

Ready to Eat Curd-A Step towards Rural Transformation

Gowri Sukumar and Asit Ranjan Ghosh*

Centre for Infectious Diseases and Control, School of Biosciences and Technology, VIT University, Vellore-632014, Tamil Nadu, India

Abstract

Pure culture of *Pediococcus* spp GS4 isolated from khadi was used as inoculum for curd preparation. 1% of the bacterial culture having viable cell count of 1.24×10^9 CFU/ml was inoculated, and curdling was observed after 18 hours of incubation at 37°C. The cell viability in the curdled sample was determined to be 2.46×10^9 CFU/ml. Physico-chemical analysis of the curd showed its moisture content to be 90.36%, free amino acids amounted to a concentration of 710 µg/µl, and protein and carbohydrate concentration in the curd was determined to be 460 µg/µl and 0.86 mg/ml, respectively. The free fatty acid content was estimated to be 6.77 g/100g as oleic acid equivalence. The confirmation of probiotic properties showed acid and bile tolerance with the percentage survivability of 88.01 and 113.33%, respectively. Antimicrobial activity of the 100 µl of the cell free extract gave maximum inhibition against *Staphylococcus aureus*, with the Zone of inhibition (ZOI) of 13.9 ± 0.32 mm, followed by *Pseudomonas aeruginosa* (12.2 ± 0.45 mm), and the least with *Escherichia coli* and *Listeria monocytogenes* with the average ZOI of 11.9 ± 0.25 and 10.6 ± 0.85 mm, respectively. The concentration of lactic acid was determined to be 2.43 ± 0.01 g/20ml of supernatant. The viable counts upon lyophilisation showed a decrease in viability and the counts dropped to less than 10^8 CFU/ml after the 6th day of storage at room temperature. Organoleptic evaluation of the reconstituted curd was judged as acceptable. The curd thus prepared possessed the health beneficial and organoleptic property to support and supplement the rural health and economy.

Keywords: *Pediococcus* spp GS4, Probiotic; Curd; Viable counts; Organoleptic

Introduction

Consumers are increasingly interested in their personal health, and expect foods to be safe and healthy. Such functional foods or neutraceuticals are of great demand in present time. The growing scientific evidence suggests that food supplements containing beneficial bacteria can provide an array of health benefits to the host. One such group of bacteria is commonly known as Probiotic bacteria. The realisation of importance of gut microflora in health restoration and maintenance kindled the interest in probiotics. Probiotics is defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" [1]. Beneficial effects related to probiotics include antagonistic effects, competition, enhancement of digestion, strengthening of the immune system and stimulation of vitamin production [2]. At present, probiotic bacteria are widely used in human and animal nutrition because they beneficially influence the balance of the intestinal flora of the host. Probiotic therapy has been used to treat several gastrointestinal disorders, delay development of allergies in children, and also treat and prevent vaginal and urinary infections in women. The information on the recommend minimum concentrations of probiotic bacteria for effective function is still insufficient. However, adequate numbers of viable cells, namely the "therapeutic minimum" needs to be consumed regularly for the "probiotic" effect. Shah [3] has suggested a minimum viable number of 106 CFU ml⁻¹ or gram, but recommends 108 CFU g⁻¹ to compensate for reduction while passing through the gut. Controlled trials have shown that Lactobacillus GG can shorten the course of infectious diarrhea in children. Studies have also documented other health benefits relating to bioavailability of the nutrients, alleviation of lactose intolerance and maintenance of intestinal homeostasis [4].

Fermentation is an effective method of preservation, and contributes to the digestibility and the nutritive value of the final product [5]. The origin of cultured dairy products dates back to the dawn of civilization, and it has been mentioned in Bible and the sacred books of Hinduism [6]. But the scientific interest in this area was boosted after the work of Metchinkoff in 1908. Among the bacteria associated with food fermentation, lactic acid bacteria (LAB) is of predominantly important. LAB has contributed to the increased volume of fermented foods worldwide, especially in foods containing probiotics or health promoting bacteria. The fundamental reason for the development and acceptance of fermented foods can be ascribed to several safety and functional benefits. According to Steinkrause [7], the traditional fermentation of food serves several functions, i) enrichment of diet through the development of diversity of flavours, aroma, texture in food substrates, ii) preservation of substantial amounts of foods through lactic acid, acetic acid, alcoholic and alkaline fermentations, iii) enrichment of food substrates biologically with protein, essential amino acids, essential fatty acids and vitamins, and iv) detoxification during fermentation processing and decrease in cooking time, and thus, reduced fuel requirements.

It has been reported that chance contamination, favourable environmental and climatic conditions and serendipity, together played a role in the development of many of the cultured dairy products [8]. The Indian economy is agriculture based since the Vedic civilization. Cow and land were the major sources of economy. Fermented milk and milk products are, thus, intimately associated with Indian society. Indians have been consuming dahi or curd for centuries as a part of their traditional diets. In India, curd is essentially a vegetarian preparation. Dairy milk is used for curd preparation. Milk is boiled and is brought to temperature around 40°C; then an inoculum, as starter culture is

*Corresponding author: Asit Ranjan Ghosh, Centre for Infectious Diseases and Control, School of Biosciences and Technology, VIT University, Vellore-632014, Tamil Nadu, India, Tel: 0416-2202618; 09790238701; E-mail: cidcvit@gmail.com; asitranjanghosh@vit.ac.in

Received May 22, 2013; Accepted July 19, 2013; Published July 24, 2013

Citation: Sukumar G, Ghosh AR (2013) Ready to Eat Curd-A Step towards Rural Transformation. J Prob Health 1: 111. doi: 10.4172/2329-8901.1000111

Copyright: © 2013 Sukumar G, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

introduced. The starter culture mainly composed of yeast or bacteria, or in combination. The preparation is then kept at room temperature (25-35°C), for 3-5 hours in summer and overnight during winter. The mode of preparation is found same as yoghurt. Curd is known for its characteristic taste and consistency. The word Dahi is derived from Sanskrit word Dadhi. Dadhi is one of the five elixirs (Panchamrita), often used in Hindu rituals [4]. However, the quality of dahi may vary with the type of starter culture used. The use of wild type of starter culture gives rise to poor grade dahi having less shelf-life and less effective. Therefore, a need was felt to formulate a probiotic curd (using the monoculture of the Probiotic strain Pediococcus spp GS4), with an extended shelf life by manipulating processing conditions to reduce the moisture content of curd, and also maintain the minimum viable cell population of the probiotic microorganism to attain the desirable health benefits. A practical approach to develop a Probiotic curd with a potential probiotic strain for extended shelf life and health benefit was the primary aim of the current study.

Materials and Methods

Standardisation of inoculum size for curd preparation

Pediococcus spp GS4 strain (NCBI Genbank: HM044322) isolated from Indian fermented food Khadi (Delicacy of Gujarat), with probiotic potentials [9], anti oxidative [10] and conjugated linoleic acid (CLA) producing [11], was used as a starter culture for curd preparation (Figure 1). Sterile MRS (de Mann Rogosa Sharpe) broth with final pH 6.5 ± 0.2 (at 25° C) was inoculated with overnight grown *Pediococcus* spp GS4 culture (1% v/v), and incubated for 18 hours at 37° C. The viable count of the overnight grown culture was enumerated by serial dilution method.

Preparation of curd

Fifteen grams of skim milk powder (Everyday, India) was aseptically added to 60 ml of sterile water and mixed. The contents were placed in a water bath set to 70°C for 15-20 minutes to dissolve the contents thoroughly. It was then inoculated with 1% v/v of the inoculum containing the standard viable cells (10^8 CFU/ml). It was then incubated at 37°C for 18 hours, and observed for curdling. Enumeration of the viable cells in the curd was determined by serial dilution.

Physico-chemical analysis of the prepared curd

Moisture content of curd: Moisture content of curd was determined following the method described elsewhere [12]. In brief, Pre-dried beaker was labeled and weighed accurately, and the mass



Figure 1: Microscopic view (1000X magnification) of *Pediococcus* spp GS4 used in curd preparation (Tetrads).

was recorded. Small quantity of curd sample was placed and reweighed accurately. The moisture content was then evaporated by placing in Hot air oven at 90°C for 3 hours. The sample was then cooled and weighed. Percentage of moisture was calculated using the following formula:

Page 2 of 5

% of moisture wt / wt =
$$\frac{\text{wt of wet sample} - \text{wt of dried sample}}{\text{wt of wet sample}} \times 100$$

Estimation of total free amino acids: The free amino acids present in the curd sample were estimated colorimetrically at 570 nm using ninhydrin reagent [13]. Ninhydrin is a powerful oxidizing agent, which decarboxylates the α -amino acids to yield a bluish purple coloured product. The concentration of free amino acids in the curd sample was determined from the standard graph plotted with the water soluble amino acid, Leucine (1 mg/ml) as the reference standard.

Protein estimation: The total protein content in the curd sample was estimated colormetrically at 660 nm using bovine serum albumin (BSA) as the protein reference standard [14]. The concentration of protein in the curd sample was calculated from the standard graph using BSA as standard.

Estimation of total carbohydrates: The concentration of the total carbohydrates present in the curd sample was determined by phenol sulphuric method colorimetrically at 490 nm, using glucose as reference standard. The concentration of the total carbohydrate was estimated from the standard graph as glucose equivalent.

Determination of free fatty acid value: Five grams of curd sample was weighed accurately and placed in 250 ml Erlmnyer flask [12]. To the sample, 100 ml of neutralized ethanol and 2 ml of phenolphthalein indicator was added and titrated against 0.1 N NaOH. The end point of titration was indicated by a slight change to pink colour that persisted for 30 seconds. The volume of the titrant at the end point of titration was used to calculate the free fatty acid (FFA) value, using the formula given below:

%FFA (as Oleic acid) =
$$\frac{(V \times N \times 282)}{(W \times 100)}$$

Where %FFA=percent free fatty acid in g/100 g expressed as oleic acid equivalent

V=Volume of titrant in ml (NaOH)

N=Normality of NaOH titrant (mol/1000 ml)

282=Molecular weight of oleic acid (g/mol)

W=sample mass (grams)

Formulation of curd into powder form for extended shelf life: The curd sample was formulated into powder form by subjecting samples to freeze drying (Lyophilization). The powdered curd was then evaluated for the shelf life by enumeration of viable count of *Pediococcus* spp GS4.

Evaluation of the shelf life of the lyophilised probiotic curd: The viable cell count of the lyophilized samples, stored at room temperature (28°C), and refrigerated temperature (10°C) was performed at two day intervals from 0 day (day of lyophilisation). 1 gram each of powdered curd stored was serially diluted using sterile normal saline (0.84% sodium chloride), and a 100 μ l was plated on MRS agar and incubated at 37°C for 24 h. The cells were then enumerated accordingly.

Confirmation of the probiotic potential of *Pediococcus* spp GS4 after lyophilization: 1 g of the powdered curd was serially diluted to an appropriate dilution and plated onto MRS agar. The single colony was selected and inoculated into MRS broth for testing probiotic properties. The probiotic potential evaluated included: acid tolerance, bile tolerance, quantification of Lactic acid production and antimicrobial production.

Acid tolerance: Acid tolerance capability of the isolate was confirmed by viable count method [15]. One ml of GS4 grown in MRS broth for three generations having an optical density of 0.280 (at 660 nm) was inoculated into 9 ml of sterile MRS broth, with the pH adjusted to 3.5 with 1 N HCl, and was incubated at 37°C for 4 hours. At 0th hour and 4th hour, 1 ml of the sample was taken for enumeration by serial dilution using sterile normal saline (0.84% NaCl). A 100 µl of the appropriate dilution was plated onto MRS agar and incubated for 24-48 hours at 37°C. Colonies were enumerated using a colony counter. The reduction in log value after exposure to pH stress as compared to control was calculated to evaluate the survivability in the acid tolerance test using the formula: % survivability= (log CFU 4th hour/log CFU 0th hour)×100

Bile salt tolerance: Survivability on exposure to bile stress was evaluated by viable count method [15]. Like acid tolerance test, 1 ml of GS4 isolate was inoculated into 9 ml of MRS broth supplemented with bile salt (0.3% sodium thioglycollate), and incubated for 24 hours at 37°C. At 0th hour and 24th hour, 1 ml of the sample was taken, serially diluted, and 100 μ l of the appropriate dilution was plated onto MRS agar. The plates were incubated at 37°C for 24 hours. The viable cell count was carried out using a colony counter, and the percentage survivability in the presence of 0.3% bile salt was calculated using the formula:

% Survivability =
$$\frac{\log \text{CFU } 24^{\text{th}} \text{hour}}{\log \text{CFU } 0^{\text{th}} \text{hour}} \times 100$$

Determination of lactic acid production: The 24 h spent broth of the strain GS4 was collected by centrifuging at 10,000 rpm for 15 minutes at 4°C, and used for the quantitative estimation of the organic acid present. Few drops of phenolphthalein were added as an indicator to 20 ml of spent broth in the conical flask, and titrated against 0.1N NaOH. It has been standardised that one ml of 0.1 N NaOH is equivalent to 90.08 mg of lactic acid [16]. Amount of lactic acid produced was calculated from the standards provided. The experiment repeated and the average value was calculated.

Antimicrobial potential: The antimicrobial activity of the cell free extract (CFE) of the GS4 (10,000 rpm for 5 min at 4°C) was investigated. Neutralised CFEs were evaluated against the reference strains of *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 25619), and *Listeria monocytogenes* (ATCC 15313), by agar well diffusion method [17]. Inocula of reference strains from log phase (0.450-0.500 at 650 nm) were seeded onto Muller Hilton agar (pH 6.5 ± 0.2 at 25°C), using sterile swab. Wells were made using incinerated metal agar borer (8 mm). A 100 µl CFE was aseptically transferred to the wells along with ampicillin (10 µg/100 µl) and Nisin (100 µg/100 µl) as positive control, and negative control with un-inoculated sterile broth. The ZOI were observed after 24 hours of incubation at 37°C. The experiment was repeated thrice. The diameters of the ZOI were measured in mm and the average diameters were computed and compared with controls [18].

Organoleptic evaluation: The reconstituted curd sample (powdered curd mixed with appropriate quantity of water) was evaluated by ranking method for sensory characteristics, and overall

acceptability by a random population of varying age groups selected from Vellore Institute of Technology University campus, Vellore.

Results and Discussion

The information on the recommend minimum concentrations of probiotic bacteria for effective function varies from study to study. The recommended "therapeutic minimum" needs to be consumed regularly for the "probiotic" effect. The study shows the survivability of *Pediococcus* spp GS4 strain qualified the "therapeutic minimum" [3]. The viable cells in the inoculum used for curd preparation was determined to be 1.24×109 CFU/ml, while the viable count of *Pediococcus* spp GS4 was calculated to be 2.46×109 CFU/ml of curd. This satisfies the minimum number of viable cells of probiotic microorganism in food carrier [3].

The prepared curd showed satisfactory physico-chemical parameters (Table 1). The study also qualifies the restoration of probiotic property after lyophilization. Acid tolerance is important not only for withstanding gastric stresses, but is also a prerequisite for their use as dietary adjuncts. It enables the strain to survive longer periods in high acid carrier foods, without reduction in their numbers [19]. Table 2 shows the results of the acid tolerance test. The *Pediococcus* spp. GS4 strain exhibited a survivability of 88.01%, when subjected to acidic pH of 3.5 for duration of four hours. Bile salt tolerance is also an important factor that affects the viability of LAB cells [20]. Bile plays a fundamental role in specific and non-specific defense mechanisms of the gut. Havennar et al. [21] has pointed out the tolerance to bile salt is a prerequisite for colonization and metabolic activity of the probiotic bacteria in the small intestine of the host. Table 3 shows the results of the bile salt tolerance potential of the isolate. In this study, the strain

Physico-chemical parameters of prepared curd				
Moisture content	Noisture content 90.36%			
Ash content		9.64%		
	Total protein	460 mg/ml		
	Total reducing sugar	0.86 mg/ml		
	Free fatty acid	67.7 mg/g		

Table 1: Physico-chemical parameters of prepared curd.

Strain	0 hours		4 hours		Survivability	
Strain	CFU/ml	Log CFU/ml	CFU/ml	Log CFU/ml	%	
Pediococcus	1.36×10 ⁸	8.125	1.42×10 ⁷	7.151	88.01	

Table 2: Survivability in condition of acidic PH.

Strain	1 O H	nours	24	hours	Survivability %
Strain	CFU/ml	Log CFU/ml	CFU/ml	Log CFU/ml	
Pediococcus spp GS4	1.59×10 ⁸	8.20	1.95×10 ⁹	9.29	113.3%

Table 3: Survivability in condition of 0.3% bile salt (Sodium thioglycollate).

Duration of Storage	Viable cell count CFU/ml under Storage conditions		
(Days)	Room temperature	Refrigerated temperature	
0 (Day of Lyophilisation)	2.11×10 ⁸	3.27×10 ⁸	
2	1.21×10 ⁸	1.21×10 ⁸	
4	1.01×10 ⁸	1.01×10 ⁸	
6	6.30×10 ⁷	1.00×10 ⁸	

Table 4: Viable counts of the powdered curd stored at room temperature (28°C) and refrigerated (10°C) conditions.

exhibited good bile tolerance potential with an increase in log value, and hence, a potential probiotic candidate.

The concentration of lactic acid was found to be 2.43 ± 0.01 g/20 ml of the supernatant. Antimicrobial potential of LAB displays a wide range of antimicrobial activities. Among these, the production of lactic acid is of primary importance. Bhatia et al. [22] has also proposed that the production of organic acid by LAB is responsible for the inhibition of gastrointestinal pathogens. The ability to inhibit the growth of harmful bacteria is also considered as a desirable feature of probiotic bacteria [23]. The results of antimicrobial potential showed the maximum antimicrobial activity against S. aureus, with the average zone of inhibition of 13.9 ± 0.32 mm, moderate with *P. aeruginosa* with the average zone of 12.2 \pm 0.45, and minimal inhibitory activity was observed with E. coli and L. monocytogenes, with the average zones of 11.9 \pm 0.25 and 10.6 \pm 0.85 mm, respectively. The positive control ampicillin (10 μ g/100 μ l) showed average zones of 39.10 \pm 1.05, 17.6 ± 0.58 , 28.5 ± 0.98 , 36.7 ± 0.00 mm, respectively for *S. aureus*, *P.* aeruginosa, E. coli, and L. monocytogenes, respectively. Positive control with Nisin gave zone of inhibition only against S. aureus (19.5 \pm 0.71 mm). The inhibition of reference bacterial member by neutralized CEF has been demonstrated by the production of a cationic antimicrobial peptide of 9.57 kDa, and found to be better candidate than Nisin (unpublished data).

The result of the viable count to evaluate the survivability of the *Pediococcus* spp GS4 cells is shown in Table 4. The shelf life of the powdered curd when stored at room temperature (28°C) was determined to be 5 days, and a decline in cell number was observed beyond the 6th day of storage. The viable cell count dropped to a 10⁷ CFU/ml on the 6th day, which is below the recommended dose (10⁸ CFU/ml) for the desirable health benefits of the probiotic inoculum. However, the viable count showed viability of 10⁸ CFU/ml after 6 days of storage in refrigerated conditions (10°C). Hence, it can be inferred that shelf life of powdered curd formulation stored at room temperature is about 5 days, and an extended shelf life of 6 days was observed when stored in refrigerated conditions.

Organoleptic survey showed overall acceptance of the reconstituted curd, with reference to factors such as taste, colour, aroma and texture.

Conclusion

Analysis of published literature reveals the various health promoting benefits of probiotic bacteria in fermented foods. There is good evidence that specific strains of probiotics are safe for human use and able to confer some health benefits on the host, but such benefits cannot be extrapolated to other strains without experimentation. The health benefits for which probiotics can be applied include conditions such as gastrointestinal infections, certain bowel disorders, allergy and urogenital infections, which afflict a large portion of the world's population. The application of probiotics to prevent and treat these disorders should be more widely considered by the medical community. In addition, there is emerging evidence to indicate that probiotics can be taken by otherwise healthy people as a means to prevent certain diseases and modulate host immunity.

Fermented foods occupy one third of the diet worldwide. Fermentation process reduces the toxicity of the food along with the increased digestibility of raw materials. Different fermented foods are prepared worldwide using different substrates. Curd is one of the popular traditional food in southern India, commonly consumed by the rural folk. The functional value of this fermented food was improved by using the probiotic strain *Pediococcus* spp GS4 as inoculum/starter culture. This probiotic strain has been found to exhibit the key probiotic potentials such as acid tolerance, bile tolerance, production of lactic acid, anti-oxidative, as well as antimicrobial potential [9,10] and CLA production [11]. The demonstration of these properties has also been confirmed in this study. Therefore, content of the curd is capable of playing vital role in the maintenance of probiotic property and shelf life [24].

Curd is a low cost food that satisfies the rural need, and is nutritionally recognized. However, the lack of adequate scientific knowledge on fermentation process, absence of control over the fermentation variables, poor hygienic practices and the non-availability of refrigeration facilities lead to the variability/ poor shelf life of the fermented preparation. This study has fulfilled this objective by the use of lyophilization technique. This is a widely used preservation technique in making liquid suspensions to powder form through the process of sublimation. The prerequisite for the probiotic inoculum to have a viable count of 108 CFU/ml was considered as criteria for assessing the shelf-life of the product. The viable count showed a decline in cell numbers when stored at room temperature, and the count dropped below 108 CFU/ml after the 6th day of storage. However the viable counts of 108 CFU/ml was observed on the 6th day of storage in refrigerated condition. Another study with GS4 showed satisfactory viability in gelatin capsule [25]. Results of a pilot study have shown the possibility of reducing the moisture content of the curd, and also to maintain its shelf life for about 5 days when stored at room temperature. This pilot study can be scaled up for the large scale production of the ready to eat Bio yoghurt with extended shelf life, which would make technology come possible in rural India.

Acknowledgement

Authors thank to VIT-TBI and Villgro Student Innovation Programme, which has given funds to GS to carry out her MS and the probiotic project at Centre for Infectious Diseases and Control, VIT University. GS also acknowledges the sincere cooperation extended by all the participants of VIT University, who took part in the survey for the organoleptic evaluation of the product.

References

- FAO/WHO (2001) Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Report from joint Food and Agriculture Organization (FAO) of the United Nations/World Health Organization (WHO) expert consultation, Argentina 1-34.
- De Baets L, Van Iwaarden P, Meeus N, Schimmel H, Philipp W, et al. (2009) First certified reference materials for molecular fingerprinting of two approved probiotic *Bacillus* strains. Int J Food Microbiol 129: 16-20.
- Shah NP (2000) Effects of milk-derived bioactives: an overview. Br J Nutr 84: S3-S10.
- Pande R, Bagad M, Dubey V, Ghosh AR (2012) Prospectus of probiotics in modern age diseases. Asian Pacific J Trop Biomed S1963-S1974.
- Board RG, Jones D, Jarvis B (1995) Microbial fermentation: Beverages, foods and feeds. J Appl Bacteriol 79: 505.
- Nakazawa Y, Hosono A (1992) Fermented milk in the orient in functions of fermented milk: Challenges for the Health Sciences. Elsevier Science Publishers Ltd, Barking, UK 61-78.
- Steinkrause Keith H (1995) Handbook of indigenous fermented foods. Marcel Dekker, NewYork, UK 76.
- Shortt C (1999) The probiotic century: Historical and current perspectives. Trends Food Sci Tech 10: 411-417.
- Gowri S, Ghosh AR (2010) *Pediococcus* spp.-A potential probiotic isolated from Khadi (an Indian fermented food) and identified by 16S rDNA analysis. Afr J Food Sci 4: 597-602.

- 10. Gowri S, Ghosh A R (2011) Antioxidative potential of probiotic bacteria from Indian fermented food. Int J Res Ayur Pharma 2: 983-986.
- Dubey V, Ghosh AR, Mandal BK (2012) Appraisal of conjugated linoleic acid production by probiotic potential of *Pediococcus* spp. GS4. Appl Biochem Biotechnol 168: 1265-1276.
- 12. Suzanne N (2003) Food analysis. Plenum Publishers, Purdue University West Lafayette, Indiana 119.
- Sadasivam S, Manickam A (2008) Biochemical methods. (3rd Edn), New Age International Publishers, India.
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265-275.
- Gilliland SE, Staley TE, Bush LJ (1984) Importance of bile tolerance of LactoBacillus acidophilus used as a dietary adjunct. J Dairy Sci 67: 3045-3051.
- AOAC (1990) Official Methods of Analysis. (15th Edn), Association of Official Analytical Chemistry, Washington DC, USA.
- Lasta S, Fajloun Z, Darbon H, Mansuelle P, Andreotti N, et al. (2008) Chemical synthesis and characterization of J46 peptide, an atypical class IIa bacteriocin from Lactococcus *lactis* subsp. cremoris J46 Strain. J Antibiot (Tokyo) 61: 89-93.
- Andrews JM, BSAC Working Party on Susceptibility Testing (2008) BSAC standardized disc susceptibility testing method (version 7). J Antimicrob Chemother 62: 256-278.

- Minelli EB, Benini A, Marzotto M, Sbarbati A, Ruzzenente O, et al. (2004) Assessment of novel probiotic *LactoBacillus casei* strains for the production of functional dairy foods. Int Dairy J 14: 723-736.
- 20. Yu B, Tsen HY (1993) *LactoBacillus* cells in the rabbit digestive tract and the factors affecting their distribution. J Appl Bacteriol 75: 269-275.
- Havenaar R, Ten Brink B, Huis In't Veld JHJ (1992) Selection of strains for probiotic use. Probiotics-the Scientific Basis. Chapman & Hall, London, UK 209-221.
- Bhatia SJ, Kochar N, Abraham P, Nair NG, Mehta AP (1989) LactoBacillus acidophilus inhibits growth of Campylobacter pylori in vitro. J Clin Microbiol 27: 2328-2330.
- Lorca GL, Wadström T, Valdez GF, Ljungh A (2001) LactoBacillus acidophilus autolysins inhibit Helicobacter pylori in vitro. Curr Microbiol 42: 39-44.
- 24. Bari MR, Ashrafi R, Alizade M, Rofchgarineghad L (2009) Effect of different contents of yogurt starter/probiotic bacteria, storage time and different concentration of cysteine on the microflora characteristics of bio-yogurt. Res J Bio Sci 4: 137-142.
- 25. Bagad M, Pande R, Ghosh AR (2012) Determination of viability of *Pedicoccus* spp. GS4 after storage into hard gelatin capsule and its survival under *in vitro* stimulated gastrointestinal condition. Int J Res Ayurv Pharma 3: 233-237.

Page 5 of 5