PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

Pakistan Journal of Nutrition 6 (6): 647-652, 2007
ISSN 1680-5194

Probiotic Characteristics of Lactic Acid Bacteria Isolated from Traditional Fermented Milk 'Dahi' in Bangladesh

Md. Harun-ur-Rashid[1], Kaname Togo[1], Minoru Ueda[2] and Taku Miyamoto[1]
[1]Department of Dairy Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh
[2]Graduate School of Natural Science and Technology, Okayama University,
1-1, Naka 1-chome, Tsushima, Okayama 700-8530, Japan
[3]Foods R & D Center, Kaneka Corporation, Takasago, Hyogo 676-8688, Japan

Abstract: We evaluated the probiotic characteristics (pH and acid production, acid and bile acid tolerance and Angiotensin I-converting enzyme (ACE) inhibitory activity) of lactic acid bacteria isolated from traditional fermented milk 'Dahi' in Bangladesh. Among the tested strains, *Lactobacillus delbrueckii* subsp. *bulgaricus* M3 40-3 displayed more tolerance at acidic medium as well as highest acid production (2.13%). Whereas, highest ACE inhibitory activity (72.09±1.07%) was found in skim milk fermented with *Streptococcus bovis* J2 40-2. On the other hand, *Lactobacillus fermentum* B5 40-2 (95.53±0.79%) was exhibited strong bile acid tolerance followed by *Enterococcus faecium* D3 25-1 (88.66±0.76%), *Lactococcus lactis* subsp. *lactis* B4 25-3 (74.40±1.09%), *Lactococcus raffinolactis* D4 25-3 (72.34±1.20%) and *Pediococcus pentosaceus* B2 25-5 (65.67±1.58%). From the results obtained, *Lactobacillus delbrueckii* subsp. *bulgaricus* M3 40-3 might be used as probiotic starter culture for milk fermentation due to high acid production and tolerance at high acidic medium. *Streptococcus bovis* J2 40-2 is a unique strain detected as highest ACE inhibitory activity and suggest that it should be used as starter culture for probiotic fermented dairy foods.

Key words: Probiotics, acid and bile acid tolerance, ACE inhibitory activity

Introduction

It is well known that lactic acid bacteria (LAB) have been widely used as starter culture for the manufacturing of various fermented foods such as dairies, beverages, meat and vegetables etc. LAB and their food products are thought to confer a variety of important nutritional and therapeutic benefits and have many documented health promoting or probiotic effects in human such as inhibition of pathogenic organism, antimutagenic and reduction of blood cholesterol (Salminen *et al*., 1996). Those LAB with scientifically supported health claims define as probiotic and have an increasingly high market potential.

Fuller, 1989 define of probiotics are live microbial food supplements, which beneficially affects the host animal by improving its intestinal microbial balance. Later, the definition of probiotics have expanded to include food and non-food use and the use of mono and mixed starter cultures (Havenaar and Huis int Velt, 1992). Recently a European expert group proposed a definition that also included mechanisms not mediated by microflora change (Salminen *et al*., 1998). Most of the definitions of probiotics, emphasize that the microorganisms should be viable and reach at their site of active alive. Several studies have reported that non-viable probiotics are able to adhere to tissue culture cells indicating that viability is not necessary for adhesion (Hood and Zottola, 1998; Coconnier *et al*., 1993). When selecting probiotics, some criteria must have to be met by the probiotics organisms such as resistance to the enzymes in the oral cavity (e.g. lysozyme) and should also have the ability to resist the digestion process in the stomach and intestinal tract and arrive at the site of action in a viable physiological state and adhesion to mucosal surfaces. If they are to be used as probiotics, cultures must have Generally Regarded as Safe (GRAS) status and also meet a number of good technological properties e.g. easy propagation and incorporation into foods and long term survival and safe in food products and clinically validated and documented health effects (Stanton *et al*., 2003).

LAB for use as a probiotic culture or as food adjunct must be tolerant to acid and bile, which enables selected strains to survive, grow and perform its therapeutic benefits in the intestinal tract (Gilliland and Walker, 1989; Salminen and von Wright, 1993; Usman and Hosono, 1999). Some LAB have been performed *in vitro* acid and bile acid tolerance, which were isolated from Indonesian Dadih (Usman, 2003; Surono, 2003). Fermented milk has been reported to posses a range of beneficial properties to humans, including assimilation of cholesterol (Noh *et al*., 1997), enhanced resistance to tumergenesis (Saito *et al*., 1987), gastrointestinal bacterial infections (Shackelford *et al*., 1983) and lowering blood pressure (Yamamoto *et al*., 1994). The blood pressure mediated through Angiotensin Converting Enzyme (ACE) and this enzyme play a key role in the regulation of blood pressure. Angiotensin

Table 1: pH and acidity (%) of selected LAB strains isolated from traditional fermented milk 'Dahi' in Bangladesh

	pH			Acidity (%)		
Name of species	24 h	48 h	72 h	24 h	48 h	72 h
Streptococcus bovis J2 40-2 (37°C)*	4.27	4.18	4.15	1.09	1.14	1.14
Streptococcus thermophilus M1 40-2 (37°C)	3.92	3.90	3.72	1.26	1.48	1.52
Lactobacillus fermentum B5 40-2 (37°C)	5.48	4.98	4.55	0.53	0.76	0.80
Lactobacillus delbrueckii subsp. *bulgaricus* M3 40-3 (37°C)	3.63	3.61	3.35	1.87	2.09	2.13
Lactobacillus del. subsp. *lactis* M6 40-3 (37°C)	3.68	3.63	3.45	1.64	2.00	2.04
Lactobacillus species D6 40-4 (37°C)	4.58	4.63	4.08	0.70	0.73	0.77
Enterococcus faecium D3 25-1 (30°C)	4.80	4.74	4.68	0.74	0.76	0.77
Leuconostoc mesenteroides subsp. *mesenteroides* M8 25-4 (30°C)	–	5.34	5.32	–	0.68	0.70
Leuconostoc mes. subsp. *dextranicum* M7 25-1 (37°C)	–	5.33	5.20	–	0.67	0.75
Lactococcus lactis subsp. *lactis* B4 25-3 (30°C)	4.84	4.68	4.57	0.76	0.83	0.90
Lactococcus raffinolactis D4 25-3 (30°C)	4.85	4.52	4.50	0.80	0.89	0.90
Pediococcus pentosaceus B2 25-5 (30°C)	–	5.95	5.83	–	0.64	0.66

*Parenthesis indicates appropriate growth temperature

Converting Enzyme (ACE) is found in the rennin angiotensin system and several researchers have been reported that fermented milk with probiotics LAB can exert ACE inhibitory effect.

To our knowledge, only one finding has been reported on identification and characterization of lactic acid bacteria isolated from traditional fermented milk 'Dahi' in Bangladesh[17] and noted that LAB isolates had contained of twelve species. However, there is no existing any evidence to evaluate the probiotic characteristics of these LAB isolates. To date, people living throughout the country have been consumed dahi mainly as a desert food after typically Bangladeshi Polao dishes. Also this product is not commercially produced and marketed in only products areas. Moreover, people do not know the potential health benefits of dahi and also other indigenous fermented milk products in Bangladesh yet. Thus, it is essential to investigate of such probiotics properties of isolated LAB from dahi. The aim of this work was to assess the probiotic characteristics such as production of acid and pH, acid and bile acid tolerance and ACE inhibitory activity of the selected LAB strains isolated from traditional fermented milk 'Dahi' in Bangladesh.

Materials and Methods

Sources and maintenance of cultures: The twelve selected LAB strains such as *Streptococcus bovis* J2 40-2, *Streptococcus thermophilus* M1 40-2, *Lactobacillus fermentum* B5 40-2, *Lactobacillus delbrueckii* subsp. *bulgaricus* M3 40-3, *Lactobacillus delbrueckii* subsp. *lactis* M6 40-3, *Lactobacillus* species D6 40-4, *Enterococcus faecium* D3 25-1, *Leuconostoc mesenteroides* subsp. *mesenteroides* M8 25-4, *Leuconostoc mesenteroides* subsp. *dextranicum* M7 25-1, *Lactococcus lactis* subsp. *lactis* B4 25-3, *Lactococcus raffinolactis* D4 25-3 and *Pediococcus pentosaceus* B2 25-5 isolated from traditional fermented milk 'Dahi' used in the study were obtained from the stock cultures of Animal Food Function Laboratory, The Graduate School of Natural Science and Technology, Okayama University, Japan. All strains were preserved in skim milk containing 0.1% glutamic acid (Nacalai Tesque Co. Japan) and stored at-20°C and-80°C for further investigation. Each strain was subcultured twice in TYLG broth [per L: 10 g tryptone (Difco), 5 g yeast extract (Difco), 5 g lactose (Nacalai), 5 g glucose (Nacalai), 1 g Tween 80 (Nacalai), 0.1 g L-cysteine HCl (Nacalai)] at their appropriate growth temperatures (Table 1) prior to experimental use.

Determination of acidity and pH: A 24 h old active culture of LAB strains were inoculated (1%, v/v) into 10% sterile reconstituted skim milk and incubated at 30 or 37°C depending on their optimal growth temperatures for 72 h. Samples were withdrawn every 24 h, 48 and 72 h interval of incubation period. The pH of cultured reconstituted skim milk was measured by using Horiba pH meter F.8 (Horiba Ltd. Japan) and acidity was determined of cultured reconstituted skim milk against the 0.1 N NaOH.

Assay for acid tolerance: For the purpose of acid tolerance test, TYLG broth was adjusted to various pHs such as 3.0, 3.5, 4.0, 4.5 and 5.0 by using 0.1 N HCl and then sterilized by autoclave at 121°C for 15 min. Active cultures of LAB strains were inoculated (1%, v/v) into pH adjusted TYLG broth medium and incubated at 30 or 37°C depending on their optimal growth temperatures for 72 h. After that cell growth was observed into the medium by periodically and designated positive growth as (+) and no growth as (–).

Assay for bile acid tolerance: The effect of bile acid tolerance on the growth rate of selected LAB according to the method described by Walker and Gilliland (1993). All cultures were evaluated for growth in MRS broth with 0.3% (w/v) Oxgall (Difco, Sparks, MD. USA). Freshly prepared active cultures were inoculated (1%, v/v) into medium and incubated at their optimal growth

Table 2: Acid and bile acid tolerance of selected LAB isolated from traditional fermented milk 'Dahi' in Bangladesh

	pH tolerance					
Name of species	3.0	3.5	4.0	4.5	5.0	Bile acid tolerance (%)
Streptococcus bovis J2 40-2 (37°C)*	–	–	–	–	+	9.07±0.38
Streptococcus thermophilus M1 40-2 (37°C)	–	–	–	+	+	27.13±1.76
Lactobacillus fermentum B5 40-2 (37°C)	–	–	+	+	+	95.53±0.79
Lactobacillus delbrueckii subsp. *bulgaricus* M3 40-3 (37°C)	+	+	+	+	+	8.21±0.55
Lactobacillus del. subsp. *lactis* M6 40-3 (37°C)	–	+	+	+	+	6.38±0.28
Lactobacillus species D6 40-4 (37°C)	–	–	–	+	+	16.82±1.77
Enterococcus faecium D3 25-1 (30°C)	–	–	+	+	+	88.66±0.76
Leuconostoc mesenteroides subsp. *mesenteroides* M8 25-4 (30°C)	–	–	+	+	+	39.26±0.76
Leuconostoc mes. subsp. *dextranicum* M7 25-1 (37°C)	–	–	+	+	+	39.92±0.68
Lactococcus lactis subsp. *lactis* B4 25-3 (30°C)	–	–	+	+	+	74.40±1.09
Lactococcus raffinolactis D4 25-3 (30°C)	–	–	+	+	+	72.34±1.20
Pediococcus pentosaceus B2 25-5 (30°C)	–	–	+	+	+	65.67±1.58

*Parenthesis indicates appropriate growth temperature

temperatures (30 or 37°C) and determined their cell growth by optical density per hourly by spectrophotometrically at 620 nm. The growth was monitored by followed 6 h incubation.

Measurement of ACE inhibitory activity: The selected twelve LAB strains isolated from traditional fermented milk 'Dahi' were used for the measurement of ACE inhibitory activity. We routinely propagated lactobacilli in MRS (Oxoid, Hampshire, England) and lactococci in GM17 broth (Difco, England) for 24 h at their optimal growth temperatures (30 or 37°C). Twenty-four-hour old active cultures of selected LAB was used to inoculate (1%, v/v) into 10 ml of 10% reconstituted skim milk media, which was incubated at 30 or 37°C for 72 h.

ACE inhibitory activity was measured according to the method of Nakamura *et al.* (1995) with some modifications. Fermented skim milk was adjusted to pH 4.6 by adding 1 N NaOH or 1 N HCl and then centrifuged at 12,000×g for 20 min at 4°C. The whey supernatant was adjusted to pH 8.3 with 5 N NaOH and was given 12,000×g for 20 min in a bench centrifuge. The supernatant was used in the assay of ACE inhibitory activity. Hip-His-Leu was dissolved (50 mM) in 100 mM Na-borate buffer (pH 8.3) containing 300 mM NaCl. A sample (60 µl) was mixed with 200 µl of Hip-His-Leu solution and 40 µl of ACE (25 mM). The mixture was incubated at 37°C for 1 h. After incubation 250 µl of 1 N HCl was added to stop the reaction. The hippuric acid was liberated by ACE was extracted with 1.7 ml of ethyl acetate. After centrifugation (5,000×g for 20 min) 0.5 ml of the upper layer was transferred into a test tube and evaporated at room temperature for 20 min in rotary vacuum evaporator. The hippuric acid was diluted in 1.0 ml sterile distilled water and the absorbance was measured at 228 nm using on UV spectrophotometrically (U-1800 Spectrophotometer, Hitachi Co. Ltd. Japan). The percentage of ACE inhibitory activity was calculated by following formula:

ACE inhibitory activity (%) = [(B-A) / (B-C)] ×100

Where,

A is the optical density in the presence of both ACE and ACE inhibitory compounds

B is the optical density without ACE inhibitory compounds

C is the optical density without ACE and ACE compounds

Results

Acid production and pH of skim milk fermented by LAB: Table 1 showed the pH and acidity of skim milk fermented with selected 12 LAB species isolated from traditional fermented milk 'Dahi'. Among them, *Lb. delbrueckii* subsp. *bulgaricus* M3 40-3 could produced the highest acid production (2.13%) and at the same time decreased their pH from 6.0 to 3.35 in fermented skim milk when incubated at 37°C for 72 h. However, highest pH (5.83) and lowest acidity (0.66%) were recorded in skim milk fermented with *P. pentosaceus* B2 25-5, incubated at their optimal growth temperature for 72 h. *Leuc. mesenteroides* subsp. *mesenteroides* M8 25-4 and *Leuc. mesenteroides* subsp. *dextranicum* M7 25-1 could unable to coagulate the skim milk up to 24 h incubation, while hard coagulation was observed in skim milk fermented with both *Lc. lactis* subsp. *lactis* B4 25-3 and *Lc. raffinolactis* D4 25-3 at early 24 h incubation.

Acid tolerance of selected LAB isolated from traditional fermented milk 'Dahi': Acid tolerance of 12 selected LAB species are showed in Table 2. TYLG broth with various initial pH inoculated (1%, v/v) by active cultures of the selected LAB were found a variable results after 72 h incubation. *Lb. delbrueckii* subsp. *bulgaricus* M3 40-3 was exhibited to growth at the wide ranges of pH (3.0 to 5.0). While, *S. bovis* J2 40-2 could growth only at pH 5.0. On the other hand, *S.*

Table 3: ACE inhibitory activity of selected LAB strains isolated from traditional fermented milk 'Dahi' in Bangladesh

Name of species	ACE inhibitory activity (%)	ACE inhibitory activity (%) Pre-incubation method
Streptococcus bovis J2 40-2 (37°C)*	72.09±1.07	45.37±0.62
Streptococcus thermophilus M1 40-2 (37°C)	18.19±0.41	–
Lactobacillus fermentum B5 40-2 (37°C)	50.60±1.14	15.37±0.83
Lactobacillus delbrueckii subsp. *bulgaricus* M3 40-3 (37°C)	31.54±0.95	–
Lactobacillus del. subsp. *lactis* M6 40-3 (37°C)	31.57±1.09	–
Lactobacillus species D6 40-4 (37°C)	47.55±0.76	16.90±1.29
Enterococcus faecium D3 25-1 (30°C)	52.37±1.01	–
Leuconostoc mesenteroides subsp. *mesenteroides* M8 25-4 (30°C)	47.40±0.96	22.46±1.11
Leuconostoc mes. subsp. *dextranicum* M7 25-1 (37°C)	30.35±0.79	20.48±1.01
Lactococcus lactis subsp. *lactis* B4 25-3 (30°C)	–	–
Lactococcus raffinolactis D4 25-3 (30°C)	–	–
Pediococcus pentosaceus B2 25-5 (30°C)	–	–

*Parenthesis indicates appropriate growth temperature

thermophilus M1 40-2, *Lb.* species D6 40-4, *Lb. fermentum* B5 40-2, *Ec. faecium* D3 25-1, *Leuc. mesenteroides* subsp. *mesenteroides* M8 25-4, *Leuc. mesenteroides* subsp. *dextranicum* M7 25-1 and *P. pentosaceus* B2 25-5 could not grow at pH 3.0 to 4.0 but well grown at pH 4.0 to 5.0.

Bile acid tolerance of selected LAB isolated from traditional fermented 'Dahi': Bile acid tolerances (0.3% Oxgall) of selected LAB stains are shown in Table 2. From the results, *Lb. fermentum* B5 40-2 was exhibited the highest bile acid tolerance (95.53±0.79%) followed by *Ec. faecium* D3 25-1 (88.66±0.76%), *Lc. lactis* subsp. *lactis* B4 25-3 (74.40±1.09%), *Lc. raffinolactis* D4 25-3 (72.34±1.20%) and *P. pentosaceus* B2 25-5 (65.67±1.58%). Lowest bile acid tolerances were observed by *Lb. delbrueckii* subsp. *lactis* M6 40-3 (6.38±0.28%), *Lb. delbrueckii* subsp. *bulgaricus* M3 40-3 (8.21±0.55%), *S. bovis* J2 40-2 (9.07±0.38%) and *Lb. species* D6 40-4 (16.82±1.77%).

ACE inhibitory activity: Table 3 represented the ACE inhibitory activity of selected LAB strains isolated from traditional fermented milk 'Dahi'. Of the selected LAB isolates, *S. bovis* J2 40-2 was exhibited the highest ACE inhibitory activity (72.09±1.07%) of fermented skim milk in respects to all strains. No ACE inhibitory activity was determined by the species of *Lc. lactis* subsp. *lactis* B4 25-3, *Lc. raffinolactis* D4 25-3 and *P. pentosaceus* B2 25-5 in the fermented skim milk. ACE inhibitory activity could not measure when ACE determined by preincubation method of the strains of *Lb. delbrueckii* subsp. *bulgaricus* M3 40-3, *Lb. delbrueckii* subsp. *lactis* M6 40-3, *S. thermophilus* M1 40-2 and *Ec. faecium* D3 25-1. However, there was no significance difference of ACE inhibitory activity of *S. bovis* J2 40-2 by determined of different methods such as preincubation method.

Discussion

Rashid *et al.* (2007) reported in our previous study that 12 species of lactic acid bacteria were isolated from traditional fermented milk 'Dahi' in Bangladesh. One representative strain from each species group was selected for the evaluation of probiotic characteristics in the present study. Results from this study revealed that *Lb. delbrueckii* subsp. *bulgaricus* M3 40-3 was produced highest acid production among the lactobacilli and *S. thermophilus* M140-2 among the lactococci. Bacteria would contact pH values ranging from 2.0 to 8.0 in the gastrointestinal tract if consumed (Hood and Zottola, 1988). On the other hand, *Lb. delbrueckii* subsp. *bulgaricus* M3 40-3 could able to survive more acidic medium among the tested strains of LAB. Besides, *S. bovis* J2 40-2 could produced low acid production and exhibited low bile acid tolerance, while *Lb. fermentum* B5 40-2 was exhibited the highest bile acid tolerance. Thus probiotic cultures must survive in the environment with gastric and bile acids, when viable cells go through the gastrointestinal tract. Resisting at pH 3.0 and growing in the medium containing 0.1% bile acids are considered as standards for acid and bile acid tolerance of probiotic culture (Itoh, 1992; Gohran, 1994). Meanwhile, *Lb. delbrueckii* subsp. *bulgaricus* M3 40-3 has good acid tolerance and less bile acid tolerance (0.3% Oxgall). Thus this strain must be survived at high acidic environment in the stomach and low concentration of bile components in the intestine when consumed fermented foods containing of this strain.

Milk fermented with *S. bovis* J2 40-2, *Lb. fermentum* B5 40-2, *Lb.* species D6 40-4, *Ec. faecium* D3 25-1 and *Leuc. mesenteroides* subsp. *mesenteroides* M8 25-4 were exhibited higher ACE inhibitory activity. It has been reported that unfermented milk apparently exhibited slight ACE inhibitory activity and the ACE inhibitory activity was markedly increased during fermentation by probiotics LAB (Nakamura *et al.*, 1995). Studies to demonstrate that probiotics supplementation can affect plasma cholesterol concentrations and consequently the incidence of blood pressure and coronary heart disease. These results clearly indicated that fermentation plays an important role to release of ACE inhibitory peptides from milk proteins by microbial

proteinases. Fuglsang *et al.* (2003) reported that two strains of *Lb. helveticus* capable of producing fermented milk with *in vivo* activity against ACE have been identified and highly significant blood pressure effects were observed. However, the activity and thus the *in vivo* potential of the fermented milk, varies with the strain. Similarly, in the present study, some strains of LAB are able to produce ACE inhibitory compounds during milk fermentation. It has been reported that milk fermentation with *Lb. helveticus* proved highly hypotensive in a cuff study, with a drop of up to about 30 mm Hg in systolic blood pressure in spontaneous hypertensive rats (Yamamoto *et al.*, 1994; Nakamura *et al.*, 1995). Therefore, inhibition of ACE results in promoting the vasodilatory effect, leading to the treatment of high blood pressure in human and animals. LAB are known to produce that ACE inhibitors of the enzyme in various amounts during milk fermentation (Yamamoto *et al.*, 1994; Meisel *et al.*, 1997; Gobbetti *et al.*, 2000). Several researchers suggested that probiotics LAB can be used for producing potent ACE inhibitory peptides from milk proteins for the regulation of blood pressure.

Conclusions: To our knowledge, this is the first report to evaluate the probiotic properties of lactic acid bacteria isolated from traditional fermented milk 'Dahi'. Among them, *Lb. fermentum* B5 40-2, *Lb. delbrueckii* subsp. *bulgaricus* M3 40-3 and *S. bovis* J2 40-2 were exhibited good probiotic characteristics that might be used for food or dairy fermentation and contribute health benefits to consumers. The strain of *S. bovis* J2 40-2 showed with certainty the production of ACE inhibitory compounds followed by *Ec. faecium* D3 25-1, *Lb. fermentum* B5 40-2, *Lb.* species D6 40-4 and *Leuc. mesenteroides* subsp. *mesenteroides* M8 25-4 and can be used for producing potent ACE inhibitory peptides. Further work will be addressed the purification and amino acid sequences of ACE inhibitory peptides/compounds in milk fermentation with these strains. Moreover, beneficial health effect of dahi and its lactic acid bacteria is not understood well by the maximum Bangladeshi yet. Therefore, results from the present study are expected to encourage the people to consume more fermented milk dahi, as it was revealed that dahi contain some probiotic lactic acid bacteria which play major role for beneficial health effects of consumers.

Acknowledgements

The authors wish to thank the Ministry of Education, Culture, Sports, Science and Technology and Foods Research and Development Center, Kaneka Corporation, Japan for financial support.

References

Salminen, S., E. Isolauri and E. Salminen, 1996. Clinical uses of probiotics for stabilizing the gut mucosal barrier: successful stains and future challenges. *Antonie van Leeuwenhoek*, 70: 251-262.

Fuller, R., 1989. Probiotics in man and animals. J. Appl. Bacteriol., 66: 365-378.

Havenaar, R. and J. Huis in't Velt, 1992. Probiotics: A general view. In wood ed. The lactic acid bacteria in health and disease. London Elsevier Appl. Sci., pp: 209-224.

Salminen, S., A.C. Ouwehand and E. Isolauri, 1998. Clinical applications of probiotic bacteria. Inter. Dairy J., 8: 563-572.

Hood, S.K. and E.A. Zottola, 1988. Effect of low pH on the ability of Lactobacillus acidophilus to survive and adhere human intestinal cells. J. Food Sci., 53: 1514-1516.

Coconnier, M.H., M.F. Bernet, S. Kerneis, G. Chauviere, J. Fourniat and A.L. Servin, 1993. Inhibition of adhesion of enteroinvasive pathogens to human intestinal Caco-2 cells by *Lactobacillus acidophilus* strain LB decreases bacterial invasion. FEMS Microbiology Letters, 110: 299-305.

Stanton, C., C. Desmond, M. Coakley, J.K. Collins, G. Fitzgerald and R.P. Ross, 2003. Challenges facing development of probiotic-containing functional foods. In Handbook of feremented functional foods. Ed. Farnworth, E.R. Florida, CRC press, pp: 27-58.

Gilliland, S.E. and D.K. Walker, 1989. Factor to consider when selecting a culture of *Lactobacillus acidophilus* as a dietary adjunct to produce a hypocholesterolemic effect in humans. J. Dairy Sci., 73: 905-911.

Salminen, S. and A. von Wright, 1993. Lactic acid bacteria. Marcel Dekker, Inc., New York, Basel, Hong Kong.

Usman, P. and A. Hosono, 1999. Bile tolerance, taurocholate deconjugation and binding of cholesterol by *Lactobacillus gasseri* strains. J. Dairy Sci., 82: 956-961.

Surono, S.I., 2003. *In vitro* probitic properties of indigenous dadih lactic acid bacteria. Asian-Australasian J. Anim. Sci., 16: 726-731.

Usman, P., 2003. Bile and acid tolerance of lactic acid bacteria isolated from Dadih and their antimutagenicity against mutagenic heated Tauco. Asian-Australasian J. Anim. Sci., 16: 1680-1685.

Noh, D.O., S.H. Kim and S.E. Gilliland, 1997. Incorporation of cholesterol into the cellular membrane of *Lactobacillus acidophilus* ATCC 43121. J. Dairy Sci., 80: 3107-3113.

Saito, H., H. Tomioka and K. Nagashima, 1987. Protective and therapeutic efficacy of *Lactobacillus casei* against experimental infections due to *Mycobacterium fortuitum* complex. J. Gen. Microbiol., 133: 2843-2851.

Shackelford, L.A., D.R. Rao, C.B. Chawan and S.R. Pulusani, 1983. Effect of feeding fermented milk on the incidence of chemically induced colon tumors in rats. Nutr. Cancer, 5: 159-164.

Yamamoto, N., A. Akino and T. Takano, 1994. Antihypertensive effect of different kinds of fermented milk in spontaneously hypertensive rats. Biosci. Biotechnol. Biochem., 58: 776-778.

Rashid, M.H., K. Togo, M. Ueda and T. Miyamoto, 2007. Identification and characterization of lactic acid bacteria isolated from traditional fermented milk 'Dahi' in Bangladesh. World J. Microbiol. Biotechnol., 23: 125-133.

Walker, D.R. and S.E. Gilliland, 1993. Relationship among bile tolerance, bile salt deconjugation and assimilation of cholesterol by Lactobacillus acidophilus. J. Dairy Sci., 76: 956-961.

Nakamura, Y., N. Yamamoto, K. Sakai, A. Okubo, S. Yamazaki and T. Takano, 1995. Purification and characterization of angiotensin I-converting enzyme inhibitors from sour milk. J. Dairy Sci., 78: 777-783.

Itoh, T., 1992. Functional benefits from lactic acid bacteria used in cultured milk. Anim. Sci. Technol., 63: 1276-1289.

Gohran, M., 1994. Lactobacillus with the ability of colonization and establishing in the intestinal tracts. Japan Patent, 6: 501-624.

Fuglsang, A., F.P. Rattray, D. Nilsson and N.C.B. Nyborg, 2003. Lactic acid bacteria: inhibition of angiotensin converting enzyme *in vitro* and *in vivo*. *Antonie van Leeuwenhoek*, 83: 27-34.

Gobbetti, M., P. Ferranti, E. Smacchi and F. Goffredi, 2000. Production of angiotensin-I converting enzyme inhibitory peptides in fermented milks started by *Lactobacillus delbrueckii* subsp *bulgaricus* SS1 and *Lactococcus lactis* subsp *cremoris* FT5. Appl. Environ. Microbiol., 66: 3898-3904.

Meisel, H., A. Goepfert and S. Gunther, 1997. ACE inhibitory activities in milk products. *Milchwissenschaft*, 52: 307-311.