to the findings of Chowdhury *et al*. (2012).

In the present study, isolates M1, M2, M3 and S2 showed satisfactory results concerning bacteriocidal and bacteriostatic activity. Isolate M1 was bactericidal to *Bacillus megaterium*, *Vibrio cholera*, *Shigella sonnei* and bacteriostatic to *Shigella dysenteriae*, *Bacillus cereus*, *P. aeruginosa*, *S. aureus* and *E. coli.* Isolate M2 was bactericidal to *Bacillus cereus*, *Shigella sonnei* and bacteriostatic to *Shigella dysenteriae*, *Pseudomonas aeruginosa*, *Bacillus megaterium*, *Staphylococcus aureus*, *Vibrio cholerae*, *Escherichia coli*. Isolate M3 was bactericidal to *Shigella dysenteriae*, *Bacillus megaterium* and *Staphylococcus aureus* and bacteriostatic to *Bacillus cereus*, *Pseudomonas aeruginosa*, *Vibrio cholerae*, *Escherichia coli* and *Shigella sonnei* and isolate S2 was bactericidal to *Shigella dysenteriae*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Vibrio cholerae* and *Shigella sonnei* and bacteriostatic to *Bacillus megaterium* and *Escherichia coli*.

CONCLUSION

In this study the *Lactobacillus* spp., were isolated and identified from selective regional yoghurts. For the identification of bacteria several bacteriological and biochemical tests were performed. Beside this a PCR was conducted using the genus specific primers (LbLMA-rev) and universal (primer R16-1) corresponding to the 16-23S rRNA intergenic spacer region of *Lactobacillus* spp., to confirm the bacteriological identification. The isolated *Lactobacillus* spp. were able to tolerate inhibitory substances like NaCl (1-9%) and bile salt (0.05-0.3%) and also able to survive in alkaline condition (pH 8.0). All these isolates (M1, M2, M3 and S2) were capable of utilizing carbohydrates such as glucose, xylose, sucrose, fructose, galactose, lactose, maltose, ribose, rhamnose, mannitol and dextrose. Furthermore, the isolated *Lactobacillus* spp., from all 4 isolates (M1, M2, M3 and S2) was able to produce organic acid in milk. This investigation indicates that there is a minor variation in organic acid production by *Lactobacillus* due to their regional variation. The presence of antibacterial activities of all 4 isolates (M1, M2, M3 and S2) against eight common human pathogenic bacteria was found satisfactory and the isolates produce bacteriocin extra cellularly. A probiotic must be able to inhibit pathogenic organisms and should be able to tolerate the harsh conditions of human gut such as high salt, low pH and high bile salt concentration. All of the isolated *Lactobacillus* spp., fulfilled these criteria and hence they may be considered as potential probiotic for human health.

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