World Applied Sciences Journal 3 (1): 40-44, 2008
ISSN 1818-4952

A Perception to Survival of *Bifidobacterium* Spp. In Bioyoghurt, Simulated Gastric Juice and Bile Solution

[1]F. Shahidi, [2]A.F.Mendosa,
[2]T. Boylstone and [1]M. Mohebbi

[1]Department of Food Science and Technology, Ferdowsi University, Iran
[2]Department of Food Science and Human Nutrition, Iowa State University, USA

Abstract: Due to special properties of fermented milks containing probiotics, such as improving protein and vitamin metabolisms, antibacterial activities, treatment of liver disease and reduction of the risk of colon cancer, in recent years, special emphasize is placed on shelf-life evaluation of these products. The effects of acid-adaptation treatment of bifidobacteria on the viability of these microorganisms in bioyoghurt during storage were evaluated in this research. Also, viability of bifidobacteria in simulated gastric juice and bile solution was assessed. The results showed that while *B. breve* survived better during storage of refrigerated bioyoghurt, *B. infantis* strains were more resistant to simulated gastric juice and the survival rate of these strains under alkaline condition of bile solution was approximately 70%. Since the survival of probiotics throughout the shelf-life of the product and at the time of consumption as well as tolerating the extreme pH conditions are important factors for exertion of health benefits in the host, both aspects must be considered in shelf-life evaluation of commercial bioyoghurt.

Key words: Bioyoghurt · *Bifidobacterium* · gastric juice · bile solution · viability · shelf-life

INTRODUCTION

Yoghurt is produced worldwide as fermented milk with the co-culture of two species of Lactic Acid Bacteria; *Streprococcus thermophilus* and *Lactobacillus delbrukii* spp. *bulgaricus*. Recently emphasis has been placed on developing new fermented milks containing probiotics [1-5]. Probiotics are commonly defined as live microorganisms that impart a health benefit to the consumer. Many bacterial genera and species are commonly used in commercial products, including species of *Lactobacillus* and *Bifidobacterium.*

These kinds of products have been developed and marketed in Europe, North America and the Far East [6-10]. Properties of *Lactobacillus* spp. is well documented, but possible roles of ingested bifidobacteria have been evaluated less. The importance of bifidobacteria with respect to the healthy operation of the human digestion system encourages consumption of such products [6, 11].

The health benefits of bifidobacteria may be listed as follows:

- Improved protein and vitamin
- Prevention of constipation;
- Antibacterial activity;
- Treatment of liver damage;
- Anti-tumor activity;
- Stimulation of immune system response;
- Reduction of blood cholesterol levels;
- Improvement of lactose digestibility metabolisms [12-19];

The incorporation of probiotic bacteria as dietary adjuncts in different fermented milk products has reinforced the acclaimed healthy properties and given rise to a tremendous consumption of the so-called value-added products in developed countries [20-22]. Among the dairy products, yoghurt considered as ideal vector for the delivery of these probiotic bacteria to consumers[9].

Corresponding Author: Dr. M. Mohebbi, Department of Food Science and Technology, Ferdowsi University, Iran

For any such product, the survival of probiotics throughout the shelf life of the product and at the time of consumption in order to exert the healthy benefits in the host, is very important [20, 22, 23].

While fortifying products with probiotics, have long been carried out by manufacturers, they have been encountered with several processing challenges. In fact, many active milk cultures may die even before the consumer receives any of the health benefits, as the result of processing, storage and transportation conditions. A number of different brands of commercial yoghurt have been analyzed for the adequate presence of *Lactobacillus* and *Bifidobacterium* species and the results indicated that most of the yoghurts contained very low number of these organisms [24-26]. On the other hand, functional therapeutic effects of food may be reserved only if the probiotic bacteria survive the passage from the mouth to the intestines. Specifically, these organisms are susceptible to environmental factors such as water, oxygen, temperature and acidity. Therefore the ingested probiotic bacteria must tolerate the acidic condition of stomach, while resisting against bile produced by small intestine. Large variations in *Bifidobacterium* spp. ability to resist these conditions have been reported [27-29].

The objective of this study was to evaluate the growth, survival and viability of acid adapted and non acid adapted cells of *Bifidobacterium infantis*, *Bifidobacterium breve* and *Bifidobacterium longum* in yoghurt during storage. Viability of bifidobacteria in simulated gastric juice and bile solution was evaluated in the second part of this work.

MATERIALS AND METHODS

Materials: Lyophilized cultures of *B. infantis*, *B. breve* and *B. longum* were obtained from Lallemand Institute Rosell. Plain yoghurt was obtained from US local market. Culture media including TSB and MRS were purchased from Difco CO.

Methods: Acid adaptation as a pretreatment was carried out by adding 1 g of each culture to TSB+0.6% yeast extract. Rezazurin (0.2 g/100 ml) and L-Cystein (25 g/25 ml) were also added under aseptic conditions. Addition of 10% dextrose to the half of the tubes resulted in the acid-adaptation (AA) pretreatment which was compared with non acid-adapted bacteria.

Also, under the same condition, N_2 (2 psi) was added to each tube for 25 seconds. Tubes were capped immediately and wrapped in four layer of parafilm. Tubes were incubated at 30°C for 24 h. At the end of incubation pH of the samples was measured. Cells were harvested by centrifugation at 10000 ×g at 4°C for 10 min and the pellets were resuspended in 10 ml of saline (0.85% w/w). 25 g of plain yoghurt was added to each tube. Stomacher bags manufactured from food grade polythene were sealed under vacuum and stored at 4°C. Every week the pH, the total counts and the viability of bifidobacteria were evaluated for a period of 42 days.

For enumeration of bifidobacteria, filtered (0.22 μm) streptomycin was added to MRS agar.

Simulated gastric juice and bile solution were used to determine the susceptibility of *Bifodbacterium* species to pH. For this purpose, 1 g of lyophilized culture was inoculated to TSB. After 24 h, bacteria were inoculated in TSB with 10% dextrose and without dextrose. Cells were harvested after 24 hours. 2 ml of cell suspension was added to 18 ml simulated gastric juice (3g pepsin/ 1000 ml saline 0.5 %w/w). After incubation at 37°C, viability of *Bifidobacterium* species was evaluated for 6 h with 1.5 hours interval.

Bile solution was prepared by dissolving 10 g Oxgall (Difco) in 90 ml distilled water and addition of 1 g bile solution (Difco) and viability was examined at 0, 4, 8 and 12 hours after incubation at 37°C.

Statistical analysis: Experiments were carried out in a randomized block design and a factorial model was used. The means were compared by Dunckans test. Analysis was accomplished with Mstatc (Ver 2.0) software.

RESULTS AND DISCUSSION

Other than selection of probiotic organisms with high potential in producing metabolites with desired health or nutritional benefits, survival of the strain during fermentation and storage at 4°C must be considered. While no general agreement has been reached on the recommended level for the minimum number of probiotic bacteria at consumption time, different ranges from 10^5 to 10^8 have been suggested in literature. However recent market surveys indicated that the viability of probiotic organisms in commercial preparation is commonly lower [13, 30, 31].

Several factors affect the viability of probiotic bacteria, including an increase in the acidity of the product during storage which has an adverse effect on the viability of bifidobacteria in fermented products[20, 32-34].

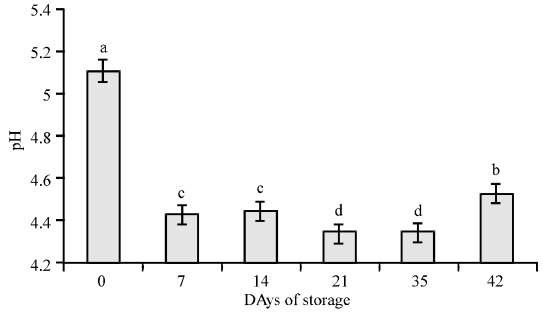

Fig. 1: The effect of storage time at 4°C on pH of samples inoculated with different bifidobacteria species

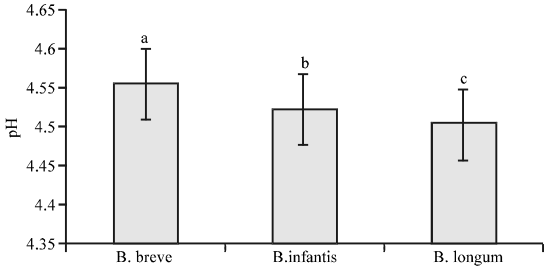

Fig. 2: Changes in the pH of samples inoculated with different bifidobacteria species

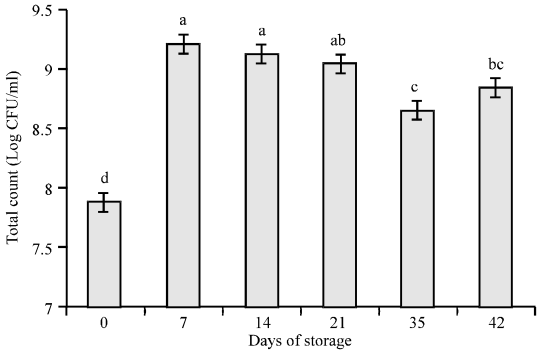

Fig. 3: The effect of storage time on total counts of the samples

As the Fig. 1 shows, the pH significantly declined at 4°C over the period of 21 days ($P<0.05$). The lowest level of pH was observed in the samples kept for 21 days in refrigerator.

Also Fig. 2. shows that samples inoculated with *B. longum* had a lower pH, while there was a non significant reduction in the pH of bioyoghurt inoculated with *B. breve.*

The effect of storage time on total counts (Log CFU/g) is given in Fig. 3. The viability of bifidobacteria is shown in Fig. 4. Low numbers of bifidobacteria after inoculation, compared to the first 3 weeks, might be due to the reduction in the number of

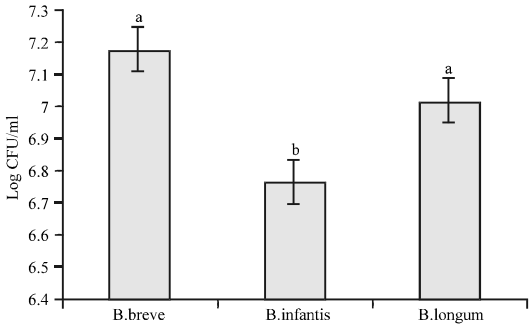

Fig. 4: Survival of different strains during storage

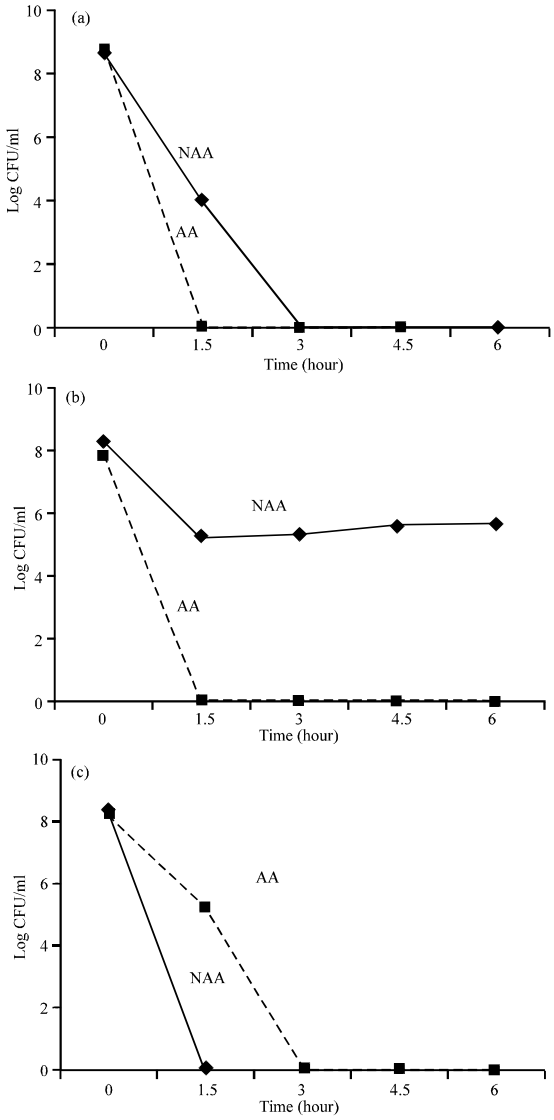

Fig. 5: Viabilty of (a*) B.breve* (b) *B. infantis* (c) *B. longum* in simulated gastric juice

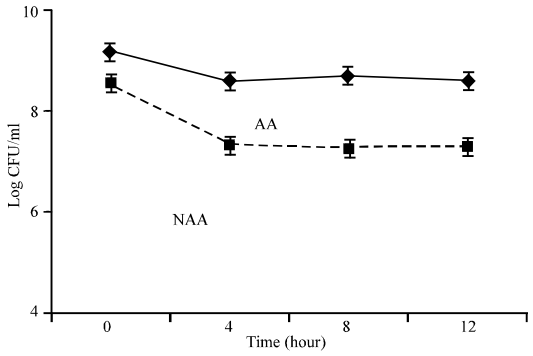

Fig. 6: Viability of *B. Infantis* in simulated bile solution

bifidobacteria in medium containing dextrose which resulted in acidic condition.

The results of experiment shows that *B. breve* can survive in refrigerated bioyoghurt better than two other species (Fig. 5).

The lowest level of pH was measured in the acid-adapted treatment inoculated with *B. longum*. On the other hand, non-acid adaptation and inoculation with *B. breve* resulted in the highest level of pH.

It is reported that delivery of viable *bifidobacterium* sp. to large intestine, the main location of their functional properties, is limited as a result of extreme acidity in human stomach [35]. In this study, counts of viable *B. infantis* (ATCC 15697) remained significantly higher ($P<0.01$) compared to other strains, while they were exposed to simulated gastric juice at pH 3.0. The viable counts of cells in acid-adapted bifidobacteria dropped to an undetectable level in 1.5 h. Non-acid adapted strain of *B. infantis* was the most acid-resistant species (Fig. 5). It seems that due to acidic condition in acid-adapted samples, the growth of bacteria were slower as compared to non acid-adapted, which affects the final counts.

Based on the results obtained in this experiment, viability of *B. infantis* in simulated bile solution was evaluated. Non-acid adapted *B. infantis* was more resistance to alkaline pH, but a significant difference was not observed (Fig. 6).

CONCLUSION

For probiotic products, special emphasis is put on culture viability throughout the shelf life of the product and at the time of consumption and therefore proper selection of bifidobacteria is a key factor. On the other hand, success of bifidobacteria containing food products depends on the resistance of bacteria to the system existing in the upper gastrointestinal tract. Therefore, it can be concluded that viability of *Bifidobacterium* strains under different conditions must be modeled. Derivative-free, multi-objective optimization (such as MOGA) according to definition of fitness function including indicator terms of resistance to simulated gastric juice and bile, survival during storage of refrigerated products, in addition to organoleptical properties, will be a powerful and reliable procedure for shelf-life determination in products containing bifidobacteria.

REFERENCES

1. Arunachalm, K.D., 1999. Role of bifidobacteria in nutrition, medicine and technology. Nutr. Res., 19: 1559-1597.
2. Chen, R.M., J.J. Wu and S.C. Lee, 1999. Increase of intestinal *Bifidobacterium* and suppression of coliform bacteria with short-term yoghurt ingestion. J. Dairy Sci., 82: 2308-2314.
3. Kaltz, F., 1999. Top product development trends in Europe. Food Technol., 53: 38-42.
4. Shah, N.P., 2000b. Probiotic bacteria: selective enumeration and survival in dairy foods. J. Dairy. Sci., 83: 894-907.
5. Kailasapathy, K., 2006. Survival of free and encapsulated probiotic bacteria and their effect on sensory properties of yoghurt. LWT, 39: 1221-1227.
7. Gilland, S.S., G.B. Reilly and H.S. Kim, 2002. Viability during storage of selected probiotic lactobacilli and bifidobacteria in a yoghurt-like product. J. Food Sci., 67: 3091-3095.
8. Hekmat, S. and D.J. McMahon, 1992. Survival of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in ice-cream for use as a probiotic food. J. Dairy Sci., 75: 1415-1422.
9. Hughes, D.B. and D.G. Hoover, 1991. Bifidobacteria: their potential for use in American dairy products. Food Technol., 45: 74-83.
10. Vinderola, C.G., N. Bailo and J.A. Reinheimer, 2000. Survival of probiotic microflora in Argentinian yoghurts during refrigerated storage. Food. Res. Intl., 33: 97-102.
11. Young, P.S.M., M.E. Sanders, G.L. Kitts, R. Cano and P.S. Tong, 2002. Species-specific identification of commercial probiotic strains. J. Dairy Sci., 85: 1039-1051.
12. Chou, L.S. and B. Weimer, 1999. Isolation and characterization of acid and bile tolerant isolates from strains of Lactobacillus acidophilus. J. Dairy Sci., 82: 23-31.

13. Ishibashi, N. and S. Shimamura, 1993. Bifidobacteria: Research and Development in Japan. Food Technol., pp: 126-135.
14. Lapierre, L., P. Undeland and L.J. Cox, 1992. Lithium chloride-sodium propionate agar for the enumeration of bifidobacteria in fermented dairy products. J. Dairy Sci., 75: 1192-1196.
15. Minervini, F., F. Algaron, C.G. Rizzello, P.F. Fox, V. Monnet and M. Gobbetti, 2003. Angiotension I-converting-enzyme-inhibitory and antibacterial peptides from Lactobacilus helveticus PR4 proteinase-hydrolyzed caseins of milk from six species. A pplied and Environmental Microbiol., 69: 5297-5305.
16. Mitsuoka, T., 1990. Bifidobacteria and their role in human health. J. Ind. Microbiol., 6: 263-268.
17. Mugueraza, B., M. Ramos, E. Sanchez, M.A. Manso, M. Miguel, A. Aleixandre, M.A. Delgado and I. Recio, 2006. Antihypertensive activity of milk fermented by Entrococcus faecalis strains isolated from raw milk. Intl. Dairy J., 16: 61-69.
18. Roy, D., 2001. Media for the isolation and enumeration of bifidobacteria in dairy products. Intl. J. Food.. Micobiol., 69: 167-1822.
19. Sanders, M.E., 1993. Effect of Consumption of lactic cultures on human health. Adv. Food. Nutr., 37: 67-130.
20. Adhikari, K., A. Mustapha and I.U. Grun, 2000. Viability of microencapsulated bifidobacteria in set yoghurt during refrigerated storage. J. Dairy Sci., 83: 1946-1951.
21. Bruno, F.A., W.E.V. Lankaputhra and N.P. Shah, 2002. Growth, viability and activity of *Bifidobacterium* spp. in skim milk containing probiotics. J. Food Sci., 67: 2740-2744.
22. McCann, T., T. Egan and G.H. Weber, 1996. Assay procedures for commercial probiotic cultures. J. Food Prot., 59: 41-45.
23. Sheu, T.Y. and R.T. Marshall, 1993. Micro entrapment of *lactobacilli* in calcium alginate gels. J. Food Sci., 54: 557-561.
24. Dave, R.I. and N.P. Shah, 1997. Viability of yoghurt and probiotic bacteria in yoghurts made from commercial starter cultures. Intl. Dairy J., 7: 31-41.
25. Donkor, O.N., A. Henriksson, T. Vasiljevic and N.P. Sha, 2006. Effect of acidification on the activity of probiotics in yoghurt during cold storage. Intl. Dairy J., 16: 1181-1189.
26. Donkor, O.N., S.L.I. Nilmini, P. Stolic, T. Vasiljevic and N.P. Shah, 2007. Survival and activity of selected probiotic organisms in set-type yoghurt during cold storage. Intl. Dairy J., 17: 657-665.
27. Clark, P.A. and J.H. Martin, 1994. Selection of Bifidobacteria for use as dietary adjuncts in cultured dairy foods: III Tolerance to simulated bile concentration of human small intestines. Cult. Dairy Prod. J., 29: 18-21.
28. Chung, H.S., Y.B. Kim, S.L. Chun and G.E. Ji, 1999. Screening and selection of acid and bile resistant bifidobacteria. Intl. J. Food Microbial., 47: 25-32.
29. Kim, H.S., B.J. Kamara, I.C. Good and G.L. Enders Jr., 1988. Method for the preparation of stabile microencapsulated lactic acid bacteria. J. Ind. Micobiol., 3: 253-257.
30. Calicchia, M.L., C.I.E. Wang, T. Nomura, F. Yotsuzuka and D.W. Osato, 1993. Selective enumeration of *Bifidobacterium bifidum*, *Enterococcus faecium* and strepto resistant *Lactobacillus acidophilus* from a mixed of mixed probiotic product. J. Food Protect., 56: 954-957.
31. Dave, R.I. and N.P. Shah, 1998. Ingredients supplementation effects on viability of probiotic bacteria in yoghurt. J. Dairy Sci., 81: 2804-2816.
32. Lourens-Hattingh, A. and C.B. Viljeon, 2001. Yoghurt as probiotic carrier food. Intl. Dairy J., 11: 1-17.
33. Shah, N.P., 2001. Functional foods: probiotics and prebiotics. Food. Tech., 55: 46-53.
34. Talwalkar, A. and K. Kailasapathy, 2004. Comparison of selective and differential media for the accurate enumeration of strains of Lactobacillus acidophilus, Bifidobacterium spp. And Lactobacilus Casei complex from commercial yoghurts. Intl. Dairy. J., 14: 143-149.