British Microbiology Research Journal
6(1): 24-31, 2015, Article no.BMRJ.2015.055
ISSN: 2231-0886

SCIENCEDOMAIN *international*
www.sciencedomain.org

Identification and Characterization of *Lactobacilli* from Rajshahi Traditional Curds

M. Khairul Islam[1], M. Abdul Alim Al-Bari[1*], Alam Khan[1], M. Kudrat-E-Zahan[2], and M. Anwar Ul-Islam[1]

[1]Department of Pharmacy, University of Rajshahi, Rajshahi-6205, Bangladesh.
[2]Department of Chemistry, University of Rajshahi, Rajshahi-6205, Bangladesh.

Authors' contributions

This work was carried out in collaboration between all authors. Author MKI was responsible for conducting the experiments. Author AAB designed the study, wrote the protocols for different experiments and the first draft of the manuscript and managed literature searches. Authors AK and MKZ analyzed the data and supported author AAB to write the manuscript. Author AI helped to identify and characterize all bacteria. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BMRJ/2015/14424
Editor(s):
(1) Yonca Karagul Yuceer, Department of Food Engineering, Canakkale Onsekiz Mart University, Turkey.
Reviewers:
(1) Solakunmi Oluwajoba, Biological Science, Yaba College of Technology, Yaba Lagos Nigeria.
(2) Anonymous, Nigeria.
(3) Anonymous, Spain.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=827&id=8&aid=7137

Original Research Article

Received 29th September 2014
Accepted 17th November 2014
Published 8th December 2014

ABSTRACT

The popular food, dairy products like curds are consumed by many people in its various forms in Bangladesh. Infact, these different types of curds are made without real respect for basic standards of technology and hygiene. This situation will be improved if lactic ferments are well-identified and their synergy taken into consideration. Not much information has been reported about the *Lactobacilli* species responsible for curd production in Bangladesh. In this context, this study was conducted to identify and characterize lactic acid bacteria in curds to provide scientific data needed for improvement of the fermented product. Twenty samples were collected from the producers of curds in the supermarket of Shaheb Bazar, Rajshahi. The culture media used were Czapek Dox (pH 5.3), M17, MRS and nutrient broth and agar for the investigation of lactic acid bacteria. The identified lactic acid bacteria were *Lactobacilli* including *Lactobacillus casei* (sample 1), *Lactobacillus delbrueckii* (sample 3), *Lactobacillus plantarum* (sample 4) and *Lactobacillus helveticus* (sample 6) based on morphological characteristics, catalase activities, milk coagulation, growth at different temperature, growth in medium with different NaCl concentrations, growth at

**Corresponding author: E-mail: alimalbari347@ru.ac.bd;*

different pH, carbohydrate fermentation profiles, exopolysaccharides production, resistance to antibiotics and other biochemical tests. The results of this work will help in the production of dairy products with better quality and longer shelf-life. The isolation of lactic acid bacteria particularly *Lactobacilli* could be a good tool for information to improve the characteristics of curds and hygienic quality of the dairy products.

Keywords: Doi; Lactobacilli; antibiotic resistance; identification; Rajshahi.

1. INTRODUCTION

In 1989, Fuller defined the term probiotics as live microbial food supplements, which beneficially affect the host by improving the intestinal microbial balance [1]. Among these microorganisms, lactic acid bacteria are regarded as major groups of probiotic bacteria. Highly nutritious food, milk and dairy products are considered as ideal for humans due to their richness in carbohydrates, fats, vitamins and minerals [2,3]. In addition to their nutritional values, the consumption of probiotics in fermented dairy products is also associated with beneficial effects on health [4]. Lactic acid bacteria in dairy products are used as remedy for the gastrointestinal disorders such as irritable bowel syndrome [5,6], chronic idiopathic constipation [7], controlling blood pressure [8], and breast cancer [9] and colon cancer [10,11], reducing cholesterol level & allergic diseases [10,12] and modulating immune responses [13]. Indeed, the dairy products come in many forms depending on the region and varied tastes. Fermented dairy products in particularly curds are very popular with Bangladeshi consumers because their tastes and flavors. Most studies on traditional fermented dairy products showed that lactic acid bacteria are the main causing agents of these fermentations groups [14]. The presence of lipase in lactic acid bacteria can decompose fat and free fatty acids in milk, resulting in the appearance of rancid odor in the dairy product [15].

The interesting groups of microorganisms, lactic acid bacteria are characterized by their abilities to ferment carbohydrates into lactic acid, aweak acid conducive to the conservation and improvement of the organoleptic quality of food. Lactic acid bacteria comprise diverse groups of Gram-positive, non-spore-forming micro-organisms. The interest of lactic acid bacteria in the food industry lies mainly in their ability to convert some sugars into lactate and soto acidify the surrounding environment. Fermentable carbohydrates are used as energy sources and are degraded to lactate (homofermentatives) or to lactate and additional products such as acetate, ethanol, carbon dioxide, formate, or succinate (heterofermentatives) [16]. Thelactic acid bacteria produced as commercial starter cultures are pure culture soramix belonging to the genera *Streptococcus*, *Lactobacillus*, *Enterococcus*, *Leuconostoc*, *Aerococcus Lactococcus*, *Streptococcus*, *Pediococcus* and *Bifidobacterium* [17]. Thus, lactic acid bacteria especially *Lactobacilli* species are widely used in food technology. The manufacture of high-quality dairy products requires close attention to characterization, differentiation, and maintenance of *Lactobacilli* starter culture strains.

The interest in microorganisms occurring in foods is primarily due to the biotechnological potential of new bacterial species and strains. Here, we selected the dairy product, curd, as a potential source of new species or types of lactic acid bacteria because curd, which is a low-pH product, also provides an added carbohydrate source. The study supported the predominance of lactic acid bacteria in curd. In Bangladesh, especially in Rajshahi, no study has so far been reported on this subject. This lack of scientific information led us to seek and identify these bacteria in different varieties of traditional dairy food curds (locally called '*doi*'), which is very well consumed by the population.

2. MATERIALS AND METHODS

2.1 Sampling and Isolation

Twenty samples of curds were procured randomly from retailed market in the Shaheb Bazar area of Rajshahi, aseptically and in sterile conditions. The collected samples were stored in a refrigerator to stop the growth of microorganisms. Total viable lactic acid bacteria counts in each sample were analyzed by spread plating the serially diluted samples onto nutrient agar. After incubation at 15-37ºC for 72 hours, colonies with clear zones were counted. Some of these colonies were selected and purified on nutrient agar medium and preserved the slants for a long time.

2.2 Identification of Bacterial Strains

For morphological identification of lactic acid bacteria, at first all isolates were used for microscopic observations such as colony characteristics (spore formation, elevation margin, surface, pigmentation, opacity, whether grown inside, at the bottom or on the surface of the medium and the rate of growth). The cultures were identified according to their physiological and biochemical characteristics up to genetic level [18,19]. The biochemical tests used were Gram reaction; production of catalase; growth at different temperatures (10ºC, 15ºC, 37ºC, 40ºC and 45ºC) for 3 days; growth resistance to 60ºC for 30 min (Sherman test); growth in the presence of 2, 3, 4 and 6.5% NaCl and different pHs (4.5 and 6.5); fermentation profile of different sugars (such as arabinose, fructose, galactose, glucose, lactose, mannose, mannitol, maltose, sorbitol, sucrose or xylose in 1% w/v) [20], lactic acid production from 1% w/v lactose and motility. For catalase test, in brief, growth from an overnight culture of the microbe was smeared on a microscope slide. A drop of 3% hydrogen peroxide was added. If copious bubbles were observed, the microbe was positive for catalase otherwise negative. Colonies were characterized on nutrient, MRS and M17 agar. Strains with gram positive and catalase negative reactions were finally used for further identification [21].

2.3 Testing for Resistance to Antibiotics

Bacterial antibiotic resistance was determined on solid nutrient agar by the use of 11 different antibiotic discs (Table 1). The results (average of 3 reading) were expressed as sensitive (S) or resistant (R) by following the standard disc diffusion method [22]. Antibiotics such as penicillin G (10 µg), amoxicillin (30 µg), cefixime (5 µg), ceftazidime (30 µg), Kanamycin (30 µg), tetracycline (30 µg), streptomycin (10 µg), erythromycin (15 µg) and ciprofloxacin (5 µg) were used for this study [23].

2.4 Exopolysaccharides (EPS) Production

The procedure used consisted of revealing the presence of diffuse capsules surrounding bacteria cells. The strain producing capsules were also tested for slime formation. Exopolysaccharide production was evaluated as reported by Azadnia et al. [24]. Briefly, on a clean slide, a loop of broth culture was mixed with a drop of India ink, covered with a cover glass and examined under a microscope. For slime production, strains were streaked on the suitable media and incubated at 37ºC for 24h. Ropiness of colonies on agar surfaces was tested with a loop to observe the formation of slimy filaments.

3. RESULTS

Here, we isolated lactic acid bacteria which were potential for lactic acid production in nutrient agar. Lactic acid bacterial colonies were marked on the surface of nutrient agar plates. Most of the cases more than one colony was recognized. With the help of a microscope, bacterial cultural and morphological characteristics were examined. A wide variety of microorganisms were distinguished, majority of them associated with the Gram positive rods and *cocci* shaped bacteria. The purification step of bacteria was performed by shifting Gram positive rods shaped bacteria to the plates of nutrient agar media separately and repetitively. These processes were continued until pure cultures of bacteria were obtained. After initial identification of lactic acid bacteria, the representative family, *Lactobacillaceae* was determined and the genus, *Lactobacillus* was confirmed and segregated from other referred genus *Lactococcus, Streptococcus, Pediococcus, Leuconostoc* and some of *Bifidobacteria, Staphylococcus* etc. [25,26]. Here we investigated to identify rod shaped lactic acid bacteria only. Finally, four strains of pure cultures were selected for various biochemical and physiological tests and the results were given in Table 1. These tests displayed that all the four strains were Gram positive, non-spore rods, and motility negative. Moreover, there were no strains presented any catalase activity. Sample 3 showed proper growth at low level of NaCl concentration (2%). It was observed that samples 1and 4 could survive in presence of up to 4% but not 6.5% NaCl concentration. Sample 3 could grow only at 2% NaCl concentration. Sample 6 showed a moderate growth in 6.5% NaCl solution in media. Four selected strains were characterized further by sugar fermentation tests and the results were appeared in Table 1. These results indicated that sample 1 showed positive reactions for fructose, galactose, glucose, lactose, mannose, mannitol, maltose, sorbitol, sucrose and xylose, except arabinose. The results for sample 3indicated that the strain fermented lactose, glucose and fructose only but notother sugars (Table 1). Sample 4 showed positive reactions with all tested sugars while sample 6 showed negative

reactions with all tested sugars except lactose, glucose and galactose. Table 1 showed the results obtained for antibiotic susceptibility of the 11 different antibiotics tested. All strains were susceptible to amoxicillin, kanamycin, tetracycline, streptomycin, erythromycin and ciprofloxacin. Most strains showed resistance to 3 of the 11 antibiotics tested that is, to penicillin G (3 out of 4 strains), cefixime (all strains) or ceftazidime (all strains) (Table 1). Exopolysaccharides (EPS) are extracellular polymeric substances that are found either attached to the cell walls as capsular forms or secreted by bacteria into the extracellular environment as slimes. For EPS-production, lactic acid bacteria have gained a great attention in food research because of the interesting protection properties of these polymers from pathogenic bacteria and the Generally Recognized as Safe (GRAS) status. The presence of EPS-polymers, lactic acid bacteria improve the quality and texture of the curds and prevent the syneresis [27,28]. Finally, all of the four strains showed exopolysaccharide positive reaction indicating that the strains were safe for curd fermentation.

4. DISCUSSION

Curd is one of the most popular fermented dairy products of diet in the Indian subcontinent. It is formed by fermentation of milk with the help of lactic acid bacteria. Curdis reported to have a greater therapeutic value and digestibility than milk [29]. Probioticfoods, includingdairy products like curds, have been classically definedas food scontaining live microorganisms believedto active lyenhance health benefit to the host [30]. The beneficial effects of lactic acid bacteria with probiotic properties are suggested to be due to a number of factors including regulation of intestinal microbial homeostasis, maintenance of the gastrointestinal barrier function that prevents pathogens from adhering, interference with the ability of pathogens to colonize, changes in total enzyme activity in the colon contents, changes in the availability of nutrients and finally modulation of local and systemic immune responses [11,31-35]. Most of the probiotic microorganisms were lactic acid bacteria which have a long history in preserving foods from spoilage microorganisms through inhibition of pathogenic bacteria and preservation of nutritive qualities of raw food material for an extended shelf life [36]. Therefore, it is important to identify and characterize lactic acid bacteria in order to improve the technological and hygienic quality of the dairy products as well as the extension of shelf life.

The results showed in Table 1 that all the four strains were non spore-forming rods, gram positive, non-motile and non-catalase activity. So, these stains were fallen in the family *Lactobacillaceae* and genus *Lactobacillus*. The results of growths at different NaCl concentrations suggested that sample 3 showed proper growth at low level of NaCl concentration (2%). However, samples 1 and 4 showed proper growth in 4% NaCl while no growth was observed in 6.5% NaCl except sample 6. Based on the sugar tests, sample 1 showed positive reactions for fructose, galactose, glucose, lactose, mannose, mannitol, maltose, sorbitol, sucrose and xylose, except arabinose. The results for sample 3indicated that the strain gave positive reactions with lactose, glucose and fructose while negative reactions were observed with other sugars. Sample 4 gave positive reactions with all tested sugars while sample 6 gave negative reactions with all tested sugars except lactose, glucose and galactose. The results obtained from antibiotic susceptibilities of different antibiotics tested indicated that all strains were susceptible most of the antibiotics including (amoxicillin, kanamycin, tetracycline, streptomycin, erythromycin and ciprofloxacin). However, the strains showed resistance to antibiotics such as penicillin G (3 out of 4 strains), cefixime (all strains) or ceftazidime (all strains). As the widespread antibiotic resistance could be attributed to excessiveor in discriminate use of antibiotics in this part of the world ordueto chromosomal resistance, all the lactic acid bacteria isolated were susceptible for different antibiotics except penicillin G, ceftazidime or cefixime. The major results obtained from our group are consistent with the reports of Azadnia et al. [24]. The *Lactobacilli* group of bacteria was divided into three subgroups, mesophilic facultative heterofermentative, thermophilic obligate homo-fermentative and mesophilic obligate hetero-fermentative bacteria. Our morphological, biochemical and physiological results were consistent with some observations in reports of [25,37,38]. All the isolated strains were further confirmed by sugar tests and the results are presented in Table 1. These results indicate mesophilic facultative heterofermentative *Lactobacillus casei* (sample 1) and *Lactobacillus plantarum* (sample 4) and thermophilic obligate homo-fermentative *Lactobacillus delbrueckii* (sample 3) and *Lactobacillus helveticus* (sample 6).

Table 1. Physiological and biochemical characteristics of isolated strains

Characteristics		Strain			
		Sample 1	**Sample 3**	**Sample 4**	**Sample 6**
Morphology		Rods pairs	Rods pairs	Rods pairs	Rods pairs
Gram stain reaction		+	+	+	+
Catalase activity		-	-	-	-
Motility		-	-	-	-
Glucose fermentation		+	-	+	+
Milk coagulation		+	+	+	+
	10	+	-	+	-
	15	+	-	+	-
Growth at temperature (ºC)	37	±	+	+	+
	40	-	+	-	+
	45	-	+	-	-
	2	+	+	+	+
	3	+	-	+	+
Growth in a medium with NaCl (%)	4	+	-	+	+
	6.5	-	-	-	+
Growth at pH	4.5	+	+	+	+
	6.5	+	+	+	-
Heat resistance 63.5ºC for 30 min		-	-	-	+
Sugar fermentation profile					
Arabinose		-	-	+	-
Fructose		+	+	+	-
Galactose		+	-	+	+
Glucose		+	+	+	+
Lactose		+	+	+	+
Mannose		+	-	+	-
Mannitol		+	-	+	-
Maltose		+	-	+	-
Sorbitol		+	-	+	-
Sucrose		+	-	+	-
Xylose		+	-	+	-
Production of CO_2 from Glucose		-	-	-	-
Production of lactic acid		+	+	+	+
Production of H_2O_2		+	+	+	+
Production of diacetyl		+	+	+	+
Exopolysaccharides (EPS) production		+	+	+	+
Resistance to antibiotics (µg/dl)					
Penicillin G	10	R	S	R	R
Cefixime	5	R	R	R	R
Ceftazidime	30	R	R	R	R
Kanamycin	5	S	S	S	S
Tetracycline	30	S	S	S	S
Streptomycin	10	S	S	S	S
Erythromycin	15	S	S	S	S
Ciprofloxacin	5	S	S	S	S
Amoxicillin	30	S	S	S	S

+: positive reaction; -: negative reaction; R: resistance and S: sensitive

Traditional probiotic dairy strains of lactic acid bacteria have a long history of safe use. There is considerable interest in extending the range of foods incorporating probiotic organisms from dairy foods to infant formulae, baby foods, fruit juice based products and cereal-based products and pharmaceuticals [39-41] due to the ability of antimicrobial compound production by lactic acid bacteria. Thus, understanding the interactions between the involved microorganisms, in different food systems, is very important in successful application of lactic acid bacteria to control other pathogenic microorganisms. This information is important in identification and selection of lactic acid bacteria for mix cultures as well as in the selection of stimulant bacteria in antimicrobial compound production using lactic acid bacteria.

5. CONCLUSION

This work has identified and characterized lactic acid bacteria specifically *Lactobacilli* in curds available in the supermarket of Shaheb Bazar, Rajshahi. The identified lactic acid bacteria include *Lactobacillus casei, Lactobacillus delbrueckii*, *Lactobacillus plantarum* and *Lactobacillus helveticus* based on carbohydrate fermentation profiles, catalase activities and other biochemical tests. The total number of identified bacteria especially lactic acid bacteria in curds still remains to be investigated because the super market of Rajshahi is too large and the agro-ecological zones of curd productions are varied in tastes, flavors and appearances. However, the knowledge gained from this study will help in the production of dairy products with longer shelf-lives and pleasant delicious food. Other studies, including the characterization of molecular biology of lactic acid bacteria are necessary to provide sufficient information to improve the technological and hygienic quality of dairy products. This study provides a first time investigation of isolation and characterization of lactic acid bacteria from Rajshahi traditional curds. Because of an increased demand for traditional curds, the results of the present study might be able to improve the hygienic quality and retain the characteristic delicious taste in curds.

ACKNOWLEDGEMENTS

This study was supported by a research grant from Faculty of Sciences, University of Rajshahi. We thank M. Aktar Uzzaman Choudhuri and M. Mamunur Rashid for technical assistance.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Fuller R. Probiotics in man and animals. J Appl Bacteriol. 1989;66(5):365-378.
2. Aggad H, Mahouz F, Ammar YA, Kihal M. Evaluation of the hygienic quality of milk in western Algeria. Revue de Médecine Vétérinaire. 2009;160(12):590-5.
3. Ahmed AI, Mohammed AA, Faye B, Blanchard L, Bakheit SA. Assessment of quality of camel milk and gariss, north Kordofan State, Sudan. Res J Animal Vet Sci. 2010;5(1):18-22.
4. Takahiro M, Nobuhiko K, Toshinao G. Milk consumption does not affect body mass index but may have an unfavorable effect on serum total cholesterol in Japanese adults. Nutri Res. 2007;27(7):395-9.
5. Lee KN, Lee OY. Intestinal microbiota in pathophysiology and management of irritable bowel syndrome. World J Gastroenterol. 2014;20(27):8886-97.
6. Wall GC, Bryant GA, Bottenberg MM, Maki, ED, Miesner AR. Irritable bowel syndrome: A concise review of current treatment concepts. World J Gastroenterol. 2014;20(27):8796-8806.
7. Ford AC, Quigley EM, Lacy BE, Lembo AJ, Saito YA, Schiller LR, Soffer EE, Spiegel BM, Moayyedi P. Efficacy of Prebiotics, Probiotics and Synbiotics in Irritable Bowel Syndrome and Chronic Idiopathic Constipation: Systematic Review and Meta-analysis. Am J Gastroenterol; 2014. DOI: 10.1038/ajg.2014.202.
8. Khalesi S, Sun J, Buys N, Jayasinghe R. Effect of probiotics on blood pressure: A systematic review and meta-analysis of randomized, controlled trials. Hyperten; 2014. pii:HYPERTENSIONAHA.114.03469.
9. Aragón F, Perdigón G, de Moreno de LeBlanc A. Modification in the diet can induce beneficial effects against breast cancer. World J Clin Oncol. 2014;5(3):455-464. DOI: 10.5306/wjco.v5.i3.455.
10. Ouwehand AC, Salminen S, Isolauri E. Probiotics: An overview of beneficial effects. Antonie van Leeuwenhoek. 2002;82(1-4):279-289.

11. Rastall RA, Gibson GR, Gill HS, Guarner F, Klaenhammer TR, Pot B, Reid G, Rowland IR, Sanders ME. Modulation of the microbial ecology of the human colon by probiotics, prebiotics and synbiotics to enhance human health: An overview of enabling science and potential applications. FEMS Microbiol Ecol. 2005;52(2):145-152.
12. Guarner F, Schaafsma GJ. Probiotics. Int J Food Microbiol. 1998;39(3):237-238.
13. Marteau P, Rambaud JE. Potential of using lactic acid bacteria for therapy and immune modulation in man, FEMS Microbiol Reviews. 1993;12:207-220.
14. Ben Amor K, Vaughan EE, de Vos WM. Advanced molecular tools for the identification of lactic acid bacteria. J Nutri. 2007;137:741S–747S.
15. Vignola CL. Science and technology of the milk: Milk processing - Quebec: Foundation and dairy technology of the Quebec. 2002;640.
16. Kandler O, Weiss N. Regular, non-sparing Gram- positive rods. U: Bergey's Manual of Systematic Applied Microbiology. Sneath PHA, Mair N, Sharpe ME, Hott JG. (ured.), Williams i Wilkins. Baltimore, str. 1986;2:1209-1234.
17. Luquet F, Corrieu MG. Lactic acid bacteria and probiotics. Ed. 8C Tec Doc Lavoisier. Paris. 2005;307.
18. Harrigan WF, McCance ME. Laboratory methods in food and dairy microbiology. Rev. Ed., London, New York, Academic Press. 1976;33-200.
19. deVos WM, Kleerebezem M, Kuipers OP. Lactic acid bacteria - genetics, metabolism and application. FEMS Microbiol reviews. 2005;29(3):391.
20. Tserovska L, Stefanova S, Yordanova T. Identification of lactic acid bacteria isolated from Katyk, goat's milk and Cheese. J Culture Collections. 2002;3:48-52.
21. Sharpe ME. Identification of the lactic acid bacteria. In: Skinner FA, Lovelock DW (Eds.). Identification methods for microbiologists. London, Academic Press. 1979;233-259.
22. NCCLS (National Committee for Clinical Laboratory Standards). NCCLS document M-100-S9. Performance standards for antimicrobial susceptibility testing. 9th Ed. Information supplement. NCCLS: Wayne Pa; 1999.
23. Mourad K, Nour-Eddine K. In vitro preselection criteria for probiotic *Lactobacillus plantarum* strains of fermented olives origin. Int J Probiotics Prebiotics. 2006;1(1):27-32.
24. Azadnia P, Khan Nazer AH. Identification of lactic acid bacteria isolated from traditional drinking yoghurt in tribes of Fars province. Ira J Vet Res. 2009;10(3):235-40.
25. Mane VN, Gandhi MB. Studies on proteolytic thermoduric psychotrophic bacteria in milk and fermented milk products. J Environ Res Develop. 2011;5(2):384-392.
26. Gonzalez CJ, Encinas JP, Gracia Lopez ML, Otero A. Characterization and identification of lactic acid bacteria from freshwater fishes. Food Microbiol. 2000;17(1):383-91.
27. Sutherland IW. Food usage of polysaccharides, in Biotechnology of Microbial Polysaccharides (Sutherland, I. W., ed.,), Cambridge University Press, Cambridge, UK. 1990;117–125.
28. Perry DB, McMahon DJ, Oberg CJ. Effect of exopolysaccharide producing cultures on moisture retention in low-fat mozzarella cheese. J Dairy Sci. 1997;80(5):799–805.
29. Varalakshmi S, Balasubramanyam BV, Surendranath B, Bagath M, Rajendran D. Use of novel lactic acid bacterial strains with antagonistic activity for the preparation of safe indigenous fermented dairy foods (dahiand raita). J Food Safety. 2014;34:26–33. DOI: 10.1111/jfs.12091.
30. Tamine AY, Deeth HC. Yoghurt: technology and biochemistry. J Food Protect. 1980;43:939-941.
31. Goossens D, Jonkers D, Stobberingh E, Van Den Bogaard A, Russel M, Stockbrugger R. Probiotics in gastroenterology: Indications and future perspectives. Scand J Gastroenterol. 2003;38:15–23.
32. Schultz M, Strauch UG, Linde HJ, Watz lS, Obermeier F, Göttl C, Dunger N, Grunwald N, Schölmerich J, Rath HC. Preventive effects of *Escherichia coli* strain Nissle1917onacuteandchronic intestinal inflammation in two different murine models of colitis. Clin Diagn Lab Immunol. 2004;11:372–378.
33. Quigley EM. Bacteria: A new player in gastrointestinal motility disorders-

infections, bacterial overgrowth, and probiotics. Gastroenterol Clin North Am. 2007;36:735-48.

34. Lavasani S, Dzhambazov B, Nouri M, Fak F, Buske S, Molin G, Thorlacius H, AlefallJ, Jeppsson B, Westrom B. A novel probiotic mixture exerts a therapeutic effect on experimental autoimmune encephalomyelitis mediated byIL-10 producing regulatory Tcells. PLoS One. 2010;5:e9009.
DOI: 10.1371/journal.pone.0009009.

35. Miller LE, Ouwehand AC. Probiotic supplementation decreases intestinal transit time: meta-analysis of randomized controlled trials. World J Gastroenterol. 2013;19:4718-4725.

36. Heller KJ. Probiotic bacteria in fermented food: Product characteristics and starter organism. Am J Clin Nutr. 2001;73: 374–379. Maciel J, Teixeira MA, Moraes CA, Gomide AM. Antibacterial activity of lactic acid cultures isolated of Italian salami. Braz J Microbiol. 2003;34:121-122.

37. Stiles ME, Holzapfel WH. Lactic acid bacteria of foods and their current taxonomy. Int J Food Microbiol. 1997;36(1):1-29.

38. Ammor S, Rachman C, Chaillou S, Prévost H, Dousset X, Zagorec M, Dufour E, Chevallier I. Phenotypic and genotypic identification of lactic acid bacteria isolated from a small-scale facility producing traditional dry sausages. Food Microbiol. 2005;22(5):373-382.

39. Kaur IP, Chopra K, Saini A, Probiotics: Potential pharmaceutical applications. Eur J Pharm Sci. 2002;15:1-9.

40. Bansal T, Garg S. Probiotics: From functional foods to pharmaceutical products. Curr Pharm Biotechnol. 2008;9:267-287.

41. Sreeja V, Prajapati JB. Probiotic Formulations: Application and Status as Pharmaceuticals. Probio Antimicrob Prot. 2013;5:81-91.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=827&id=8&aid=7137