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Original Research Article

Lactic Acid Bacteria Growth in Indian Fermented Foods Available in Delhi Markets

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ABSTRACT

Probiotic as functional foods is gaining popularity in India. They are food which provides special beneficial effect to the host along with the basic nutrition. Probiotic organism grows in products prepared by spontaneous fermentation as well. Not many Indian products have been explored till now. In the present study the fermented products available in the market of Delhi were tested for lactic acid bacteria content which could have a potential to produce probiotic properties. Only few products were reflective of probiotic nature. Due to improper conditions during the supply chain, the lactic acid content depletes. Thus proper guidelines should be formulated so that a uniform lactic acid bacterial population is maintained throughout the shelf life of the fermented products.

Keywords: Probiotics, fermented foods, market survey, lactic acid bacteria

1. INTRODUCTION

The term *Probiotics*, meaning "for life," is derived from the Greek language. It was first used by Lilly and Stillwell [(1)] in 1965 to describe "substances secreted by one microorganism which stimulates the growth of another" and thus was contrasted with the term *antibiotic*. In 1971 Sperti [(2)] applied the term to tissue extracts that stimulate microbial growth. Parker [(3)] was the first to use the term *Probiotics* in the sense that it is used today. He defined Probiotics as "organisms and substances which contribute to intestinal microbial balance." Fuller [(4)] attempted to improve Parker's definition of Probiotics with the following distinction: "A live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance." This revised definition emphasizes the requirement of viability for probiotics and introduces the aspect of a beneficial effect on the host, which was, according to his definition, an animal.

To have a therapeutic effect a probiotic strain need to resist the manufacturing process and secondly they should remain viable during the storage period in the commercial products until the end of the shelf-life. [(5)] However, many studies have shown low viability of probiotics in market preparations. [(6)] Several factors such as oxygen and salt concentrations, temperature, pH, acidity, the presence of other microorganisms and nutritional resources influence probiotic growth rates and total cell yields. [(7-8)] When probiotics are added to fermented foods, several factors must be considered that may influence the ability of the probiotics to survive in the product. These factors include a) the physiologic state of the probiotic organisms added (preservation techniques used) *b*) the physical conditions of product storage (e.g., temperature, packaging

material), *c*) the chemical composition of the product to which the probiotics are added (e.g., acidity, pH, water activity, and oxygen content), d) possible interactions of the probiotics with other product components (e.g., food additives, prebiotics, food matrices) [(9)]

In USA**,** probiotics are considered to be "complementary" or "alternative" medicine. In India Amul, Nestle and Mother diary are contributing a lot to probiotics dairy products and urban population acceptance to these products is helping to increase companies focus to produce more and more probiotic products. [(10)]

Above all Indian market also has some probiotic based pharmaceutical formulations named as Sporolac, ViBact, Darolac, Biglac, Bifilac etc. ICMR formulated the guidelines which deal with the use of probiotics in food and provide requirements for assessment of safety and efficacy of the probiotic strain and health claims and labelling of products with probiotics. [(11)] In the present study the lactic acid bacteria content in fermented Indian foods available in the market is being explored.

2. METHODOLOGY

2.1 Fermented product samples

All the samples were procured from local grocery shop in Delhi. Lemon pickle, amla pickle, stuffed red chilli pickle, amla murabba, carrot murabba, fermented idli and dhokla batter, probiotic curd 1,2 and 3, packaged curd 4, curd 5 from open dairy, packaged chachh 1 and chhach 2 from open dairy samples were collected and analyzed for microbial counts and for pH and acidity measurements. pH and TA were determined according to AOAC. [(12)]

2.2 Viable cell enumeration

LAB count was analysed using standard procedure on MRS agar procured from Hi Media (India) (IS: 14844- 2000). [(13)] Appropriate dilutions were plated in triplicates. Pour plate method was used for plating and after incubation period of 2 days at 30°C, colonies were counted in appropriate dilutions to calculate cfu/g of sample. Biochemical tests were carried out by picking up the representative colonies. Bacterial colonies were identified by gram staining, catalase test and indole test as per protocol described by Aneja. [(14)]

Other Microbiological counts were determined according to the procedure given in IS: 5402-2002 for total plate count, [(15)] IS:5401 (part I) – 2002 for coliforms count, [(16)] IS: 5403-1999 for yeast and mould count, [(17)] and Compendium of Methods for the Microbiological Examination of foods by American Public Health Association for E.coli. [(18)]

3. RESULTS AND DISCUSSION

Fermented food samples were collected and analysed for various parameters. Various studies have highlighted the presence of lactic acid bacteria in fermented pickles. Lactobacillus plantarum G63 was isolated from traditional Chinese pickles made by a local restaurant. [(19)] Nowadays, the lactic acid fermentation of vegetables has an industrial significance mainly for cabbages, cucumbers and olives. [(20)] We need to explore this aspect for Indian fermented products as well.

With above mentioned studies as reference the pickles and murabba available in the market was analysed for its lactic acid bacteria content. As observed from table 1 in amla pickle the pH was 3.0, Titratable acidity 3.06%, total plate count was 3.626 log cfu/g while coliform, e.coli, yeast and mold and lactic acid bacteria count was not detected. Similar results were observed in lemon pickle and red chilli stuffed pickle as well. In carrot murabba the pH was 3.16, Titratable acidity 0.7%, total plate count was 1.778 log cfu/g while coliform e.coli, yeast and mold and lactic acid bacteria count was not detected. The recommended acidity for pickles according to IS:3501-1966 [(21)] is 3 (acidity as acetic acid) and 1.2 (acidity for pickles in citrus juices). The pH of pickles and murabba was higher and Titratable acidity is also higher, this could be due to use of high amount of

preservatives. To maintain the quality of the product the Titratable acidity is maintained at high level to minimize the growth of coliform and yeast and mold. Maybe very high temperature processing of vegetables was carried to minimize bacterial load and thereby affecting the Lactic acid bacteria growth. But at very high Titratable acidity the lactic acid bacteria growth is also affected. High Titratable acidity also affects the flavour of the product and it could be observed that home fermented pickles gives better flavour and natural taste to the pickle with freshness of vegetables maintained for longer period. After spontaneous fermentation pH of the pickle could be maintained at a stable rate thereby minimizing lactic acid bacteria loss. Lactic acid bacteria are generally mesophilic and can grow at wide range of temperature ranging from 5°C-45°C and they are tolerant to acidic conditions thereby optimal pH is 5.5-6.5. [(22)]

Fermented idli and dhokla batter also provides a good substrate base for the growth of lactic acid bacteria. The pH of idli batter was 4, Titratable acidity was 0.92%, total plate count was 6.159 log cfu/g, yeast and mold count was 3.861 log cfu/g, lactic acid count was 4.062 log cfu/g, while coliform and e.coli was not detected. Similarly for fermented dhokla mix the pH was 3.85, Titratable acidity was 1.92%, total plate count was 6.605 log cfu/g and lactic acid bacteria count was 4.470 log cfu/g. Coliform, e.coli and yeast and mold count was not detected. A study was conducted and eight *L. plantarum* strains were isolated from fermented idli batter and they possessed probiotic properties. [(23)] Many studies have shown a very high growth rate when extrinsic lactic acid bacteria was added to the batter. But during the study it was observed that the growth is less than the desirable level as spontaneous fermentation is taking place and the lactic acid bacteria may not be viable at steady rate.

In probiotic curd 1 the pH was 4.1, Titratable acidity was 0.81%, total plate count was 5.313 log cfu/g while coliform, e.coli and yeast and mold count were not detected. The lactic acid bacteria content was 4.278 log cfu/g, which is lower than the desirable range. Studies indicate that to show the beneficial effects the product should contain the lactic acid bacteria count at 10^6-10^7 cfu/g level.

Similar results were obtained for probiotic curd 2, while in probiotic curd 3 the pH was 4.1, Titratable acidity was 0.74%, total plate count was 5.740 log cfu/g, e.coli, coliform and yeast and mold count was not detected and the LAB count although it was less than the claimed value but still lying in desirable range at 6.899 log cfu/g. While in curd 4 the pH was 4.44 and lactic acid bacteria count at 4.913 log cfu/g. In curd 5 obtained from open dairy the pH was 3.84 and Titratable acidity was 0.80% with a total plate count of 7.644 log cfu/g which may be due to the mixed culture used for inoculum. The lactic acid bacteria count was 5.826 log cfu/g. According to IS 9617:1980 [(24)] the Titratable acidity should lie in the range of 0.6-0.8% and every sample was complying to the standard.

Chachh a fermented Indian drink was also tested for the presence of lactic acid bacteria. In packaged chachh 1 the pH was 4.22, Titratable acidity was 0.75%, total plate count was 6.978 log cfu/ml, yeast and mold count was 1.204 log cfu/ml, lactic acid bacteria content was 4.291 log cfu/ml, while e.coli and coliform count was not detected. Similar results were obtained in chachh 2 procured from open dairy but lactic acid bacteria count was 6.792 log cfu/ml which was in the desirable range.

Packaging material also plays an important role in maintaining the viability of lactic acid bacteria. The level of oxygen within the package during storage of the product should be as low as possible in order to avoid toxicity and death of the microorganism and the consequent loss of functionality of the product. [(25)] The low level of lactic acid bacteria count in commercial products could be due to inappropriate maintenance of temperature through the supply chain. A study was

conducted on commercial fermented milks and presence and viability of probiotic lactobacilli and bifidobacteria was evaluated. Counts of Lactobacillus spp. always remained higher than 10^5 CFU/ml, whereas the population of Bifidobacterium spp. decreased below this level in two products. All the probiotics announced on the label were present in commercial products. [(26)] Similar results were obtained and presence of bifidobacteria was revealed in all the 14 commercial probiotic yoghurts. [(27)] The use of glass packages favors the survival of probiotic cultures due to its extremely low oxygen permeability. On the other hand, the high cost of glass along with the hazards inherent to its handling makes it an inappropriate choice for packaging dairy products. For that reason, the dairy industry prefers to market its products, including probiotic fermented milks and yogurts, in plastic packaging.

Table 1: Chemical and microbiological quality of fermented Indian products available in Delhi markets*

Product	pH	Titratable acidity (%lactic acid)	TPC	Coliform Count	E.coli Count	Yeast and mold	Lactic Acid Bacteria Count
			log cfu/g	log cfu/g	log cfu/g	log cfu/g	log cfu/g
Amla pickle	3.02	3.06	3.626	<10	Absent	<10	<10
Lemon pickle	2.77	3.2	3.272	<10	Absent	<10	<10
Red chilli stuffed pickle	3.56	2.62	2.658	<10	Absent	<10	<10
Carrot murabba	3.16	0.7	1.778	<10	Absent	<10	<10
Amla murabba	3.52	0.52	1.544	<10	Absent	<10	<10
Fermented Idli batter	4.00	0.92	6.195	<10	Absent	3.861	4.062
Fermented Dhokla batter	3.85	1.92	6.605	<10	Absent	<10	4.470
Probiotic curd 1	4.1	0.81	5.313	<10	Absent	<10	4.278
Probiotic curd 2	4.02	0.80	5.445	<10	Absent	<10	5.874
Probiotic Curd 3	4.1	0.74	5.740	<10	Absent	<10	6.897
Curd 4	4.44	0.80	3.591	<10	Absent	<10	4.913
Curd 5	3.84	0.80	7.649	<10	Absent	<10	5.826
Chhach 1	4.22	0.75	6.978	<10	Absent	1.204	4.291
Chhach 2	4.20	0.70	7.342	<10	Absent	2.568	6.792

**Average of three determinations*

A study was conducted on fermented dairy product which was fermented in three different package types (clay pots, plastic cups and glass bottles) and stored at two different temperatures 29 °C and 4 °C. It was found that the Bifidobacteria survived best in the glass bottles, followed by the plastic packages and the clay pots when stored at 29 °C. The results obtained suggest that the use of packaging materials with low oxygen permeability properties is required to obtain a product that produces beneficial effects on the health of the consumer and a shelf life beyond 4 days. [(28)] Active packages with incorporated oxygen barrier materials or films with selective permeability properties also have potential applications in the packaging of probiotic food products. [(29)]

Further a study was conducted and it was observed that the viable counts of all the products met the prescribed minimum viable count of 10^5 to 10^6 CFU/g for the claimed health benefits for the consumer except for 3 samples. [(30)] Probiotic market in India is most potential market with growing interest in health care and need for preventive medicine. The major challenge to study the efficacy of probiotic products is to conduct meaningful studies to show the improvement in health or, even more challenging is to study the maintenance of the same. But there is no real regulatory framework for probiotics. In an initiative from the Indian Council of Medical Research, guidelines were formulated for the evaluation of probiotic foods, their strains, efficacy and health claim labeling. [(11)] A strict monitoring of the claimed probiotic products is required and regulators need to eliminate products which do not fit the definition or that contain unsubstantiated health/benefit claims from the market and provide a workable application procedure, allowing food products to obtain the recognition they deserve, based on the research performed. Developing a new dairy-free probiotic food is an expensive

process. Food companies have traditionally funded research for new food product formulations, but the stakes are higher for lactose-free products, for both food companies and consumers. [(31)] Thus, we must maintain and preserve the quality of products which could be obtained through spontaneous fermentation and is inexpensive also.

4. CONCLUSION

Many probiotic products are now available in Indian market which is further growing exponentially. Mainly dairy products are available and fruit and vegetable based options are very less. In many countries studies have highlighted the presence of probiotic nature in locally fermented products as well. Even in India also we can follow this by maintaining the temperature and quality of the product throughout the supply chain. Very few products tested showed the presence of Lactic acid bacteria in adequate amount. Thus a strict regulation is required to be formulated for the probiotic products. The regulations should be according to the substrate and the organism present. Organisms act differently in fermented foods with varied substrate. Thus a detailed regulations are needed keeping in mind Indian environmental conditions.

Conflict of Interest

Lamba J., Goomer S. declare that they have no conflict of interest.

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REFERENCES

1. Lilly DM, Stillwell RH. Probiotics: growth-promoting factors produced by microorganisms. Science. 1965 Feb 12;147(3659):747-8.
2. Sperty GS. Probiotics. West Point, Conn. Avi Publishing. 1971, 200 p.
3. Parker RB. The other half of the antibiotics story. Anim. Nutr. Health. 1974;29:4-8.
4. Fuller R. Probiotics in man and animals. J ApplBacteriol. 1989, 66:365–78.
5. Mortazavian AM, Mohammadi R, Sohrabvandi S (2012) Delivery of Probiotic Microorganisms into Gastrointestinal Tract by Food Products. New Advances in the Basic and Clinical Gastroenterology 10.5772/47946.
6. Shah NP, Lankaputhra WE, Britz ML, Kyle WS. Survival of Lactobacillus acidophilus and Bifidobacterium bifidum in commercial yoghurt during refrigerated storage. International Dairy Journal. 1995 Jan 1;5(5):515-21.
7. Shah NP. Functional foods from probiotics and prebiotics. Food technology. 2001.
8. Mills S, Stanton C, Fitzgerald GF, Ross R. Enhancing the stress responses of probiotics for a lifestyle from gut to product and back again. Microbial Cell Factories. 2011 Aug 30;10(1):S19.
9. Heller KJ. Probiotic bacteria in fermented foods: product characteristics and starter organisms. The American journal of clinical nutrition. 2001 Feb 1;73(2):374s-9s.
10. Stanton E. Malaysia's Markets for Functional Foods. Nutraceuticals and Organic Foods: An Introduction for Canadian Producers and Exporters. 2011.
11. Ganguly NK, Bhattacharya SK, Sesikeran B, Nair GB, Ramakrishna BS, Sachdev HP, Batish VK, Kanagasabapathy AS, Muthuswamy V, Kathuria SC, Katoch VM. ICMR-DBT guidelines for evaluation of probiotics in food. The Indian journal of medical research. 2011 Jul;134(1):22.
12. Association of Official Agricultural Chemists. "Official Methods of analysis". 14th ed. Association of Official Agricultural Chemists, Inc., Virgina.1984.
13. Indian Standards. Meat and meat products-enumeration of lactic acid bacteria- colony count technique at: 30^0 C. Bureau of Indian Standards. New Delhi. IS: 14844-2000.
14. Aneja KR. Experiments in microbiology plant physiology and biotechnology. 4th

ed. New age international (P) limited, publishers, 2003; 102-278.
15. Indian Standards. General guidance for the enumeration of microorganism-colony count technique at 30^0 C. Bureau of Indian Standards. New Delhi. IS: 5402-2002.
16. Indian Standards. General guidance for the enumeration of coliforms. Bureau of Indian Standards. New Delhi. IS: 5401(part 1)-2002
17. Indian Standards. Method for yeast and mould count of foodstuffs and animal feds. Bureau of Indian Standards. New Delhi. IS: 5403-1999.
18. Compendium of methods for the microbiological examination of foods. 2nd ed. In:Speck ML editor. American public Health Association, Washington DC, 1984. 265-279.
19. Xi X, Fan J, Hou Y, Gu J, Shen W, Li Z, Cui Z. Characterization of three cryptic plasmids from Lactobacillus plantarum G63 that was isolated from Chinese pickle. Plasmid. 2013 Nov 30;70(3):321-8.
20. D. Montet, G. Loiseau, and N. Zakhia-Rozis, "Microbial technology of fermented vegetables," in Microbial Biotechnology in Horticulture, R. C. Ray and O. P. Ward, Eds., vol. 1, pp. 309–343, Science Publishers, Enfield, NH, USA, 2006.
21. Indian Standards. Specification for pickles. Bureau of Indian Standards. New Delhi. IS: 3501-1966.
22. Caplice E, Fitzgerald GF. Food fermentations: role of microorganisms in food production and preservation. International journal of food microbiology. 1999 Sep 15;50(1):131-49.
23. Agaliya PJ, Jeevaratnam K. Screening of Lactobacillus plantarum isolated from fermented idli batter for probiotic properties. African Journal of Biotechnology. 2012;11(65):12856-64.
24. Indian Standards. Specification for dahi. Bureau of Indian Standards. New Delhi. IS: 9617-1980.
25. Champagne CP, Gardner NJ, Roy D. Challenges in the addition of probiotic cultures to foods. Critical reviews in food science and nutrition. 2005 Jan 1;45(1):61-84.
26. Gueimonde M, Delgado S, Mayo B, Ruas-Madiedo P, Margolles A, de los Reyes-Gavilán CG. Viability and diversity of probiotic Lactobacillus and Bifidobacterium populations included in commercial fermented milks. Food Research International. 2004 Dec 31;37(9):839-50.
27. Fasoli S, Marzotto M, Rizzotti L, Rossi F, Dellaglio F, Torriani S. Bacterial composition of commercial probiotic products as evaluated by PCR-DGGE analysis. International journal of food microbiology. 2003 Apr 15;82(1):59-70.
28. Jayamanne VS, Adams MR. Survival of probiotic bifidobacteria in buffalo curd and their effect on sensory properties. International journal of food science & technology. 2004 Aug 1;39(7):719-25.
29. da Cruz AG, de AF Faria J, Van Dender AG. Packaging system and probiotic dairy foods. Food Research International. 2007 Oct 31;40(8):951-6.
30. Karna BK, Emata OC, Barraquio VL. Lactic acid and probiotic bacteria from fermented and probiotic dairy products. Science Diliman. 2007;19(2):23-4.
31. Walzem RL. 2004. Functional foods. Trends Food Sci Technol 15:518.

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