INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 7, No. 4, p. 139-149, 2015

RESEARCH PAPER OPEN ACCESS

A study on probiotic properties of isolated and identified bacteria from regional yoghurts

S.M. Khaledur Rahman[1,2*], Abdul Jalil[1], S.M. Mahbubur Rahman[1], Khondoker Moazzem Hossain[1]

[1]*Biotechnology and Genetic Engineering Discipline, Khulna University, Khulna-9208, Bangladesh*
[2]*Department of Genetic Engineering and Biotechnology, Jessore University of Science and Technology, Jessore-7408, Bangladesh*

Key words: Probiotic, LAB, yoghurt, cholesterol, antimicrobial.

http://dx.doi.org/10.12692/ijb/7.4.139-149 Article published on October 25, 2015

Abstract

Probiotics are living microorganisms that fall in the beneficial category, and confer health benefits for the host. Specifically, Lactic Acid Bacteria (LAB) cultures are used for the production of specific probiotic products. The aim of the present study was the isolation and categorization of probiotic bacteria from selected provincial yoghurt, and subsequently study of their probiotic properties through *in vivo* mice trial. A total of eight isolates were obtained from yoghurt, each two of *Streptococcus thermophilus, Lactobacillus acidophilus, Lactobacillus brevis,* and *Bifidobacterium* spp. According to their physiological and biochemical assay, it was pragmatic that all the species from yoghurt were gram positive, endospore negative, catalase negative and non-motile which are the characteristics of usual probiotic bacteria. Sugar fermentation outline experiments using sixteen vital sugars ensured the presumptive identification of these four species from yoghurt samples. All species were resistant to synthetic gastric juice environment and able to endure artificial bile salts. All species from yoghurt showed good acceptance against low level of phenol and sodium chloride. Isolated probiotic bacteria also able to increase body weight, decrease blood cholesterol level and show antimicrobial activity in *in vivo* mice trial. The isolated and identified probiotic bacteria of present study could be further scrutinized for probiotic product development.

* **Corresponding Author:** S.M. Khaledur Rahman ✉ khaled_shuvo@yahoo.com

Introduction

Bacteria can be both obliging and detrimental to the body. Probiotics are living bacteria that fall on the beneficial category, and afford health benefits for the host. The word probiotic is derived from the Greek that means "for life" which opposed to 'antibiotics' that means 'against life'. Lilly and Stillwell defined probiotics as "microorganisms promoting the growth of other microorganisms" (Lilly and Stillwell, 1965). According to Salminen *et al.* probiotics can be defined as 'a live microbial food ingredient that is beneficial to health' (Salminen *et al.,* 1998*).* The record of probiotics began with the history of man by consuming fermented foods which was admired by Greek and Romans (Gismondo *et al.,* 1999, Guarner *et al,.* 2005). Probiotics are microorganisms introduced orally in the gastrointestinal tract (GIT) that are able to supply positively to the activity of intestinal micro flora and consequently, to the health of its host. Human GIT has a multifarious ecosystem with a different and intense microbial population that mediates frequent interactions with the chemical environment, such as digestion, adhesion and colonization in the GIT. Up to date, many research studies paying attention on the role of probiotic bacteria in the yogurt for the augmentation of the gastrointestinal function through increasing minerals absorption, reduction in lactose intolerance (lactase deficiency) consequences from utilization of *L. acidophilus* culture yogurt (Vesa *et al.,* 2000, Martini *et al.,* 1991). Generally, lactic acid bacteria (LAB) can be defined as Gram positive, non-spore forming, catalase negative, lack of cytochromes, acid tolerant, and facultative anaerobe group that produce lactic acid as the major end-product during fermentation of carbohydrates. The biological activity of probiotic bacteria is used in the manufacture of cultured diary product and their metabolites. The main processing steps including product formulation and modification of the physiological activity of the final product (Mazza 1998).

Foods are no longer measured by consumers only in terms of savor and instant nutritional requirements, but also in terms of their capacity to provide definite health benefits outside their basic nutritional value. *Lactobacillus* and *Bifidobacterium*, both of which have been comprehensively studied and established as valuable indigenous inhabitants of the GIT (Fuller, 1989, Salminen *et al*., 1998). To fulfill the demand of new epoch isolation of suitable bacterial strains and characterization of indigenous probiotic bacteria were first apprehension towards probiotic product development. Probiotic product development involves numerous steps such as isolation, biochemical categorization, and study of probiotic properties of identified bacteria. Molecular characterization, quality assurance, laboratory trials, and ethical considerations were also performed for better product performances. Therefore, to achieve part of the probiotic product development the general goal of the study was probiotic properties analysis of isolated bacteria from selected yoghurt samples and to explore the potentialities for development of human consumable probiotic products.

Materials and methods

Four yoghurt samples were collected from Khulna region of Bangladesh. After collection, the samples were stored aseptically at 4°C in refrigerator to defend from corrosion and contagion. LABs were isolated from the sample using MRS (De Man Rogosa and Sharpe) media (De Man *et al.,* 1960). ST (*Streptococcus thermophilus*) agar media was used for the isolation of *Steptococcus thermophilus* (Saccaro *et al.,* 2011). Only MRS agar medium was used for the isolation of *L. acidophilus* and *L. brevis*. L-cysteine (0.05%) was added to MRS agar media to advance the specificity for isolation of *Bifidobacterium spp.* by providing an anaerobic condition (Zinedine and Faid, 2007). The definite incubation temperature and time for the isolation of *Lactobacillus, Bifidobacterium* and *S. thermophilus* were followed according to Saccaro *et al.* & Linn *et al.* (Saccaro *et al.,* 2011, Linn *et al.,* 2008).

Bacterial characterization

Bacterial plates were further purified by streaking continually on MRS agar media, and the colony morphologies (color, shape and size) were examined

in naked eye, sometimes microscopic inspection was needed to separate one colony from another. Gram staining and endospore test was done according to the procedure of Erkus and Schaeffer-Fulton with some modifications (Erkus, 2007, Schrezenmeir and de Vrese, 2001). For performing sugar fermentation test overnight activation of isolates were organized in 10 mL MRS media at 37°C, then centrifugation was done for 10 min at 10000 rpm. The pelleted cells were resuspended in 5 mL MRS without glucose, containing bromecresol purple. The customized MRS medium was used for carbohydrate fermentation. Centrifugation was done for 10 min at 10000 rpm; finally pelleted cells were resuspended in actual volume of 10 mL MRS without glucose containing bromecresol purple. One mL different sugar solutions were inoculated into different tubes. Then 200 µL overnight liquid cultures were inoculated into the broth. Incubation was performed anaerobically at 37°C for 24 h (Erkus, 2007). In case of motility test, Motility-Indole-Lysine (MIL) partially broth medium was prepared and dispensed in 5 mL aliquots in screw-top test tubes and additional dispensation was done according to Reller and Mirrett (Reller and Mirrett, 1995).

Assays for probiotic properties

For the purpose of testing NaCl tolerance of isolates, test tubes containing MRS broth were supplied with different concentrations (1-10%) of NaCl and test was done according to Hoque *et al.*, (Hoque *et al.*, 2010). Gastric juice resistance of isolated *Lactobacillus* from provincial yoghurts was determined using protocol described by Graciela and Maria with some modification at pH 2.2 and pH 6.6 (Graciela and Maria, 2001). For the purpose of phenol tolerance of isolates, MRS broth was adjusted with different concentration (0.1-0.4%) of phenol then inoculated with 1% (v/v) fresh over night culture. After 24 h of incubation their growth was determined by 620 nm filter absorbance of cell concentration by spectrophotometer (Hoque *et al.*, 2010). MRS broth with different concentrations (0.05%, 0.1%, 0.15% and 0.3%) of bile salt was utilized to conclude the tolerance and growth rate of isolated LABs according to Graciela and Maria (Graciela and Maria, 2001). Agar plates were prepared with 0.5% (wt/vol) of the sodium salt of taurocholic acid (TCA) to determine bile salt hydrolase activity. All plates were inoculated with overnight culture by using a 10 µL loop. Plates were incubated in glass screw-cap jars at 37°C in the anaerobic chamber for 72 h. To determine the milk coagulation pattern, 1% (v/v) culture of probiotic bacteria was inoculated into sterilized milk and incubated for 24 hr.

In vivo trial on mice

After biochemical assessment, preferred strains were tested for *in vivo* experiments. For *in vivo* experiments, seven week-old Swiss Albino mice (*Mus musculus*) were purchased from International Centre for Diarrheal Diseases Research, Bangladesh (ICDDR, B). Twenty four mice were divided into four groups; group 1, group 2 and group 3 were denoted as treatment group 1, 2 and 3, respectively, and group 4 was denoted as control. Table 1 represents the feeding design of mice groups. For preparation of yogurt, cow milk was collected from a local farm and was heated at 95°C for 15 min and then chilled to 43°C. Then inoculated with 5% (v/v) liquid culture of isolated LAB, which were characterized during biochemical assay and incubated at 37°C for 8 hr.

Individual body weight of mice was measured at day 0, 7, 14, 21, and 28. To measure weight, each mouse was placed in digital balance with the help of plastic container. Blood cholesterol level was measured on day 0 and 28. For this experiment, blood was taken from each mouse with pointed blade from the tail. When bleeding found then touched with *EasyMate*^{R} cholesterol measuring stripes. *EasyMate*^{R} cholesterol measuring stripes were previously adjusted with digital *EasyTouch*^{R} cholesterol measuring apparatus. Finally each mouse was inoculated with *Escherichia coli* by using a feeding needle with 0.1 ml of the bacterial suspension containing about 10^2 viable cells with drinking water. They were supplemented with yogurt according to earlier feeding design prior to 5 days of challenge with *E. coli* (Silva *et al.,* 1999). The feces of control infected and yogurt supplemented

infected mice were collected accordingly to group following day 2 of post infection for bacterial plate count.

Statistical analysis

After mice trial all the results were analyzed for statistical significant tests using SPSS data analysis software. At this point one way ANOVA was performed to conclude the level of significance at $P>0.05$ as well as all others data were articulated as mean with standard deviation (n = 3).

Results

Identification of lab from yoghurt sample

Colony morphology

From colony morphologies it was observed that the colony morphology of *S. thermophilus* was found medium, circular, low convex and irregular creamy color. Furthermore very small, round shaped and non transparent colonies were observed for *L. acidophilus* and small, white rod shaped and non-transparent colonies were observed for *L. brevis*. Small, triangular, watery circle with deep white center colonies were observed for *Bifidocterium spp*.

Table 1. Feeding design of mice groups.

Group Name	Feeding Schedule
Control Group	5 gm basal feed (rat pellet) /day /mouse + additional 6 gm rat pellet (to equalize with treatment group)
T1	5 gm basal feed (rat pellet) /day /mouse + 4gm yoghurt/day/mouse
T2	5 gm basal feed (rat pellet) /day /mouse + 8 gm yoghurt/day/mouse
T3	5 gm basal feed (rat pellet) /day /mouse + 12 gm yoghurt/day/mouse

NB. One gm set yoghurt contains 1.67 Kcal ME whereas, one gm rat pellet contains 3.1 Kcal ME.

Gram staining and motility test

All the isolates of bacteria were found gram positive due to grasp violet blue color which is indicative positive sign, and they were found also non-motile through growth stimulation along the inoculation line (Fig. 1). Under light microscope, vegetative cells stained as red, and both endospore and free spore stained as green. In microscopic observation, lack of endospores was indicated if green color was not observed. All isolates of bacteria were found catalase negative due to no bubble production (O_2 production) during addition of 3% H_2O_2.

Sugar fermentation profiles patterns up to species level

Isolated LAB was identified based on the origin of their sugar fermentation prototype which was determined by color change of the broth medium due to acid and gas production. The change of purple colour of the broth medium to yellow colour was the indication of fermentation performed by the isolated probiotic bacteria (due to lactic acid production) of that particular sugar. It was observed that each probiotic bacterium had definite sugar fermentation pattern of its own which has been presented in Table 2 with positive and negative signs. *L. acidophilus* fermented all the sugar except sorbitol, mannitol, rhamnose among sixteen sugars, which was allied with the study of Ghanbari *et al.*; Karna *et al.* and Azizpour *et al.* (Ghanbari *et al.*, 2009, Karna *et al.*, 2007, and Azizpour *et al.*, 2009). *L. brevis* fermented all sugars except salicin, rhamnose and sorbitol among sixteen sugars. The sugar fermentation pattern of *S. thermophilus* (isolated on ST agar medium) was found positive in all sugars except rhamnose and sorbitol, the finding was analogous to previous studies (Giraffa *et al.,* 1997, Garvie, 1984 and Teuber *et al.,* 1995). The sugar fermentation pattern of *Bifidobacterium spp*. was found positive apart from rhamnose and sorbitol, which was faintly fermented. Sugar fermentation results showed little incompatibility, which may be due to involvement of numerous environmental factors, method of yoghurt preparation, and maintenance of the yoghurt sample etc.

Determination of probiotic properties of isolated bacteria

Gastric juice resistance test

Isolates of probiotic bacteria had the capability to stay alive in artificial gastric acid environment at low pH (pH 2.2) and favorable pH (pH 6.6) but their endurance ability decreased after 24 h of incubation at 37°C. The optical density of cell concentration at 620 nm wavelength of identified probiotic bacterial culture in gastric juice environment signifying the nature of survival and multiplication ability. The aloft lines in Fig. 2 indicated more tolerance ability of the isolates at pH 2.2. Isolated *S. thermophilus, L. brevis, L. acidophilus* and *Bifidocterium spp.* were able to stay alive in gastric environment at low pH 2.2 as well as also able to proliferate in pH 6.6. The aloft lines indicate the resistance ability of the eight isolates. Data was expressed as mean with standard deviation (n = 3) was presented.

Table 2. Sugar fermentation patterns of isolated probiotic bacteria.

Isolate no.	Name of the LAB	Sugar name															
		Lactose	Mannitol	Sucrose	Fructose	Salicin	Ribose	Cellubiose	Glucose	Maltose	Xylose	Rhamnose	L-Arabinose	D-Sorbitol	D-Mannose	Raffinose	Galactose
Isolate 01	no. *L. acidophilus* (1)	+	-	+	+/-	+	+	+	+	+	+	-	+	-	+	+/-	+
Isolate 02	no. *L. acidophilus* (2)	+	+/-	+	+/-	+	+	+	+	+	+	-	+	-	+	-	+
isolate 03	no. *L. brevis* (1)	+	+	+	+	-	+	+	+	+	+	-	+	-	+	+/-	+
Isolate 04	no. *S. thermophilus* (1)	+	+/-	+	+	+	+	+	+	+	-	-	+	-	+	+	+
Isolate 05	no. *S. thermophilus* (2)	+	+/-	+	+	+	+	+	+	+	+	-	+	-	+	+/-	+
Isolate 07	no. *Bifidobacterium* (1)	+	+	+	+/-	+	+	+	+	+	+	-	+	-	+	+	+
Isolate 08	no. *Bifidobacterium* (2)	+	+	+	+/-	+	+	+	+	+	+	-	+	-	+	+	+
Isolate 10	no. *L. brevis* (2)	+	-	+	+	+	+	+	+	+	+	-	+	-	+	+	+

'+' Represents good fermentation; '+/-' represents moderate fermentation; '-' represents no fermentation.

Phenol tolerance test

The identified isolates of the species *S. thermophilus*, *L. brevis*, *L. acidophilus* and *Bifidocterium spp.* showed good acceptance against 0.1 - 0.2% phenol and moderate acceptance against 0.3% and 0.4% phenol. The optical density of cell concentration was taken at 620 nm wavelength in spectophotometer to assess bacterial isolate cell concentration. The aloft lines in Fig. 3 indicated more tolerance ability of the eight isolates in 0.3% of phenol. Data was expressed as standard value of eight isolates at different concentrations and after 12 h and 24 h of incubation at 37°C. The aloft lines indicate more tolerance ability of the eight isolates. Data was expressed as mean with standard deviation (n = 3).

Bile salt tolerance test

Isolated eight isolates of the probiotic bacteria were capable to survive in 0.05%, 0.1%, 0.15% and 0.3% inhibitory substance, bile salt. The isolates were also able to multiply in the above mentioned concentrations of bile acid after 24 h of incubation at 37°C. In the Fig. 4 the optical density of cell concentration were measured at 620 nm wavelength

in spectophotometer to assess bacterial cell concentration at 0.3% of bile salt. The optical density standards were plotted in a line diagram representing tolerance to synthetic bile salt, the aloft lines represent the more tolerance ability of the isolated bacteria. Data was expressed as mean with standard deviation (n = 3).

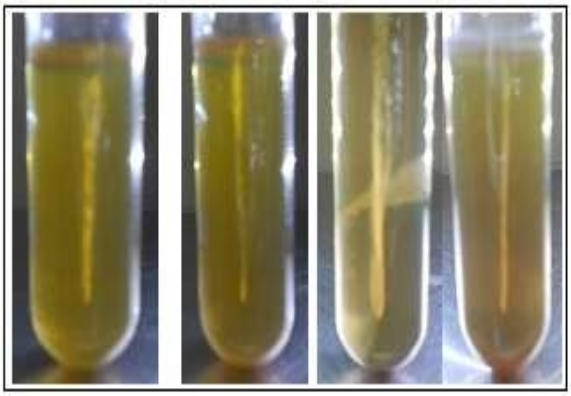

Fig. 1. Motility test of *S. thermophilus* (1), *L. acidophilus* (1), *L. brevis* (1) *and Bifidocterium spp* (1) (sequentially).

Milk coagulation test

Milk coagulation due to formation of lactic acid was observed when bacterial culture was added to fresh skim milk. All the isolated probiotic bacteria were capable to coagulate milk and turned into curd.

The isolated bacteria were able to grow at 1-7% of NaCl concentration. In case of 8 and 9% of NaCl, each isolates showed sensible growth but in 10% of NaCl, no growth was observed. Those bacteria were also good enough for producing bile salt hydrolase. The deconjugation activity of isolated bacterial colonies was turn into dense granular white colonies indicating the bile salt hydrolase active lactic acid bacteria.

Trial on mice by laboratory made yoghurt

The whole treatment group showed significant lowering of cholesterol level and increased body weight gain after 28 days of experiment. The average body weight gain of control group was nearly 8.01 gm over 28 day's treatment period. Treatment group 3 showed highest average body weight gain (16.88 G) and treatment group 1 and 2 showed nearly 10.96 G and 16.06 G weight gain, respectively, while control group showed only 8 G [Fig. 5]. Here in case of body weight gain, T2 and T3 were statistically significant (*star indication)* but T1 was insignificant. So, the overall result was significant at 0.05 level of significance. The value of the F test in this experiment was 4.237 at 23 degree of freedom and the *P* value of this test was 0.028. So the entire result was significant at 0.05 level of significance (P<α).

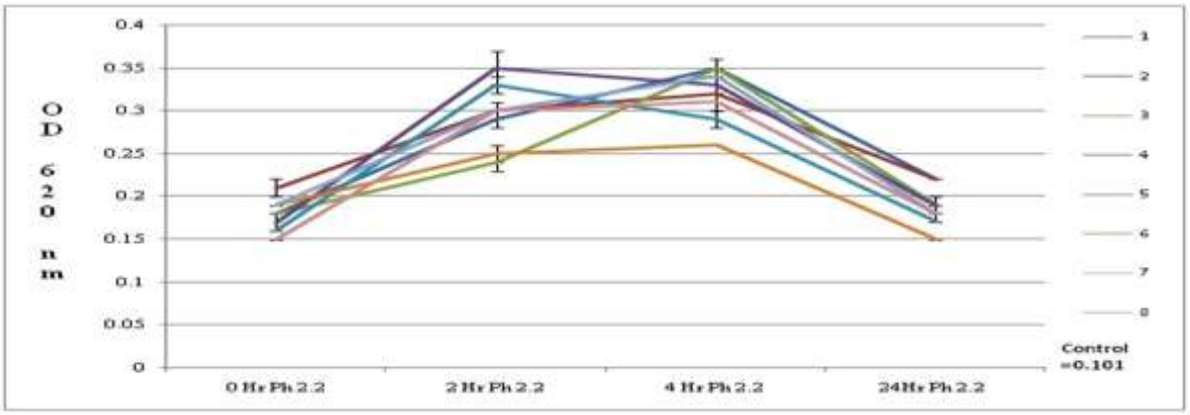

Fig. 2. Survival and multiplication abilities of identified probiotic bacteria in artificial gastric juice at pH 2.2. Here, numbering of the isolated bacteria considered according to the sequence of identification by sugar fermentation.

The average cholesterol reduction of the control group was 0.56 milimole per liter (mM/L) over 28 day's treatment episode. Treatment group 3 showed .highest average cholesterol reductions 1.16 mM/L and treatment group 1 and 2 showed 0.71 mM/L and 0.88 mM/L cholesterol reduction, respectively.

Here in Fig. 6 showed that, T2 and T3 was statistically significant (*star indication)* but T1 was insignificant. So, the overall result was significant at 0.05 level of significance. The value of the F test in this experiment was 4.097 at 23 degree of freedom and the *P* value of this test was 0.032. So the entire result was significant at 0.05 level of significance (P<α). In case of inhibition of pathogenic microorganism *in vivo,* the number of pathogenic bacteria was 5.42 million (cfu/mL) in control group. Treatment group 3 showed highest inhibition of growth of pathogenic bacteria (5.27million cfu/mL) and treatment group 1 and 2 showed significant inhibition of growth of pathogenic microbes than the control group of mice.

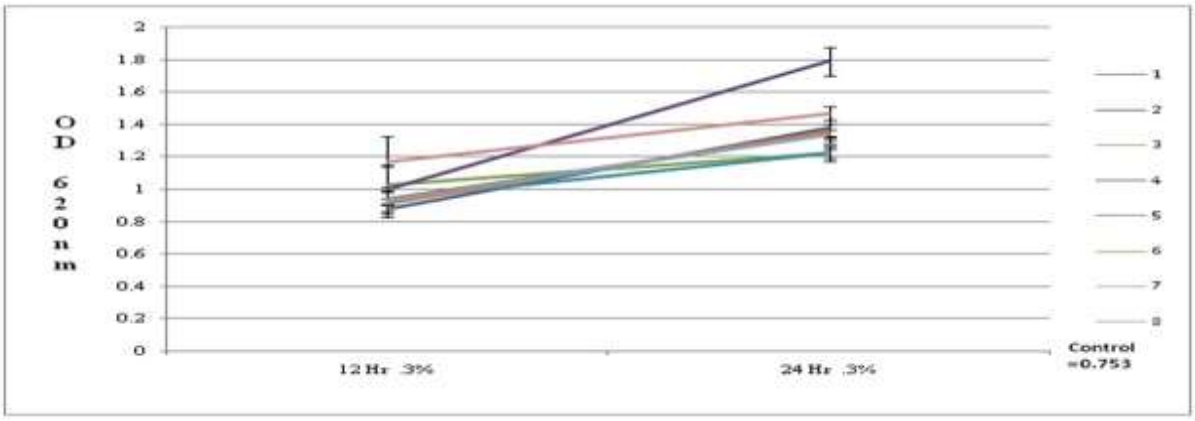

Fig. 3. Phenol tolerances of isolated probiotic bacteria from yoghurt samples at (0.3%). Here, numbering of the isolated bacteria considered according to the sequence of identification by sugar fermentation.

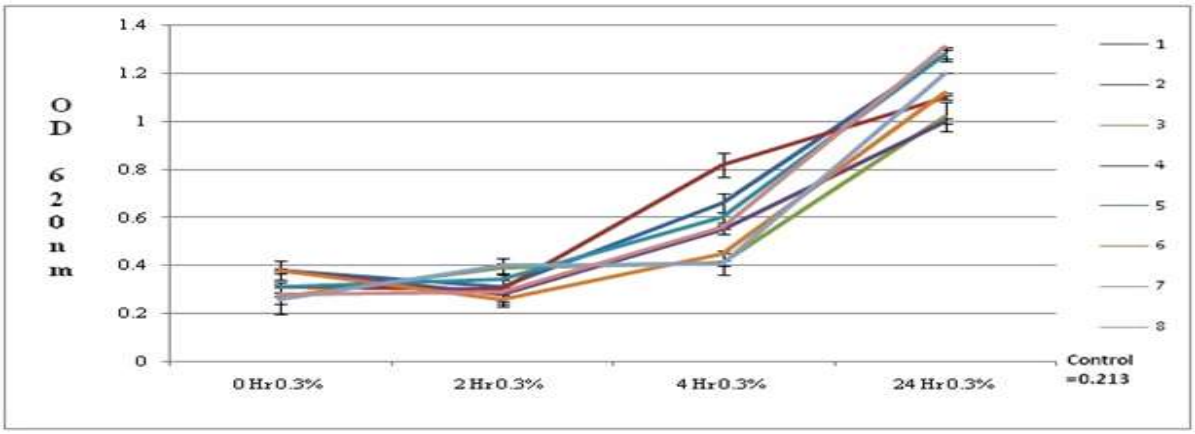

Fig. 4. Bile salt tolerances of identified probiotic bacteria from yoghurt samples at (0.3%). Here, numbering of the isolated bacteria considered according to the sequence of identification by sugar fermentation.

Discussion

Two isolates of *S. thermophilus,* three isolates of *L. acidophilus*, three isolates of *L. brevis* and two isolates of *Bifidobacterium spp.* were isolated from four yoghurt samples of Khulna district of Bangladesh. All the isolates had outstanding tolerance against 1-7% NaCl. At 8% and 9% NaCl concentrations they showed low altitude of growth and in 10% of NaCl, no growth was observed. The NaCl test results of the current study were found parallel to Hoque *et al.* who isolated *Lactobacillus spp.* (Graciela and Maria, 2001) from yoghurt and tested at diverse concentrations of NaCl (1-10%) and found 1-9% NaCl lenience of their *Lactobacillus spp.* Elizete and Carlos isolated *lactobacilli* from gastrointestinal tract of swine that were endurable to 4-8% NaCl (Elizete and Carlos, 2005). Schillinger and Lucke found the growth of *Lactobacilli* in the presence of 7.5% NaCl isolated from animal protein and meat products and the result is approximately similar to the conclusion of present study (Schillinger and Lucke, 1987).

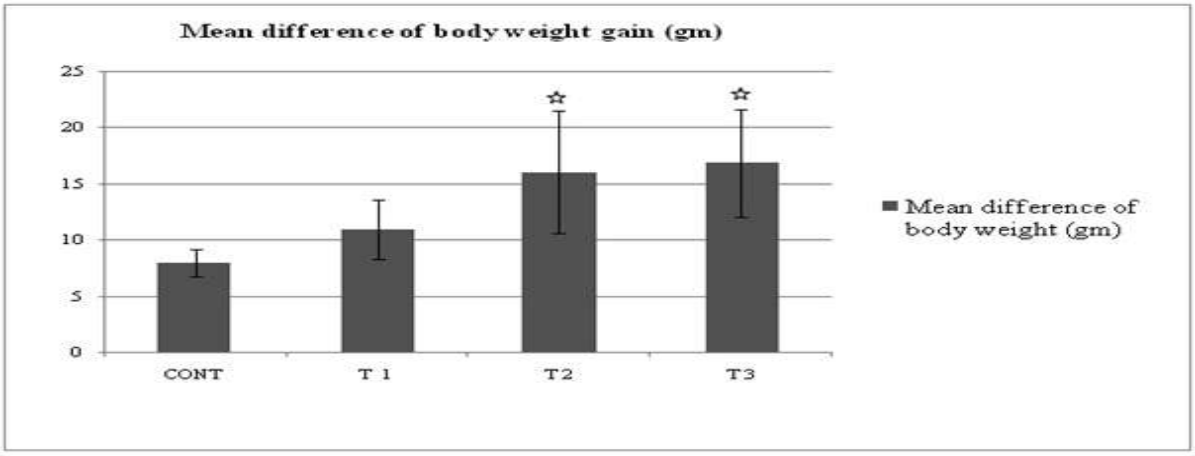

Fig. 5. Body weight measurements of mice groups after 28 days treatment with laboratory made yoghurt. Significance vs control *P<0.05. Here T1 for Treatment group 1, T2 for Treatment group 2, T3 Treatment group 3 and CONT for control group.

In artificial gastric juice they showed good acceptance but after 24 hours, at pH 2.2, all the isolates show lowest survival aptitude compared to earlier hours. In addition, at pH 6.6 after several hours of incubation, all the ten isolates showed more or less equivalent endurance and multiplication abilities. This result was analogous to Maniruzzaman *et al.* (Maniruzzaman *et al.,* 2010).

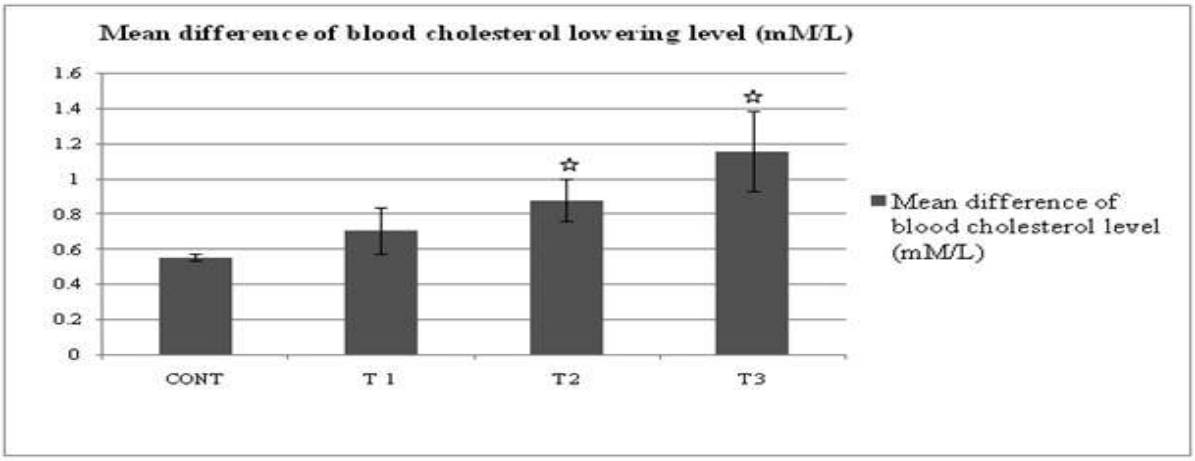

Fig. 6. Blood cholesterol level of mice groups after 28 days treatment with laboratory made yoghurt. Significance vs control *P<0.05. Here T1 for Treatment group 1, T2 for Treatment group 2, T3 Treatment group 3 and CONT for control group.

After 24 hours of incubation, improved resistance and multiplication capability were observed against 0.1 and 0.2% phenol and when the concentration enlarged to 0.3%, their tolerance were sensible and at 0.4% concentration of phenol, their tolerance were lowest, same as Xanthopoulos, *et al.* study (Xanthopoulos *et al.,* 2000). Although the bile concentration of the human GIT varies Prasad, *et al.* showed that resistance capabilities of isolates showed almost equal resistance against different concentrations of artificial bile acid after 0, 2, 4 and 24 hours of incubation correspondingly and the present study found the same consequences (Prasad *et al.,* 1998).

The bile salt hydrolase activity test outcomes of the present study were similar to Dashkevicz and Feighner who developed an agar plate assess to identify bile salt hydrolase activity in *Lactobacilli* (Dashkevicz and Feighner, 1989). In case of mouse

trial, blood cholesterol intensity, body weight , antibiotic resistance, inhibition of growth of pathogenic microorganism *in vivo* tests were performed by feeding yoghurt and compared with control group (no yoghurt) which containing all individuals species. One study on mice show that supervision of low levels of *Lactobacillus reuteri* for a week, lowered total cholesterol and triglyceride levels by 38% and 40% correspondingly, and improved LDL ratio by 20% (Taranto *et al.,* 1998). In the present study, significant level of cholesterol diminution was recorded in treatment groups that were nourished with highest amount of yogurt. In the current research the control group showed highest amount of pathogenic (*Escherichia coli*) bacterial count since here no probiotic product was applied, but in treatment groups there were significant diminution of pathogenic bacteria count, due to introduction with probiotic yogurt. Probiotic bacteria accomplish this task principally by producing substances noxious to pathogenic organisms such as lactic acid, acidic acid, formic acid, hydrogenperoxide, and bacteriocin (Rosenfeldt *et al.,* 2003).

Diverse types of probiotic bacteria originate in different types of yoghurt from different locations. The growth of probiotic bacteria varies from diverse environmental surroundings. Optimum temperature, accessibility of sugars, and pH condition considerably change the growth outline of specific strains. Bangladesh is a developing country and has a diversify climate, hence the deviation among growth prototype of probiotic bacteria are very frequent. In this country a considerable number of people are malnourished. Therefore, for removing malnutrion probiotic product can play a crucial role. With the references to World Health organization and Food and Agriculture Organization (FAO and WHO, 2002) strategy, the tentative probiotic LABs showed some surprising properties which were precious for potential experiments and some of these were very much trustworthy for probiotic product development.

Conclusion

As probiotic bacteria have potential curative or prophylactic effects, so development of numerous probiotic products such as fermented milk drinks, yoghurt, cheese, ice-cream, sausages, probiotic juice, and drinking water, etc. with distinct starter culture are obligatory. These products would be able to confer health benefits of mass and common people of Bangladesh in limited cost. Futher studies should be performed to use these isolates reliably including molecular techniques like PCR-RFLP and 16S rRNA sequencing for precise identification of lactic acid bacterial species and further revisions on human trial would be commendable for probiotic product development.

Conflict of interests

The authors declare that they do not have any conflict of interests with any third party receiving any financial gain from this study.

Acknowledgments

The author is grateful to the Ministry of Education, Government of the Peoples's Republic of Bangladesh through his honourable supervisor professor Dr. Khondoker Moazzem Hossain and honourable co-supervisor Dr. S.M. Mahbubur Rahman for providing financial "Grants for Advance Research in Science" to conduct the present research work.

References

Azizpour K, Bahrambeygi S, Mahmoodpour S, Azizpour A, Mahmoodpour S, Bahrambeygi S, Azizpour K. 2009. History and basic of probiotics. Research Journal of Biological Sciences **4,** 409-426. http://medwelljournals.com/abstract/?doi=rjbsci.2009.409.426

Karna BKL, Emata OC, Barraquio VL. 2007. Lactic acid and probiotic bacteria from fermented and probiotic dairy products. Science Diliman **19,** 23-34.

Dashkevicz M, Feighner SD. 1989. Development of a differential medium for bile salt Hydrolase-active lactobacillus spp. Applied and Environmental Microbiology **55,** 11-16.

De Man JC, Rogosa M, Sharpe ME. 1960. A

medium for cultivation of lactobacilli. Journal of Applied Bacteriology **23,** 130–135.

Erkus O. 2007. Isolation, phenotypic and genotypic characterization of yoghurt starter Bacteria. Izmir Institute of Technology, Turkey, Thesis of MS.

Elizete DFRP, Carlos SR. 2005. Biochemical characterization and identification of probiotic lactobacillus for swine. Bulletin Research Center Food Processing **4,** 299-310.

FAO/WHO. 2002. FAO/WHO Guidelines for the evaluation of probiotics in food. Report of a joint FAO/WHO working group on drafting guidelines for the evaluation of probiotics in food. FAO/WHO Working group, London, Ontario.

Fuller R. 1989. Probiotics in man and animals: a review. Journal of Applied Bacteriology **66,** 365–378.

Garvie EI. 1984. Taxonomy and identification of bacteria important in cheese and fermented dairy products, in advances in the microbiology and biochemistry of cheese and fermented milk, edited by F. L. Davies and B. A. Law. Elsevier, England. p.35-67.

Giraffa G, Carminati D, Neviani E. 1997. *Enterococci* isolated from dairy products: a review of risks and potential technological use. Journal of Food Protection **60,** 732– 738.

Gismondo MR, Drago L, Lombardi A. 1999. Review of probiotics available to modify gastrointestinal flora. International Journal of Antimicrobial Agents **12,** 287-292. http://dx.doi.org/10.1016/S0924-8579(99)00050-3

Ghanbari M, Rezaei M, Jami M, Nazari RM. 2009. Isolation and characterization of *Lactobacillus* species from intestinal contents of beluga (Huso huso) and Persian sturgeon (Acipenser persicus). Iranian Journal of Veterinary Research **10,** 152-157.

Graciela F, de V, Maria PT. 2001. Food Microbiology Protocols (J.F.T. Spencer and A.L.R.D. Spencer, Ed.), Totowa, New Jersey: Humana Press Inc., chap. 21 (Probiotic Properties of Lactobacillus). p. 173-181.

Guarner F, Perdigon G, Corthier G, Salminen S, Koletzko B, Morelli L. 2005. Should yoghurt cultures be considered probiotic? British Journal of Nutrition **93,** 783-786. http://dx.doi.org/10.1155/2014/572754

Hoque MZ, Akter F, Hossain KM, Rahman MSM, Billah MM, Islam KMD. 2010. Isolation, Identification and Analysis of Probiotic Properties of *Lactobacillus Spp.* From Selective Regional Yoghurts. World Journal of Dairy & Food Science **5,** 39-46.

Lilly DM, Stillwell RH. 1965. Probiotics: growth promoting factors produced by microorganisms. Science **147,** 747-748.

Linn ZM, Kyaw Nyein AYE, San San YU. 2008 (12-14 Nov). Processing of Probiotic Yogurt. GMSARN International Conference on Sustainable Development: Issues and Prospects for the GMS.

Maniruzzaman M, Khan MFR, Amin MM, Paul AK, Islam M. 2010. Isolation and identification of bacterial flora from milk of apparently healthy buffalo-cows. International Journal of Biological Research **1,** 13-16.

Martini MC, Kukielka D, Savaiano DA. 1991. Lactose digestion from yogurt: influence of a meal and addition lactose. American Journal of Clinical Nutrition **53,** 1253- 1258.

Mazza G. 1998. Functional Food, Biochemical and Processing Aspects. Taylo and Francis, GP. LLC. Roca Raton. 357-374 P.

Prasad J, Gill H, Smart J, Gopal PK. 1998. Selection and characterization of *Lactobacillus* and

Bifidobacterium strains for use as probiotics. International Dairy Journal **8,** 993-1002.

Reller LB. Mirrett S. 1975. Motility-Indole-Lysine medium for presumptive identification of enteric pathogens of Enterobacteriaceae. Journal of Clinical Microbiology **2,** 247-252

Rosenfeldt V, Benfeldt E, Nielsen SD, Michaelsen KF, Jeppesen DL, Valerius NH, Paerregaard A. 2003. Effect of probiotic *Lactobacillus* strains in children with atopic dermatitis. Journal of Allergy and Clinical Immunology **111,** 389-395.

Saccaro DM, Hirota CY, Tamime AY, Oliveira DE. MN. 2011. Evaluation of different selective media for enumeration of probiotic micro-organisms in combination with yogurt starter cultures in fermented milk. African Journal of Microbiology **23,** 3901-3906.

Salminen SC, Bouley MC, Boutron-Ruault JH, Cummings, Franck A, Gibson GR, Isolauri E, Moreau MC, Roberfroid M, Rowland I. 1998. Functional food science and gastrointestinal physiology and function. British Journal Nutrition **80,** 147-171.

Schillinger U, Lucke FK. 1987. Identification of *lactobacilli* from meat and meat products. Food Microbiology **4,** 199-208.

Schrezenmeir J, de Vrese M. 2001. Probiotics, prebiotics and synbiotics: approaching a definition. American Journal of Clinical Nutrition **73**, 361–364.

Silva AM, Bambirra EA, Oliveria AL, Souza PP, Gomes DA, Viera EC, Nicoli JR. 1999. Protective effect of bifidus milk on the experimental infection with *Salmonella* enteritidis subsp. *typhimurium* in conventional and gnotobiotic mice. Journal of Applied Microbiology **86,** 331-336.

Taranto MP, Medici M, Perdigon G, Ruiz Holgado AP, Valdez GF. 1998. Evidence for hypocholesterolemic effect of *Lactobacillus reuteri* in hypercholesterolemic mice. Journal of dairy science **81,** 2336- 2340.

Teuber M, Geis A, Neve H. 1995. The genus *Lactococcus* in the prokaryotes. Vol II. Balows A, Truber HG, Dworkin, Harder MW, Schleifer KH, editors. New York, NY, Springer Verlag. 1482-501 P.

Vesa TH, Marteau P, Korpela R. 2000. Lactose intolerance. Journal of the American College of Nutrition **19,** 165-175.

Xanthopoulos V, Litopoulou-Tzanetaki E, Tzanetakis N. 2000. Characterization of *Lactobacillus* isolates from infant feces as dietary adjuncts. Food Microbiology **17,** 205-215.

Zinedine A, Faid M. 2007. Isolation and characterization of strains of Bifidobacteria with probiotic properties in vitro. World journal of dairy & food science **2,** 28-34.