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Isolation, Identification and Characterization of Lactic Acid Bacteria from Milk and Yoghurts

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Research Article

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ABSTRACT

Nineteen samples of freshly drawn raw milk, pasteurized milk, locally and commercially manufactured yoghurts samples were evaluated for total viable count of Lactic Acid Bacteria (LAB) using selective media under aerobic and anaerobic conditions. Yoghurt samples showed higher LAB count ranged from 1.0×10^6 to 5.6×10^7 cfu/mL and 2.2×10^7 to 5.4×10^8 cfu/mL in comparison to milk samples varied from 1.1×10^5 to 7.2×10^6 cfu/mL and 6.1×10^5 to 5.3×10^7 cfu/mL respectively both at aerobic and anaerobic condition. In general, anaerobic LAB growth (6.1×10^5 to 5.4×10^8 cfu/mL) counted higher than the aerobic growth (1.1×10^5 to 5.6×10^7 cfu/mL). One hundred ninety-one (191) distinct colonies of LAB were isolated and categorized into13 groups on the basis of their colony morphology and microscopic features. These isolated and selected 13 LAB strains were identified on the basis of phenotypic, physiological and biochemical properties. Seven isolates (53.8%) were found to belong to the genus *Lactobacillus* while the remaining six isolates (46.2%) were under the genus *Streptococcus*. Identified *Lactobacillus* species includes *L. cellobiosus, L. delbrueckii, L. hilgardii, L. coryniformis subsp. coryniformis, L. salivarius, L. leichmanni* and *L. plantarum.* On the other hand, *Streptococcus* species were identified as *S. faecalis, S. lactis, S. thermophilus, S. faecium, S. sanguis* and *S. uberis.* Individual LAB were characterized for their ability to produce the high quality yoghurt in consideration to color, consistency, amount of curd, whey as well as presence or absence of gas. Considering every aspect, mixture of *S. lactis* and *S. uberis* were found to produce the better organoleptic characteristic yoghurt. Isolated LAB were also screened for the top acid producer. *Among the identified all isolates, S. uberis* produced the highest acid (w/v) 0.18% and *L. cellobiosus* and *L. delbrueckii* among *Lactobacillus* species produced highest quantity 0.15% after 6 hours of incubation period. While after prolong incubation period of 72 hours, S. *lactis and S. uberis* were the utmost producer of acid (w/v) 0.46% and 0.47% equivalent to 360 mg and 370 mg of lactate respectively. These two species in combination and with other all the species produced 0.50% and 0.60% acid equivalent to 394 mg and 472 mg of lactate respectively.

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INTRODUCTION

Milk contains high nutritive food value for the new borne mammal and human beings. It is also an ideal growth medium for the microbial proliferation. Fermented milk and milk based products at different formulation in different name are popular throughout the world for their taste as well as health benefits. Popular fermented milk products named, Tarag - China and Mongolia [1], Masai - East African Rift Valley between southern Kenya and northern Tanzania [2], Gariss – Sudan [3], White cheese and Rob – Sudan [4], Chal – Iran [5], Katyk – Bulgaria [6], Laban - Lebanon and some Arab countries [7], Kefir – Caucasian countries, Koumiss – Russia and Siberia, Mazun – Armenia, Leben – Egypt, Gioddu – Italy, Buttermilk – Western Europe, Villi and langfil – Scandinavian countries [8], cheese and yoghurts in many countries are produced by the activity of LAB. The nature of the fermented milk products depends on the type and temperature of the milk, pre-treatment steps, reaction conditions, technological approaches, microbial compositions or the added starter culture [8].

LAB are Gram positive, non-spore former, usually nonmotile, nonacid fast, nonrespiring rods or coccobacilli with frequently in chain, devoid of cytochrome, catalase negative. They grow well under anaerobic conditions but may grow in microaerophilic as well as aerobic conditions. They exhibit optimum growth at slightly lower acidic condition (pH 5.5 – 6.0) while growth is often restricted at neutral or somewhat alkaline condition (pH above 7.0 to 7.5). They are strictly fermentative, with lactic acid as the major end product during sugar fermentation [9,10]. LAB can be classified on the basis of their morphology (cocci or rods, tetrad formation), mode of glucose fermentation, growth at different temperatures and salt concentrations, and configuration of the lactic acid production (D, L or both) [10]. Besides, fatty acid composition and motility are also considered as the identifying tools. They have two different metabolic pathways for hexose fermentation. In homofermentative pathway, lactic acid (more than 85%) is major end product whereas in heterofermentative pathway lactic acid, ethanol/acetone and CO_2 are the terminal products [9,10].

Lactic acid bacteria (LAB) are widely distributed in nature, occur as natural adventitious contaminants in raw milk, yoghurt, etc. and sometimes with the coexistence of yeasts, moulds and some other pathogenic microorganisms. LAB as indigenous flora in raw milk acidify the milk very slowly due to their low numbers. Hence most of the dairy industries now a days use LAB as starter culture for the manufacture of fermented products including milk products such as yoghurt, cheese; meat products, bakery products, wine, and vegetables [8,10]. Besides, alive quantified LAB starter culture in some instances resolves the danger of being lost off the raw milk flora in the newer fermentation technology as well as giving conformance of uniformity [8], contribute to taste, flavor, aroma, viscosity and texture development of the product [11,12], alleviation of lactose intolerance [13,14] as well as preservative activity [15]. At present, LAB are in the focus of intensive research for their ability to promoting probiotic properties and exerting antagonistic effect on the gastro enteric pathogens - *Clostridium difficle, Campylobacter jejuni, Helicobacter pylori* and rotavirus [16], antitumoral activity [17], reduction of serum cholesterol, stimulation of the immune system [18] and stabilization of the gut microflora [19].

It is of immense importance to know the total numbers and composition of LAB in raw milk and fermented milk products, to explore the promising and beneficial strains and their usage as starter culture in fermentation technology for the newer and quality product innovation and novel applications as well. Conventional plate count methods using selective media are the gold standard for the determination of total LAB count as well as sophisticated techniques such as real-time quantitative PCR [20] is also employing now a day. Till date a lot of experimental procedures have been developed for the identification of LAB. Rapid identification through biochemical tests miniature - API 38 CHL strips, API 20 STREP and API 50 CHS [3,21-23]; Random amplified polymorphic DNA (RAPD)-polymerase chain reaction (PCR) [3] and RAPD-PCR followed by 16S rDNA gene sequencing [24], 16S rRNA gene sequences analysis and PCR-DGGE [1,25] are the updated biochemical and molecular techniques now a days employed for the rapid as well as huge number of LAB identification. On the contrary, in spite of having few limitations, the conventional phenotypic expression (cultural and microscopy), biochemical and physiological methods are still being considered the principal methods of LAB identification [2,21,23,26] with limited resources and simple instrumentations. This conventional cultural technique mainly employs selective media for the genus specific LAB, in some cases in combination with specific molecular techniques. This study has been conducted to determine the total load of LAB, isolation and taxonomic determination of the LAB from variety of samples including the fresh raw cow milk, pasteurized milk as well as local and commercially produced yoghurt samples applying the widely accepted cultural techniques using selective agar media, biochemical and physiological tests. This study was further extended to select the best organoleptic characteristic yoghurt producer in terms of color, consistency, quantity of whey, and presence or absence or gas in the yoghurt; to screen the effective lactate producer.

MATERIALS AND METHODS

Sampling

Fourteen fresh cow milk samples were collected into the sterilized screw cap conical flask from the nearby village of the University of Chittagong, Bangladesh and transferred to the Microbiology Laboratory within 30 mins of sampling. On the other hand, one commercially available pasteurized liquid milk and four yoghurt samples (local and commercially manufactured) were collected from different shops of the Chittagong city. All the sample were analyzed on the same day of sampling and kept at refrigerated (2-8°C) condition until microbiological analysis.

Microbiological analysis

Enumeration, isolation, storage and maintenance of LAB: 10 mL of sample was homogenized with 90 mL of 0.85% (w/v) sterile sodium chloride solution to make an initial dilution (10^{-1}). Serial dilutions upto 10^{-6} were made for each sample. 1 mL sample from each of the corresponding dilutions (10^{-5} and 10^{-6}) were inoculated into various selective media such as MRS agar (Oxoid, UK), Rogosa agar (Oxoid, UK), and Yeast Glucose Lemco Agar (YGLA). MRS agar (pH 6.2-6.6) and Rogosa agar (pH 5.2-5.6) for total LAB enumeration as well as lactobacilli isolation [27] while YGLA (pH 7.0) for the isolation of streptococci [28] of LAB origin. Inoculated plates were then incubated at 37°C for 48-72 hours both at aerobic and anaerobic conditions. After incubation, plates with 30–300 colonies were enumerated and the calculated results were expressed as colony forming unit (cfu) per gm or mL by multiplying the average number of colonies with the reciprocal of dilution factor [29]. Colonies with distinct morphologies such as form, elevation, margin, surface, color and consistency were selected randomly and isolated [30]. Presumptive lactobacilli colonies were purified by inoculating into MRS and Rogosa broth followed by incubation at 37°C for 24 hours as well as streaking on to Rogosa agar and MRS agar. Similar steps and conditions were followed for the purification of the streptococci using Yeast Glucose Lemco Broth (YGLB) and YGLA. Lactobacilli were streaked on MRS agar slant and streptococci on YGLA slant and kept at 4°C. Cultures were maintained by streaking fortnightly on the same media. Prior use, lactobacilli were activated in MRS broth and streptococci in YGLB at 37°C for 24 hours.

Selection of the isolates: Isolated 191 strains were evaluated and categorized into 13 groups on the basis of their colony morphology in media plates and slants, Gram staining, cell morphology, catalase activity, and motility test (summarized in Table 1). Among them, seven were selected and isolated from MRS media for *Lactobacillus* spp. and hence marked as M18A, M18B, M18C, M16C, M12A, M17B, and M14B. The remaining six colonies were isolated from YGLA for *Streptococcus* spp. and marked Y1A, Y11A, Y8, Y19B, Y-YL and Y-GW. These 13 representative isolates were identified upto species level by the traditional phenotypic methods.

Characterization of the isolates

Gram-positive, catalase negative rods were characterized according to the methods and criteria of Bergey's Manual of Determinative Bacteriology, 8th Edition [31], Axelsson [10], Kandler and Weiss [32], Schleifer and Kilpper-Bälz [33], and Bottazzi [34]. *Streptococcus* were characterized according to the criteria used by Bergey's Manual of Determinative Bacteriology, 8th Edition [31].

All strains were subjected to the common biochemical tests such as Voges-ProsKauer (VP), Methyl Red (MR) reaction test, starch hydrolysis, deep glucose agar test, CO_2 gas from glucose in Gibson's semisolid tomato juice medium, gas from citrate in semisolid citrated milk agar, NH_3 from arginine [35], and gelatin liquefaction. Carbohydrates (glucose, maltose, lactose, arabinose, rhamnose, raffinose, dextrose, sucrose, inulin, xylose, galactose, fructose, mannitol, starch) fermentation test was performed in fermentation broth medium containing 1% (w/v) carbohydrate, bromothymol blue 0.1% as pH indicator, and keeping Durhum's tube at inverted position. Results were recorded after 48 hours of incubation at 37°C.

Presumptive lactobacilli inoculated in MRS broth and streptococci in YGLB media were further characterized by conducting the following physiochemical tests. Growth response at different temperatures 5°C, 10°C, 27°C, 37°C and 45°C for 24-48 hours, pH 4.5, 6.5, and 7.2 at 37°C for 48 hours, tolerance to NaCl by growth in MRS broth containing NaCl at concentrations of 1%, 2%, 3%, 4%, 5%, 6%, 6.5%, 7% at 37°C for 48 hours, heat resistance by keeping the inoculated media in the boiling water bath at 55°C and 60°C for 30 minutes were evaluated for all of the selected 13 LAB isolates [28,36,37].

Screening of the efficient LAB strain

Potential LAB isolate in terms of yoghurt formation and acid production were studied. 10% reconstituted skimmed milk was inoculated with 0.2 mL (2%) starter culture suspension of *Lactobacillus spp.* and *Streptococcus spp.* separately and in different combinations. Inoculated tubes were incubated at 45°C. Characteristics of the yoghurts formed by the individual LAB and mixed inoculums were studied by visual observations. To get the potential lactic acid producer, 100 ml of sterile skimmed milk medium was inoculated with 1mL starter culture of the identified individual species of *Lactobacillus* and *Streptococcus* and incubated at 30°C in a water bath for 6 hours and 72 hours respectively. Total acidity was determined by titration of 10 mL of incubated sample cultures titrating with 0.1N NaOH using 0.5% phenolphthalein as indicator. Total acidity was measured after 6 hours and 72 hours of incubation period respectively and expressed in percentage as well as milligram equivalent to lactic acid [23,35].

RESULTS AND DISCUSSION

Enumeration of LAB

LAB were enumerated from the fresh raw milk, commercial liquid milk and yoghurt samples. **Table 2** shows the total viable aerobic and anaerobic count of milk and yoghurt samples. Yoghurt samples showed higher LAB count than the freshly drawn raw milks both at aerobic and anaerobic conditions. Aerobic bacterial count in milk sample ranges from 1.1×10^5 to 7.2×10^6 while anaerobic bacterial count ranges from 6.1×10^5 to 5.3×10^7. In yoghurt samples, total anaerobic bacterial count (2.2×10^7 - 5.4×10^8) is higher than that of total aerobic bacterial count (1.0×10^6 - 5.6×10^7). It has been found that both in milk and yoghurt samples, anaerobic bacterial count (6.1×10^5 to 5.4×10^8 cfu/mL) are greater than that of aerobic count (1.1×10^5 to 5.6×10^7

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cfu/mL). These findings correlate with previously examined few studies. For example, Kacem et al. provided that total LAB count in milk and yoghurt shows higher than the cow's, goat's and sheep's milk samples in Western Algeria [22]. Similar LAB count were also reported for the traditional koopeh cheese in Iran [38], camel's milk [26], yoghurt [39], and goat's yoghurt [40].

Table 1. Cultural, morphological and biochemical characteristics of bacterial isolates.

Group	Colony morphology					Slant character-istics	Microscopic observation	Staining Properties				Catalase test	Motility test
	Form	Color	Elevation	Margin	Surface		Cell form and arrangement	Gram	Acid fast	Capsule	Spore		
1	Circular	White	Pulvinate	Entire	Smooth	Echinulate	Rod, single & in chain	+	-	-	-	-	-
2	Circular	White	Flat	Undulate	Rough	Spreading	Rods with rounded end, single and in short chain	+	-	-	-	-	-
3	Circular	White	Raised	Entire	Smooth	Filiform	Rods with rounded end, single, and short to long chain	+	-	-	-	-	-
4	Circular	White	Light Convex	Entire	Smooth	Beaded	Cocco bacilli, sinlge, pair or in short chain	+	-	-	-	-	-
5	Circular	Gray	Contoured	Undulate	Wrinkled	Echinulate	Rounded end rod, single, pair & in short-long chain	+	-	-	-	-	-
6	Circular	White	Convex	Entire	Rough	Echinulate-spreading	Rounded end rods, sin-gle, and in short chain	+	-	-	-	-	-
7	Circular	White	Raised	Entire	Smooth	Spreading	Rounded end rods Single, rarely in pair & in short chain	+	-	-	-	-	-
8	Circular	White	Convex	Entire	Smooth	Filiform	Cocci, single, mostly in pairs or short chains	+	-	-	-	-	-
9	Circular	White	Convex	Entire	Smooth	Filiform	Cocci, single, mostly in pair or short chain	+	-	-	-	-	-
10	Circular	White	Light Convex	Entire	Smooth	Filiform	Cocci, single, pair & in long chain	+	-	-	-	-	-
11	Circular	White	Flat	Undulate	Smooth	Echinulate	Cocci, single, mostly in pair & occasionally in short chain	+	-	-	-	-	-
12	Circular	Gray	Convex	Entire	Smooth	Echinulate	Cocci, single, pair, medi-um-long chain	+	-	-	-	-	-
13	Circular	White	Convex	Entire	Smooth	Echinulate	Cocci, single, pair & in chain	+	-	-	-	-	-

Table 2: Total LAB count at aerobic and anaerobic condition.

No. of Sample	Name and source of Sample	Total LAB count	
		Aerobic (cfu/ml)	Anaerobic(cfu/ml)
1	Cow milk, CU campus	2.5×10^6	1.2×10^7
2	Cow milk, CU campus	1.8×10^6	5.8×10^6
3	Cow milk, CU campus	3.0×10^6	7.0×10^6
4	Cow milk, CU campus	2.8×10^6	6.8×10^6
5	Cow milk, CU campus	2.1×10^6	5.1×10^6
6	Pasteurized liquid milk, commercial	1.1×10^6	1.1×10^7
7	Cow milk, CU campus	2.1×10^5	1.2×10^6
8	Cow milk, CU campus	2.4×10^5	6.2×10^6
9	Cow milk, CU campus	1.7×10^5	8.7×10^5
10	Cow milk, CU campus	1.1×10^5	6.1×10^5
11	Cow milk, Jobra Village	3.5×10^6	1.9×10^7
12	Cow milk, Jobra Village	5.3×10^5	5.3×10^7
13	Cow milk, Jobra Village	7.2×10^6	5.0×10^7
14	Cow milk, CU campus	2.4×10^6	6.9×10^6
15	Cow milk, CU campus	8.0×10^5	7.7×10^6
16	Yoghurt, commercial	7.2×10^6	3.8×10^7
17	Yoghurt, CU campus	1.0×10^6	2.2×10^7
18	Yoghurt, Halisahar, Chittagong	5.6×10^7	2.5×10^8
19	Yoghurt, CU campus	1.5×10^7	5.4×10^8

Identification of the isolates

The 13 selected representative isolates were chosen among 191 isolates on the basis of their colony characteristics in selective media plates, various staining properties, catalase reactions, and motility **(Table 1).** These 13 isolates were categorized into two main genus Lactobacillus and Streptococcus using the selective media. Isolates were then studied extensively for various biochemical and physiochemical studies which are shown in **Table 3.** All of the Lactobacillus species shared the common staining - Gram negative, noncapsulated, nonsporing, nonacid fast and microscopic features - short rod to coccobacilli with single, pair and in chain arrangement with different colony morphology in MRS or Rogosa agar and in MRS slant. Besides, they also showed non-motility, negative result in catalase reaction, MR and VP tests, unable to hydrolyze citrate, starch, and liquefy gelatin. Isolated and preliminary identified Lactobacillus strains were primarily differentiated in some of the biochemical tests, their physiological properties and patterns of carbohydrate fermentation. However, when the fermentation patterns of these isolates were compared with the identification key in Bergey's Manual of Determinative Bacteriology, 8th edition few deviations were noted.

Strains M18A and M18B showed common biochemical reactions such as NH_3 from arginine, acid and gas from glucose fermentation, and physiological properties with few exceptions - M18B produced CO_2 from Gibson's semisolid media, growth at 45°C while M18A were unable **(Table 3).** They reflected identical fermentation pattern with few variations as per Bergey's Manual of Determinative Bacteriology, 8th edition. On the other hand, M18C and M16C both produced CO_2 in Gibson's semisolid media with no citrate utilization, NH_3 from arginine, liquefaction of gelation. Both of the strain could not tolerate high salt concentration (6.0%, 6.5% and 7%), low temperatures (4°C and 10°C). M16C produced scanty growth at 45°C while M18C could not grow at all **(Table 3).** Both of the strains produced almost typical carbohydrate fermentation results in respect to Bergey's Manual of Determinative Bacteriology. Isolates M12A, M17B and M14B produced similar biochemical reactions except NH_3 from arginine by M17B strain, showed optimum growth at 37°C - 45°C with capability to grow at 6%, 6.5% and 7% of NaCl concentrations, heat resistance at 55°C and 60°C for 30 minutes. These three isolates did not produce gas from glucose fermentation and showed characteristic carbohydrate fermentation pattern with few exceptions. Hence, according to the Bergey's Manual of Determinative Bacteriology, 8th Edition [31], Axelsson [10], Kandler and Weiss [32], Schleifer and Kilpper-Bälz [33], and Bottazzi [34] strain M18A is considered as *Lactobacillus cellobiosus,* M18B - *Lactobacillus delbrueckii*, M18C - *Lactobacillus hilgardii,* M16C - *Lactobacillus coryniformis* subsp. *coryniformis,* M12A - *Lactobacillus salivarius,* M17B - *Lactobacillus leichmannii,* M14B - *Lactobacillus plantarum*

Strain Y1A was isolated using YGLA medium. Cells of this strain were G+ve cocci, mostly in pairs or short chains, utilized the citrate and produced NH_3 from arginine, showed optimum growth at 37°C and 45°C with little growth at 10°C, heat resistance at 60°C for 30 minutes. They produced both acid and gas from glucose fermentation and showed similar sugar fermentation pattern like *Streptococcus faecalis* mentioned in Bergey's manual. Strain Y11A and Y-GW shared the almost common biochemical features including CO_2 production from Gibson semisolid medium, citrate utilization and NH_3 from arginine, growth at low temp. 10°C with optimum growth at 37°C. Strain Y-GW showed luxuriant growth at 45°C strain while Y11a failed to grow. Both strains produced acid and gas from glucose fermentation, as well as reflected similar carbohydrate fermentation pattern with exception in arabinose and inulin fermentation. According to Bergey's Manual of Determinative Bacteriology (8th edition) [31] Y11A is considered as *Streptococcus lactis* and Y-GW resembled to *Streptococcus uberis*. Isolates Y8 and Y19B produced luxuriant growth at 27°C - 45°C, pH (4.5 to 7.2) while Y8 showed positive growth at 10°C and Y19B produced no growth. Both of them showed heat resistance at 55°C and 60°C for 30 minutes, gelatin liquefaction, difference in citrate utilization and arginine hydrolysis [23]. Strain Y19B could grow at 6.5% and 7% NaCl but strain Y8 could not. These two strains were different in carbohydrate fermentation **(Table 3).** According to the Bergey's Manual [31], strain Y8 is considered as *Streptococcus thermophiles* and Y19B *as Streptococcus faecium*. Cells of the strain, Y-YL were cocci, pair and medium to long chain in arrangement, liquefied the gelatin and utilized arginine with NH_3 production. They could not grow at low temperatures (10°C and 27°C), high salt concentrations (6.5% and 7.0%), and no heat resistance at 55°C and 60°C for 30 minutes. Acid but no gas was generated from glucose fermentation with identical carbohydrate fermentation like *Streptococcus sanguis* according to Bergey's Manual of Determinative Bacteriology, 8th Edition [31].

Harun-ur-Rashid et al. [41] conducted a study in Bangladesh on the identification of the dominant LAB species from the traditional fermented milk *dahi.* Their findings are very relevant with our current study. Besides, identified same LAB species have been reported in other fermented products such bushera – a Ugandan traditional fermented beverages [21]. *L. plantarum* identified from sorghum powder, its corresponding fermented and cooked fermented samples [42], cereal fermented products – such as maize-derived products Nigerian ogi [43] and vegetable fermentations [44]. The prevalence of *S. lactis* is greater in the raw milk due to the free amino acids and the peptides initially present in the milk [45] but they can also be isolated from other sources [46]. *S. lactis* is able to produce acid from casein, hence it can be used as the starter culture for making different products including cheese [47]. *L. delbrueckii* is one best known starter for fermented dairy products because of the capability of producing large amounts of acid in milk, flavor compounds, folic acid and EPS which has effect on the rheological properties of yoghurt [26].

Determination of the efficient strain

Efficient LAB species was screened considering the parameters: formation and color of yoghurt, amount of whey and presence of gas. *L. cellobiosus* was the highest amount of whey producer **(Table 4).** Yoghurt production in different combinations were also tried and summarized in **Table 5.**

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Table 3. Biochemical and physiological characteristics of the LAB. isolates

Isolate No.	MR Test	VP test		CO_2 in Gibson medium	Hydrolysis of			Gelatin Lique-faction	Growth at different pH			Growth at different temperature (°C)					Growth at different NaCl (%)								Heat Resistance (°C)	
		Glucose Phosphate Broth	Skim milk media		Cit-rate	Argi-nine	Starch		4.5	6.5	7.2	4	10	27	37	45	1	2	3	4	5	6	6.5	7.0	55	60
M18A	-	-	-	-	-	+	ND	-	-	++	++	-	-	++	+++	-	+++	+++	++	++	++	+	+	+	+++	+++
M18B	-	-	-	+	-	+	ND	-	-	++	++	-	-	+	+++	+++	+++	+++	+++	+++	+++	++	+	+	++	++
M18C	-	-	-	+	-	-	ND	-	+	++	++	-	-	+	+++	-	+++	+++	+++	+++	++	-	-	-	++	++
M16C	-	-	-	+	-	-	ND	-	-	++	+	-	-	+	+++	+	+++	+++	+++	+++	+++	-	-	-	++	+++
M12A	-	-	-	-	-	-	ND	-	-	++	++	-	-	+	+++	++	+++	+++	+++	+++	+++	++	+	+	+	+++
M17B	-	-	-	-	-	+	ND	-	-	++	++	-	-	-	+++	+++	+++	+++	+++	+++	++	++	++	+	++	++
M14B	-	-	-	-	-	-	ND	-	-	++	++	-	-	+	+++	++	+++	+++	++	++	++	++	++	+	+++	+++
Y1A	-	-	-	-	+	+	-	-	++	++	++	-	+	++	+++	++	++	++	++	+	+	+	++	+	++	++
Y11A	-	-	+	+	+	+	-	-	++	+++	+++	-	+	++	+++	-	++	++	++	++	++	-	-	-	++	+
Y8	-	-	-	-	-	-	+	+	++	++	++	-	-	+++	+++	++	++	++	++	++	+	+	-	-	++	+++
Y19B	-	-	-	-	-	+	-	+	++	++	++	-	+	++	++	+++	+++	+++	+++	++	++	++	++	++	++	++
Y-YL	-	-	-	-	-	+	-	+	+++	++	++	-	-	+++	+++	+	+++	+++	++	++	++	++	-	-	-	-
Y-GW	-	-	+	+	+	+	+	-	+++	+++	+++	-	+	++	+++	++	+++	++	++	++	++	++	-	-	+++	++

Table 4. Carbohydrates fermentation of LAB.

Isolate No.	Glucose		Maltose	Lactose	Arabinose	Rhamnose	Raffinose	Dextrose	Sucrose	Inulin	Xylose	Galactose	Fructose	Mannitol	Starch
	A	G	A	A	A	A	A	A	A	A	A	A	A	A	A
M18A	+	+	+	-	+	-	-	+	+	+	+	+	+	-	+
M18B	+	-	+	-	-	-	-	-	+	+	+	+	+	-	+
M18C	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+
M16C	+	-	+	-	+	+	+	+	+	-	+	+	+	+	+
M12A	+	-	+	+	-	-	-	+	-	+	-	+	+	+	+
M17B	+	-	+	-	_	-	-	+	+	-	-	-	+	-	+
M14B	+	-	+	+	+	_	+	+	+	+	_	+	+	+	+
Y1A	+	-	+	+	+	-	-	+	+	-	+	+	+	+	+
Y11A	+	+	+	+	+	+	-	+	+	-	+	+	+	+	-
Y8	+	-	-	+	+	-	-	+	+	-	+	+	+	-	-
Y19B	+	-	+	+	+	-	-	+	-	-	+	+	+	+	-
Y-YL	+	-	+	+	-	-	-	+	+	+	-	+	+	-	-
Y-GW	+	+	+	+	-	+	-	+	+	+	-	+	+	+	-

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Table 5. Screening of the efficient LAB isolate.

Isolate No	Color of Yoghurt	Yoghurt Formation	Amount of whey	Gas Production
L. cellobiosus	White	+	++++	-
L. delbrueckii	Cream	+	++	-
L. hilgardii,	Cream	+	+	-
L. coryniformis subsp. *Coryniformis*	White	+	++	-
L. salivarius	White	+	++	-
L. leichmannii	Cream	+	+	-
L. plantarum	Cream	+	+	-
S. faecalis	Cream	+	+++	++
S. lactis	Cream	+	+++	++
S. thermophiles	Cream	+	++	-
S. faecium,	Light yellowish	+	+++	-
S. sanguis	Cream-light yellowish	+	++	-
S. uberis	Cream	+	+++	++
L. cellobiosus + *L. delbrueckii*	Cream	+	++	-
L. delbrueckii + *L. salivarius*	Cream	+	++	-
L. delbrueckii + *L. leichmannii*	Cream	+	+++	-
L. coryniformis subsp. *coryniformis* + *L. leichmannii*	White	+	+	-
S. lactis + *S. uberis*	Cream	+	+++	++
All bacteria + *S. lactis*	White	+	+	-
All bacteria + *S. uberis*	Cream	+	+++++	+
All bacteria + *S. lactis* + *S. uberis*	Cream	+	++++	++

Among the identified *Lactobacillus* species, none of the potential species was identified in terms of acid production both in 6 hours and 72 hours of incubation period. *L. cellobiosus* was found to be a little bit higher acid producer than the others. *S. lactis* and *S. uberis* were found as the highest acid producer both after 6 hours (0.14 and 0.18) and 72 hours (0.46 and 0.47) of incubation period **(Figure 1).** From the following data, it can be summarized that after 6 hours of fermentation period almost all the identified species of the two genus *Lactobacillus* and *Streptococcus* produced near about the equal quantity of acid except *S. uberis* (0.18). Interestingly, *S. lactis* and *S. uberis* species kept continue in acid production at longer incubation period. The total acidity equivalent to lactate (mg) for *S. uberis* was the highest 370 mg while *S. lactis* was the immediate highest lactic acid producer (360 mg) after 72 hours of incubation period at 35°C **(Figure 2).** Considering the total acidity, conversion of total acidity in equivalent to lactic acid, it is proved *S. lactis* and *S. uberis* are the homofermentative acid producer. Result from the combined activity of the LAB isolates in acid producing capability, again reflected that *S. lactis* and *S. uberis* in combination and mixed with all other lactobacilli and the remaining streptococci produced a little higher lactate than the individual species **(Figures 1 and 3).**

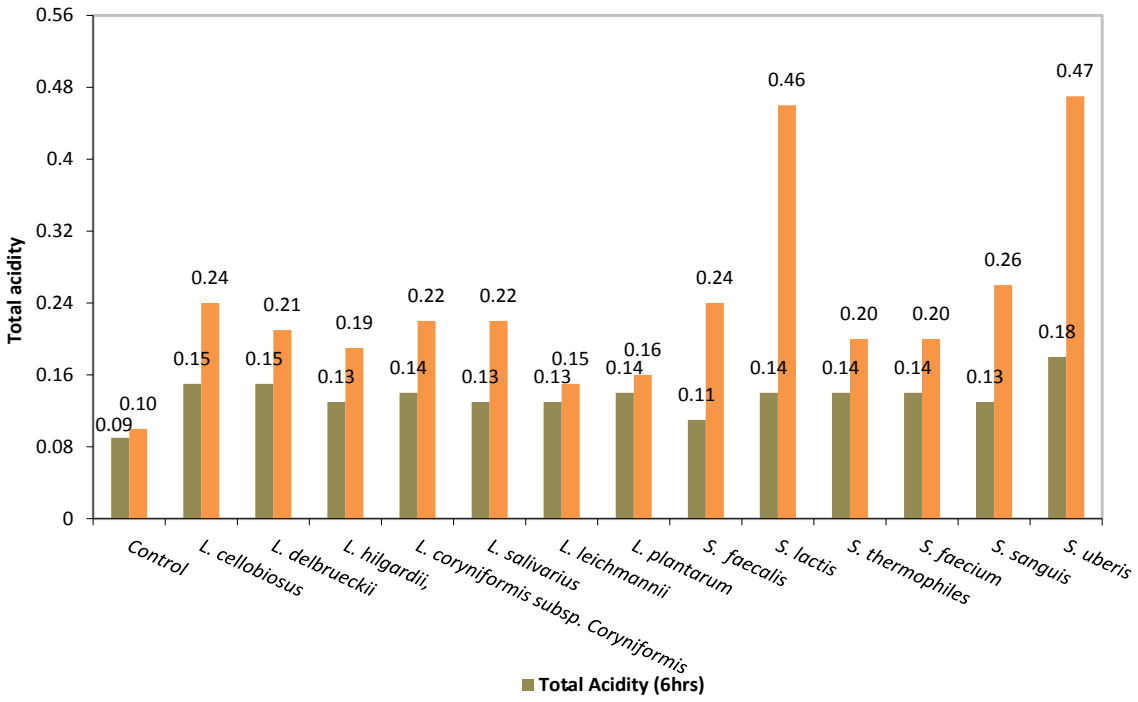

Figure 1. Total acidity at two different incubation conditions by the individual LAB isolates.

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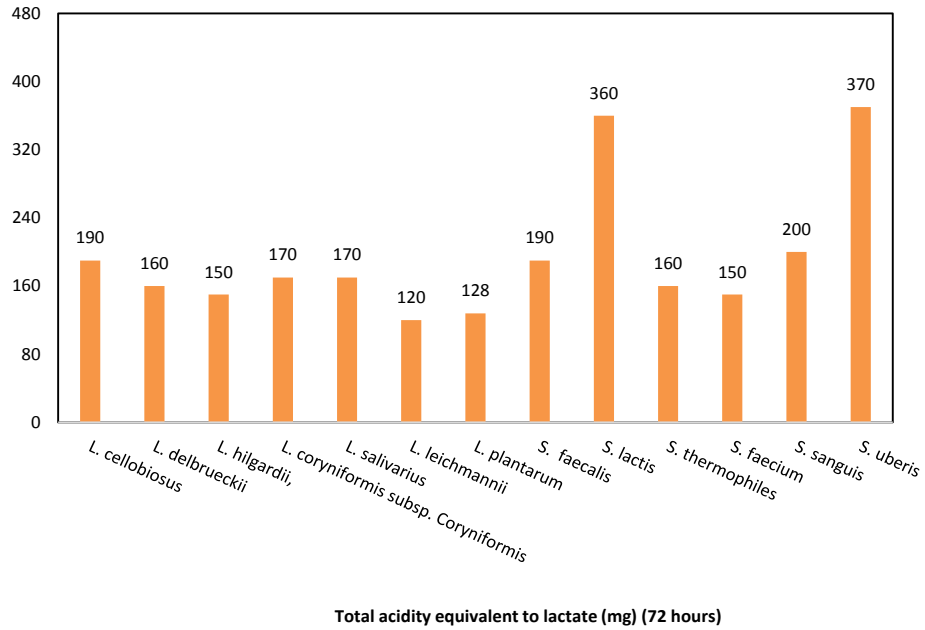

Figure 2. Total acidity (equivalent to lactate in mg) after 72 hours of incubation conditions by the individual LAB isolates.

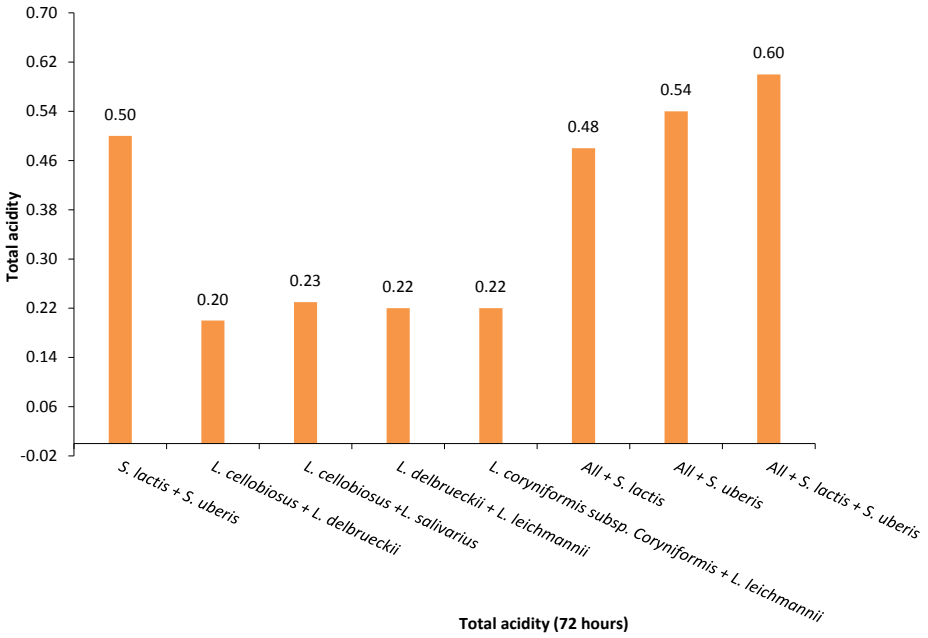

Figure 3. Total acidity by the different combinations of LAB isolates.

CONCLUSIONS

The results obtained in this study revealed the presence of a wide variety of LAB from milk and yoghurt samples. Some of the isolated and identified LAB species show outstanding performances in lactic acid production. The results of this study have indicated that several different species of LAB can be used as the starter culture for the production of yoghurt. Hence, the selection and potential use of appropriate beneficial strain or mixture of strains from the identified isolates as the starter organism for yoghurt and lactate production need more studies about the optimization process among the temperature, time, ingredient compositions as well as inoculum concentrations. Extensive study must be done to improve the technological properties of yoghurt as well as for the development of small-scale dairy industries in Bangladesh.

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