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Full Length Research Paper

Isolation and presumptive characterization of probiotic lactic acid bacteria from selected yoghurt

***Humayun Abed Ershad, Muhammed Karim and Fazlur G. Shabnam**

Life Science School, Biotechnology and Genetic Engineering Discipline, Khulna University, Khulna-9208, Bangladesh.

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Probiotics, as functional food components, are recognized as safe microorganisms of viable single or mixed cultures with claimed health promoting effects on their host by improving the properties of the indigenous intestinal microflora. In the present study, a total of ten probiotic lactic acid bacteria were isolated comprising *Lactobacillus acidophilus*, *Lactobacillus brevis*, *Bifidobacterium* sp. and *Streptococcus thermophilus*. According to morphological, physiological, and biochemical assays, all the isolates were gram positive, endospore negative, catalase negative, non-motile, and possessed bile salt hydrolase activity characteristic to probiotic bacteria. Carbohydrate fermentation profiles ensured the presumptive identification. Importantly, the isolates were resistant to artificial gastric juice environment at pH 2.2, and their resistance decreased after 24 h of incubation at 37°C. Moreover, bile salt tolerance was observed not only at 0.05, 0.1, 0.15, and 0.3% artificial bile from 0 to 4 h of incubation at 37°C, but also started multiplication after 16 h. The best phenol tolerance found at 0.1 to 0.2% phenol, very low at 0.3 and 0.4% phenol after 12 and 24 h of incubation, respectively. They also possessed excellent tolerance against 1 to 7% NaCl. Because of being probiotic potentiality, the best isolates can be used for probiotic product development in future.

Key words: Probiotics, artificial bile, artificial gastric juice, bile salt hydrolase, carbohydrate fermentation.

INTRODUCTION

The word „probiotic" derived from the Greek word „pro bios" which means „for life". According to World Health Organization (WHO)/Food and Agriculture Organization (FAO), probiotics are „live microorganisms which when administered in adequate amounts confer a health benefit on the host". According to Salminen et al. (1998), probiotics can be defined as „a live microbial food ingredient that is beneficial to health".

Lactic acid bacteria are ubiquitous in nature and their nutritional requirements are highly complex. Therefore, their predominate habitats are rich in carbohydrates, protein breakdown products, vitamins, and environments with low level of oxygen. This confirms the prevalence in various kinds of dairy products (Stiles and Holzapfel,

*Corresponding author. E-mail: humayun.abed@yahoo.com

1997). Most probiotic microorganisms belong to the group of lactic acid bacteria (LAB) that reportedly play a significant role in maintaining the intestinal ecosystem and in stimulating the immune system of the host (Saarela et al., 2002). Species of the genera *Lactobacillus* and *Bifidobacterium* are the most commonly used probiotics of human food and animal feeds (Belicova et al., 2013). The strains of *Lactobacillus, Bifidobacterium,* and *Streptococcus* have traditionally been used in the manufacture of various kinds of fermented dairy products and are generally regarded as safe (GRAS status) according to the American Food and Drug Administration due to their long history of safe use in fermented foods and feeds, and their presence in the intestinal and urogenital microbiota (O"Sullivan et al., 1992; Belicova et al., 2013). In addition, these bacteria are desirable members of the intestinal microflora (Berg, 1998). A clinical study revealed that, *Lactobacillus* species containing probiotics are associated with a reduction in antibiotic associated diarrhea and boost the immune system (Hempel et al., 2012). Lack of pathogenicity, tolerance to gastrointestinal conditions, such as gastric acid and bile, tolerance to phenol, NaCl, bile salt hydrolase activity are some of the general criteria for the selection of probiotics (Collins et al., 1998; Ouwehand et al., 2002a; Pereira et al., 2003; Hoque et al., 2010). LAB may provide beneficial health effects by modifying the host immune system by reducing the colonization of pathogenic microorganisms and promoting healing of damaged mucosa during bacterial adhesion to the epithelium (Ouwehand et al., 2002b).

Lactobacillus acidophilus and *Bifidobacterium bifidum* were used to make mildly acidified yogurts called "bio-yogurts" in Germany during late 1960s (Goktepe et al., 2006). Viable probiotic strains with beneficial functional properties are supplied in the markets as fermented food products, mainly "yogurt"-type, or in lyophilized form, both as food supplements and as pharmaceutical preparations. For many years, pharmaceutical preparations contain live microorganisms in capsules, also known as "biotherapeutics" after or during antibiotic treatment (Goktepe et al., 2006). As probiotic bacteria have potential therapeutic or prophylactic effects, so development of numerous probiotic products such as fermented milk drinks, yoghurt, cheese, ice-cream, sausages etc. with defined starter culture are quite demanding in the markets. However, industrial applications of probiotics are quite challenging.

Yoghurt is regarded as one of the most popular probiotic food worldwide with claimed health benefits, ranging from high nutrition value, reduction of duration of diarrhea, reduction of blood cholesterol, obesity, gastrointestinal disorders, diabetes, overall stimulation of immune system, control of gastrointestinal pathogens through antimicrobial compounds, improve lactose digestion in lactose intolerant individuals, etc. Yoghurt is defined by the *Codex Alimentarius* of 1992, the result of coagulation of milk, causing the lactic acid of *Streptococcus thermophilus*, *Bifidobacterium* species, *Lactobacillus bulgaricus* and other species depending on regional differences (Bourlioux and Pochart, 1988). Yogurt gels are built of clusters of aggregated case in particles formed as a result of gradual fermentation of lactose by lactic acid bacteria (Horne, 1999). According to the National Yoghurt Association, the criteria for active and live yoghurt is the finished yoghurt containing live lactic acid bacterial count of $\geq 10^8$ CFU/g at the time of production, and cultures must live and be active at the end of defined self-life (Chandan and Shahani, 1993). Consumption of milk and milk products was traced back to the time when people used domesticated mammals. It is commonly decided among historians that yogurt and other fermented milk products were discovered by chance as a consequence of milk being stored in warm climates. Most historical accounts attribute yogurt to the Neolithic peoples of Central Asia around 6000 B.C. Herdsmen began the practice of milking their animals and the natural enzymes in the carrying containers (animal stomachs) curdled the milk, essentially making yogurt. Not only did the milk then keep longer, it is thought that people preferred the taste, so they continued the practice, which then evolved over centuries into commercial yogurt making. Yoghurt was thought to be originated from the Middle-East. The word „yoghurt" is derived from the Turkish word, „yoğurt", which means "to curdle or coagulated; to thicken". The first written description of yoghurt was printed in Diwanu I-Lugat al-Turk, a Turkish dictionary written in 100 to 1073. The popularity of yogurt soared in the 50 and 60s with the boom of the health food culture and is now available in many varieties to suit every taste and lifestyle (DNR, 2014). Yogurt is also very healthy as a part of daily diet. The most common types of yogurt are set type yogurt and strained yogurt. Set type yogurt is fermented in containers and no water removal takes pace after the fermentation. Strained or Greek style yogurt is fermented in tanks under continuous mild stirring and after the completion of fermentation, a portion of the whey is removed.

Yogurt is mainly produced from bovine milk. Raw milk undergoes centrifugal clarification to remove somatic cells and solid impurities, followed by a mild heating process, known as thermalization, at 60 to 69°C for 20 to 30 s. The purpose of heat treatment is to kill any vegetative microorganisms and the partial inactivation of unwanted enzymes. Then, milk is cooled at <5°C, at that time, inoculation with lactic acid bacteria is performed for fermentation of milk lactose to produce lactic acid by the action of enzyme lactase. Lactase converts lactose into glucose and galactose, which upon glycolysis and fermentation produce lactic acid at the end. The fat content of the milk is adjusted to range from <0.5% for skim milk to 1.5 to 2% for semi-fat milk to 3.5% for full fat milk. The fat content ranges from 0.1 to 10% according to

consumer demands. Fermentation process is the starter culture that acts through biochemical reactions and inductively causes the formation of the curd and the development of flavor components when incubated overnight at 37 to ~40°C. After incubation, the fermentation period is completed by lowering the temperature to 4°C and the produced yoghurt is ready for package and commercial distribution and consumption (Sfakianakis and Tzia, 2014). It is a good source of calcium, phosphorus, riboflavin-vitamin B2, iodine, vitamin B12, pantothenic acid-vitamin B5, zinc, potassium, protein, and molybdenum. Yogurt is also high in probiotics that can help a person live longer. The bacteria can also help boost the immune system. People who have allergies to dairy products are advised to consume yogurt as it does not produce the allergy that is caused by lactose. Yogurt is also a good option for people who suffer from stomach ailments, such as diarrhea. Consumption of low-fat yogurt can also aid in weight loss. Yoghurts are distributed and consumed in three different ways depending on regional preferences. Firstly, the Balkan style or set style yoghurt with a thick texture, consumed in the Middle-East and India. Secondly, Swiss style or stirred style yoghurt, slightly thinner than Balkan-style or set yogurt can be eaten as-is, in cold beverages or incorporated into desserts or fruits. Thirdly, Greek style yoghurt, which is a very thick yogurt that is either made from milk that has had some of the water removed or by straining whey from plain yogurt to make it thicker and creamier. Yoghurts are now industrially produced and commercialized into different shapes. These are frozen type yoghurt, yoghurt drink, fat free, gluten free, artisanal type, organic type, fruits mixed yoghurt, etc.

However, Bogra district in Bangladesh is famous for the finest quality of artisanal yoghurt production in terms of taste and odor. Therefore, to accomplish part of the probiotic product development steps, such as isolation of probiotic bacteria, characterization and probiotic properties determination of isolated bacteria, selective Bogra district yoghurts of Bangladesh were considered in this study. Moreover, to the best of our knowledge, literature review revealed that no such extensive studies regarding regional yoghurt was conducted so far in Bangladesh.

Therefore, the present research work was undertaken with the following objectives:

(1) Isolation and presumptive characterization of probiotic lactic acid bacteria from selected yoghurt.
(2) Study of probiotic properties of identified probiotic lactic acid bacteria.

MATERIALS AND METHODS

Collection of samples

Four artisanal yoghurt samples were collected from Bogra district of Bangladesh. The experiments were conducted at the “Food and Molecular Biotechnology Laboratory” of “Biotechnology and Genetic Engineering Discipline”, Khulna University, Bangladesh. For collection, the newly manufactured samples were transported in ice and stored for several hours aseptically at 4°C in refrigerator to protect from deterioration and contamination. Pour-plate method was conducted immediately at the same day for colony morphology observation and isolation. The famous shops from which the samples were collected were:

1. Sample No. 01: Ruchita Hotel and Restaurant, Sheikh Sorifuddin Super Market, Bogra.
2. Sample No. 02: Gourogopal Dodhi and Mistanno Vhandar, Nabab Bari More, Bogra.
3. Sample No. 03: Mohorom Ali Dahi Ghor, Station Road, Satmatha, Bogra.
4. Sample No. 04: Sherpur Dahi Ghor, Station Road, Satmatha, Bogra.

Chemicals and equipments

All the chemicals, sugars/carbohydrates, MRS, and ST culture media, motility–Indole-Lysine (MIL) medium components, and staining kits were purchased from the Sigma-Aldrich (India). 3% H_2O_2 (Sigma-Aldrich, USA) was purchased for catalase test. Bench top centrifuge (model 5415D, Eppendorf, Hamburg, Germany) was used for centrifugation purposes, and microplate reader (Multiskan FC Microplate photometer, USA) was used for optical density measurements.

Isolation of probiotic bacteria

For each sample, 1 g of yoghurt was dissolved in 9 ml of sterile peptone water solution (0.15% peptone), and serially diluted up to ten logarithmic (10^{-10}) fold. The diluted sample was then inoculated into the De-Man Rogosa Sharpe (MRS) agar plate for *Lactobacillus* species isolation or ST agar plate for *Streptococcus* species isolation by ensuring the optimum pH, incubation temperature, and incubation time anaerobically as shown in Table 1. The isolated cultures were maintained in MRS broth as a pure culture at pH 6.5 (International Dairy Federation, 1998). *Lactobacillus* spp. was isolated from the collected yoghurt using MRS media (De Man et al., 1960). *Bifidobacterium* spp., *S. thermophilus*, *L. acidophilus,* and *Lactobacillus brevis* are the most widely used species for commercial production of yoghurt and hence were targeted for isolation in the present study (Martini et al., 1991). For isolation, *L. acidophilus* was subjected to cultivate on MRS agar medium supplemented with 0.5% salicin (Dave and Shah, 1996), while L-cysteine (0.05%) was added to MRS medium to improve the specificity for isolation of *Bifidobacterium* spp. (Zinedine and Faid, 2007). ST agar medium was used for isolation of *S. thermophilus* (Driessen et al., 1977).

Lactic acid bacteria characterization

Isolates were further purified by streaking repeatedly and colony morphologies were observed. *S. thermophilus* colonies were selected based on coccoid shape, spherical or oval and occur in chains from samples No. 01 and 03. In addition, rod shaped, regular in long chains were selected for isolation of *L. acidophilus* from samples No. 01, 02, and 04. Rod shape, regular, occur singly/chain were observed for *L. brevis* from sample No. 01, 02, 03, and 04. Tiny rod, branched, v and y arrangement in chains were observed in *Bifidocterium* spp. from sample No. 02 (Table 2). Gram staining and catalase test were performed according to standard

Table 1. Optimum conditions for growth of lactic acid bacterial (LAB) isolates (Linn et al., 2008; Hoque et al., 2010; Saccaro et al., 2011)

Species name	Medium pH	Incubation time (h)	Incubation temperature (°C)
Lactobacillus acidophilus	6.5	24	37
Bifidobacterium spp.	5.2	72	45
Lactobacillus brevis	6.5	24	37
Streptococcus thermophilus	6.8	48	37

Table 2. Relation among sample collection shop, sample number and isolate number.

Shop name	Sample number	Isolate number	Species name
Ruchita Hotel and Restaurant	01	01	*Streptococcus thermophilus*
Mohorom Ali Dahi Ghor	03	02	*Streptococcus thermophilus*
Sherpur Dahi Ghor	04	03	*Lactobacillus brevis*
Gourogopal Dodhi and Mistanno Vhandar	02	04	*Bifidobacterium* spp.
Gourogopal Dodhi and Mistanno Vhandar	02	05	*Lactobacillus brevis*
Gourogopal Dodhi and Mistanno Vhandar	02	06	*Lactobacillus acidophilus*
Ruchita Hotel and Restaurant	01	07	*Lactobacillus acidophilus*
Mohorom Ali Dahi Ghor	03	08	*Lactobacillus brevis*
Sherpur Dahi Ghor	04	09	*Lactobacillus acidophilus*
Ruchita Hotel and Restaurant	01	10	*Lactobacillus brevis*

procedures. Schaeffer and Fulton (1933) method was employed for staining endospores.

Carbohydrate/Sugar fermentation profiles were done according to Erkus (2007) and Rahman et al. (2015), using sixteen different carbohydrates. Briefly, the first step was the preparation of active cells free from sugar residues (centrifugation at 10,000 rpm for 10 min, followed by resuspending the pelleted cells in 10 ml MRS without glucose, and containing bromecresol purple). The second step was the preparation of sterile sugar solutions. Finally, sugar solutions and active cell culture without sugar were combined. 200 µl of active cell solution without sugar was used as negative control. After overnight incubation at 37°C, the turbidity and the color change from purple to yellow with respect to negative and positive controls were recorded as positive fermentation result. All the reactions were performed in triplicate using 96-well microtiter plates.

Motility-Indole-Lysine (MIL) medium was used to determine the motility of microorganisms (Difco, 1998; Reller and Mirrett, 1975). Using a sterile needle, a well-isolated colony was picked and the medium was stabbed within 1 cm of the bottom of the tube. Incubation was accomplished at 35°C for 18 h or until growth was evident. A positive motility test result was indicated by a diffuse cloud of growth away from the line of inoculation. For maintenance, subcultures were prepared every week for maintenance of lactic acid bacteria for daily or weekly use.

Study of probiotic properties of lactic acid bacteria

Gastric juice resistance and bile salt tolerance were assayed using the method of Graciela and Maria (2001) with some modification and Zinedine and Faid (2007) to some extent where it was necessary. OD_{620nm} of cell growth in gastric juice and bile salt medium were taken for detecting the cell resistance and multiplication using microtiter plate reader. At 0 h, 1% of overnight bacterial culture was inoculated to the culture medium containing gastric juice (pH 2.2), or bile salt.

MRS broth was modified with 0.1 to 0.4% phenol to determine the phenol tolerance of the isolates. At 0 h, 1% of overnight bacterial culture inoculated to the culture medium containing 0.1 to 0.4% phenol. Uninoculated phenol solution served as negative control. Different concentrations (1 to 10%) of NaCl were inoculated into MRS medium. Growths were observed based on turbidity (Hoque et al., 2010). MRS agar medium with 0.5% (w/v) of the sodium salt of taurodeoxycholic acid (TDCA) was used to prepare test plates for plate assay (Dashkevicz and Feighner, 1989).

RESULTS

All the isolates were gram positive due to retain violet blue color and catalase negative due to the absence of catalase enzyme, confirmed by the lack of production of H_2O and O_2, when colonies were treated with H_2O_2. Moreover, endospore staining is one tool in the arsenal of bacterial identification method. Bacterial endospores are differentiated cells formed within the vegetative cells. The observed vegetative cells of the isolates were brownish red to pink, and no indication of bright green endospore, and hence all were endospore negative. In addition, motility has long been recognized as an important taxonomic tool and biological characteristic of microorganisms. The isolates were observed to be non-motile, indicated by growth along the inoculation line, but not further. Physiological and biochemical characteristics are shown in Table 3. Carbohydrate fermentation tests detect the ability of microorganisms to ferment a specific

Table 3. Physiological and biochemical characteristics of isolated LAB.

Physiological and biochemical characteristics	*Streptococcus thermophilus* (Isolate No. 01 and 02)	*L. acidophilus* (Isolate No. 06 07, and 09)	*L. brevis* (Isolate No. 03, 05, 08, and 10)	*Bifidobacterium* spp. (Isolate No. 04)
Gram staining	+	+	+	+
Catalase	-	-	-	-
Endospore	-	-	-	-
Motility	-	-	-	-
Bile salt hydrolase	+	+	+	+

Table 4. Presumptive identification of isolated LAB by sugar/carbohydrate fermentation pattern.

Isolate number	Lactose	Mannitol	Sucrose	Fructose	Salicin	Ribose	Cellubiose	Glucose	Maltose	Xylose	Rhamnose	L-Arabinose	D-Sorbitol	D-Mannose	Raffinose	Galactose	Conclusion/Presumptive identification
Isolate No. 01	++	--	++	++	++	++	++	++	++	+	--	++	++	++	--	++	*Streptococcus thermophilus*
Isolate No. 02	++	--	++	++	++	+	++	++	++	+	--	++	++	++	--	++	*Streptococcus thermophilus*
Isolate No. 03	++	+	++	++	++	++	++	++	++	++	--	++	--	++	-	++	*Lactobacillus brevis*
Isolate No. 04	++	+	++	++	++	++	++	++	++	++	--	+	--	++	+	++	*Bifidobacterium* spp.
Isolate No. 05	++	+	++	++	++	++	++	++	++	++	--	+	--	++	+/-	++	*Lactobacillus brevis*
Isolate No. 06	++	+/-	++	++	++	++	+/-	++	++	++	++	++	++	++	--	++	*Lactobacillus acidophilus*
Isolate No. 07	++	--	++	++	++	++	++	++	++	++	--	++	--	++	--	++	*Lactobacillus acidophilus*
Isolate No. 08	++	+	++	++	++	++	++	++	++	++	--	++	--	++	--	+	*Lactobacillus brevis*
Isolate No. 09	++	--	++	++	++	+	++	++	++	++	--	++	--	++	--	+	*Lactobacillus acidophilus*
Isolate No. 10	++	+/-	++	++	++	++	++	++	++	++	--	++	--	+	+	+	*Lactobacillus brevis*

(++) Excellent fermentation; (+) moderate fermentation; (- -) no fermentation, (-) very low fermentation, (+/-) low fermentation. LAB species identification results in Table 4 were analyzed based on Erkus (2007), Tamime (1985), Karna et al. (2007), Erdogrul and Erbilir (2006) and Rahman et al. (2015).

carbohydrate. Fermentation patterns can be used to differentiate among bacterial groups or species (Bartelt, 2000; Forbes et al., 2007; MacFaddin, 2000). Therefore, a total of 16 carbohydrates were used to presumptively identify the species of the isolates. The change of purple color of the MRS broth medium to yellow was the indication of fermentation due to lactic acid production (Table 4).

The isolates had the ability to survive in artificial gastric acid environment at low pH (pH 2.2), but their survival ability decreased after 24 h of incubation at 37°C (Figure 1). In addition, isolates 1 and 2 of the *S. thermophilus* showed the best tolerance in the gastric juice environment after 1 and 2 h of incubation in comparison to most of the isolates. Interestingly, the isolates exhibited reduced tolerance at pH 2.2, after 3 h of incubation

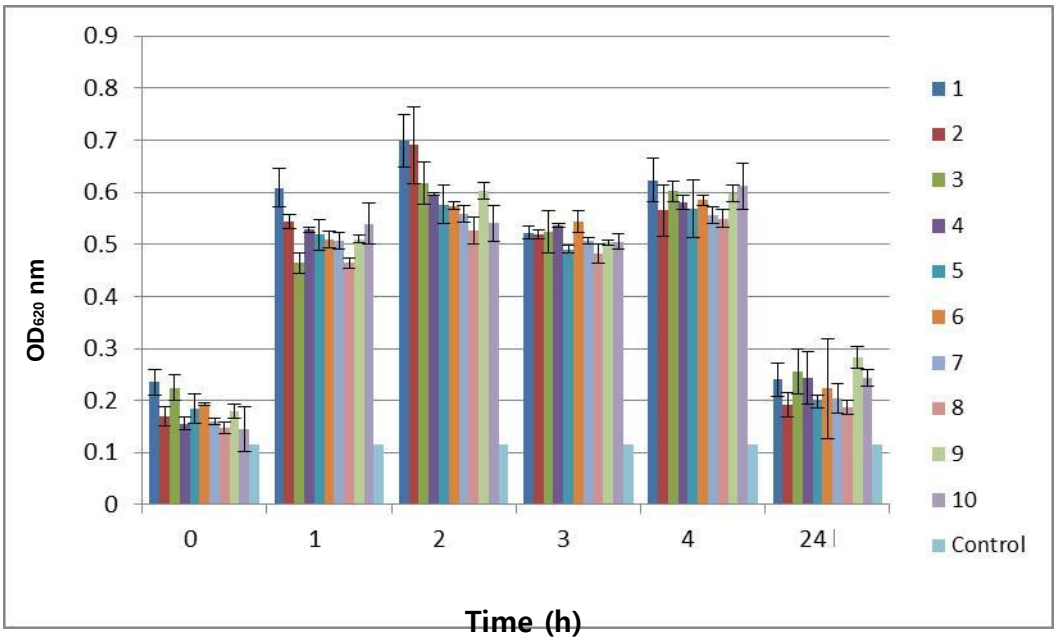


Figure 1. Survival and multiplication abilities of identified lactic acid bacterial isolates in artificial gastric juice at pH 2.2. Uninoculated medium was served as negative control. High $OD_{620\ nm}$ value bar diagram lines indicate more gastric juice resistance ability. Bars indicated standard error of the averages (n=3).

as compared to 2 h and further multiplication observed after 4 h of incubation (Figure 1). Furthermore, tolerance to 0.05, 0.1, 0.15, and 0.3% bile salt exhibited after 16 and 24 h of incubation at 37°C (Figure 2). From 0 to 4 h, the isolates were not started multiplication and as the times elapsed, they were able to grow in the bile salt environment. Importantly, after 24 h, their growth was the highest and indicated a sign of probiotic potentiality of all the isolates as excellent bile salt tolerance capability.

After 24 h, excellent tolerance and growth was detected at 0.1 and 0.2%, but very low at 0.3 and 0.4% phenol (Figure 3). Moreover, excellent NaCl tolerance was detected at 1 to 7% NaCl, moderate at 8 and 9%, but no growth occurred at 10% by observing turbidity (Table 5). At 0.1 to 0.3% phenol, isolate No. 07 showed the best tolerance ability as compared to other three isolates of *L. acidophilus*, but at 0.4% phenol, isolate No. 06 showed the best tolerance ability after 12 and 24 h of incubation (Figure 3). A 0.4% concentration of phenol causes a bacteriostatic action in some microorganisms (Xanthopoulos et al., 2000). When bile salt hydrolase-producing isolated and identified lactic acid bacteria were streaked out on MRS agar plates containing 0.5% taurodeoxycholic acid (TDCA), the taurine-conjugated bile acid was deconjugated, producing deoxycholic acid. This deconjugation activity of isolates colonies were turn into opaque granular white colonies or precipitate halos around colonies characteristic to bile salt hydrolase-activity. Copius amount of deoxycholic acid precipitated around active colonies and diffused into the surrounding medium or producing precipitate halos. The production of opaque granular white colonies or precipitate halos around colonies indicated bile salt hydrolase-active of all the isolates of the present study showing one of the most important characteristics of probiotic bacteria. A plus (+) sign indicates white granular opaque colonies (Table 3).

DISCUSSION

Recent understanding of the functions of intestinal microflora and the use of probiotic microorganisms is a novel concept to improve human health and an innovative approach for new food product development in functional foods for specific diseases. In this study, efforts were made to isolate and identify the best yoghurt lactic acid bacteria from the best artisan yoghurt production district Bogra in Bangladesh. For this purpose four different samples of yogurt were selected finally from the best shops. A total of ten lactic acid bacteria were isolated and identified and their probiotic properties were evaluated to determine which species will be the best choice for future probiotic product development attempt. The main task of carbohydrate fermentation test is to investigate the ability of bacteria to ferment different types of carbohydrate and uses as a method to identify the species. Therefore, species identification of the present study was determined using sixteen carbohydrates as the main species identification assay method. Ability to ferment carbohydrates of a particular LAB species is not exactly the same, because of geographical differences of the country, regional location

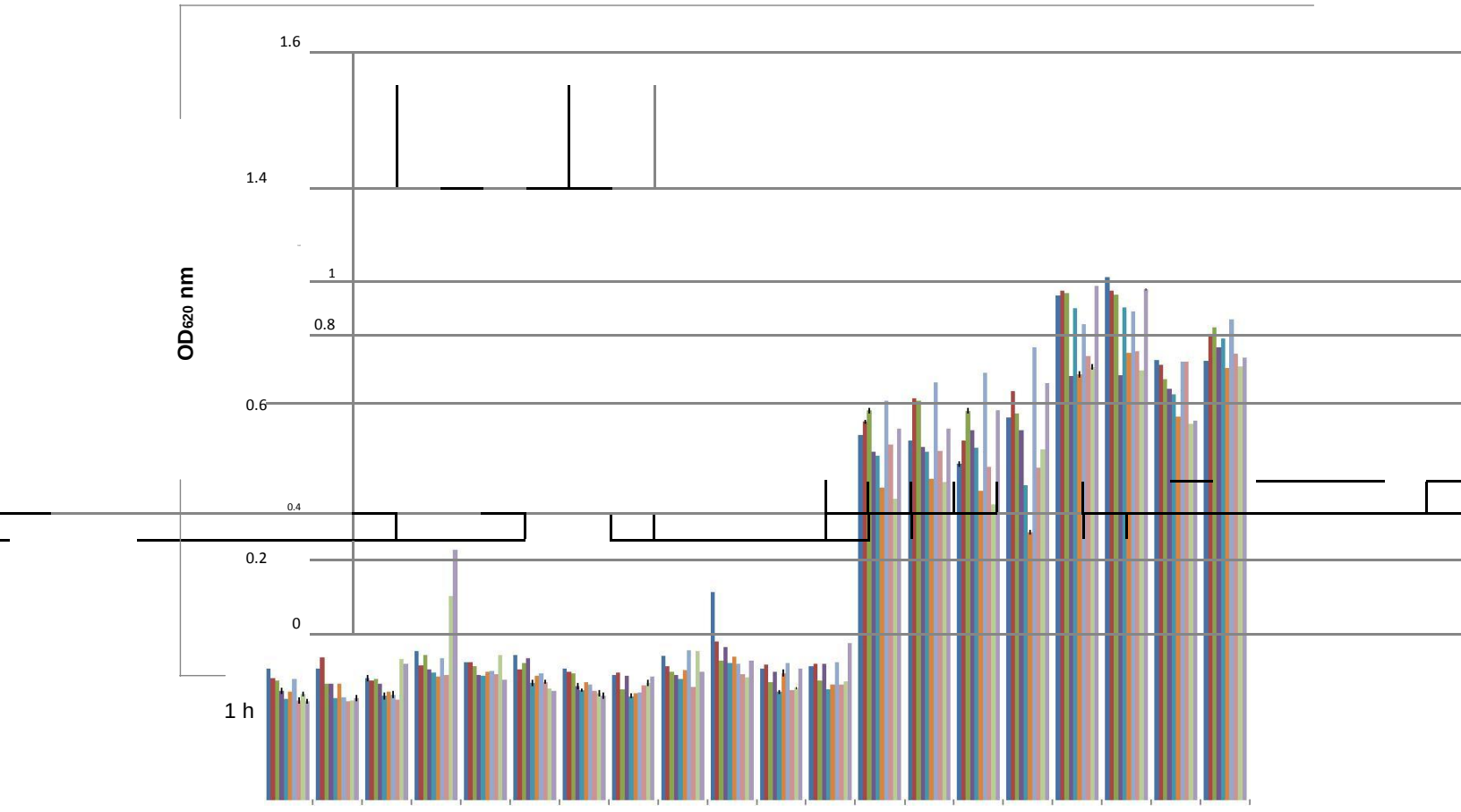
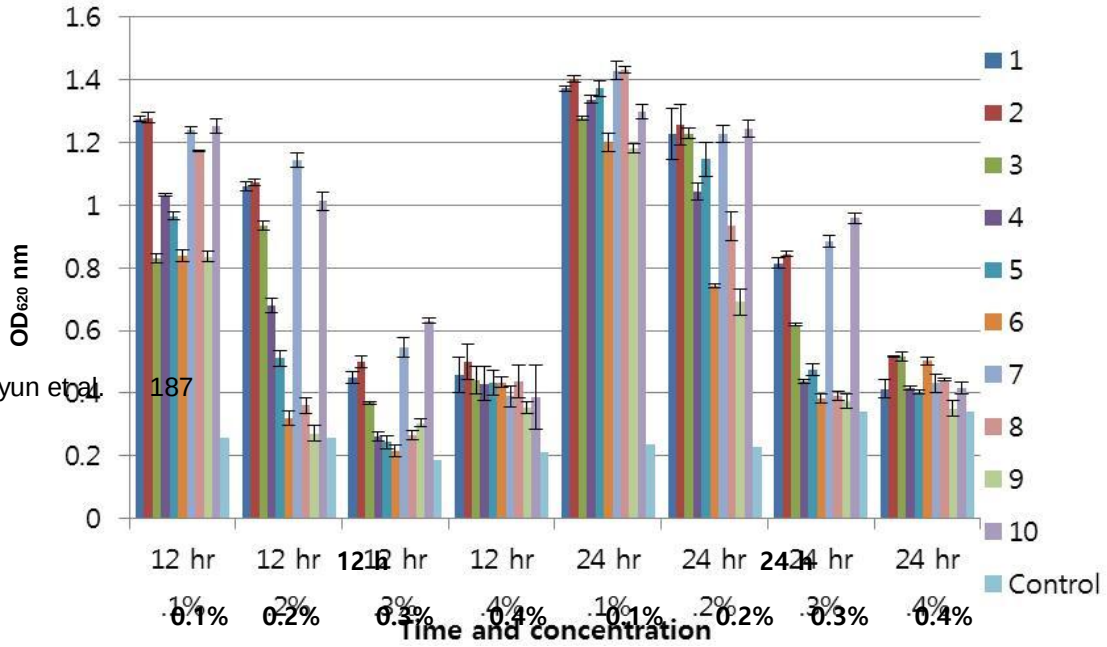


Figure 2. Bile salt tolerance of the isolates at 0.05, 0.1, 0.15, and 3% concentrations at 1, 2, 4, and 24 h of incubation at 37°C. Uninoculated bile salt medium was served as negative control ($OD_{620\ nm}$ 0.21). High $OD_{620\ nm}$ value bar diagram lines indicate more bile salt tolerance ability. Bars indicated standard error of the averages (n=3).



Time and concentration

Figure 3. Tolerance of the isolates at 0.1, 0.2, 0.3, and 0.4% phenol after 12 h and 24 h of incubation at 37°C. High $OD_{620\ nm}$ value bar diagram lines indicate more tolerance ability. Uninoculated phenol medium was used as negative control. Bars indicated standard error of the averages (n=3).

Table 5. NaCl tolerance test of isolated LAB.

NaCl concentration (%)	Isolate No. 1 (Sample No. 01)	Isolate No. 2 (Sample No. 03)	Isolate No. 3 (Sample No. 04)	Isolate No. 4 (Sample No. 02)	Isolate No. 5 (Sample No. 02)	Isolate No. 6 (Sample No. 02)	Isolate No. 7 (Sample No. 01)	Isolate No. 8 (Sample No. 03)	Isolate no. 9 (Sample No. 04)	Isolate No. 10 (Sample No. 01)
1	++	++	++	++	++	++	++	++	++	++
2	++	++	++	++	++	++	++	++	++	++
3	++	++	++	++	++	++	++	++	++	++
4	++	++	++	++	++	++	++	++	++	++
5	++	++	++	++	++	++	++	++	++	++
6	++	++	++	++	++	++	++	++	++	++
7	++	++	+	++	++	++	++	++	++	++
8	+	+	+	+	+	+	+	+	+	+
9	+	+	+	+	+	+	+	+	+	+
10	-	-	-	-	-	-	-	-	-	-

(++) Indicates excellent growth; (+) indicates moderate growth; (-) indicates no growth.

within the country, method of preparation, preservation of the yoghurt sample, etc.

According to the derived data (Figures 1, 2, and 3) on the resistance to artificial gastric juice at pH 2.2, bile salt and phenol tolerance, it was revealed that isolate No. 01 of *S. thermophilus* was better than isolate No. 02, while isolate No. 10 was the best among the four isolates of *L. brevis*, and all the three *L. acidophilus* isolates designated isolate No. 06, 07, and 09 were equally potent. The results of the gastric juice resistance were found similar to the results of Rahman et al. (2015). In addition, Hoque et al. (2010) isolated *Lactobacillus* spp. (isolate-2), that was also able to survive in gastric juice environment at pH 2.2. The bile salt resistance test results of the FSA project by Gibson et al. (year anonymous) provided evidence of the bile tolerance nature of some of the *Lactobacillus* spp. Elizete and Carlos (2005) stated that bile tolerance is an essential characteristic for better survival of LAB, not necessary for multiplication. Schillinger and Lucke (1987) found that the growth of lactobacilli occurred in the presence of 7.5% NaCl isolated from meat and meat products. The NaCl test results of the present study were also similar to Hoque et al. (2010) who isolated *Lactobacillus* spp. from yoghurt samples and tested different concentrations of NaCl (1 to 10%) and found 1 to 9% NaCl tolerance of their *Lactobacillus* spp. Rahman et al. (2015) also found the same result from chicken feces samples LAB isolates in NaCl tolerance assay. The bile salt hydrolase activity test results of the present study were similar to Dashkevicz and Feighner (1989).

According to World Health Organization (WHO) and Food and Agriculture Organization (FAO, 2002), working group guidelines, the probiotic organisms should possess the characteristics of resistance to gastric acid and bile with other attributed criteria. The present experimental outcome revealed that the isolated probiotic lactic acid bacteria have shown similar characteristics/ criteria defined by WHO and FAO standard. Furthermore, the best isolates could have the potential to be used for improved probiotic product development and community based establishment of probiotic product industries to empower the local people and poverty alleviation.

Abbreviation

MRS, De-Man Rogosa Sharpe.

Conflict of Interests

The authors have not declared any conflict of interests.

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