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International Journal of Microbiology & Advanced Immunology (IJMAI)
ISSN 2329-9967

Microbiological Considerations for Probiotic Supplemented Foods

Research Article

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Abstract

Functional properties of probiotics coupled with consumer's inclination towards healthful foods have projected probiotics as a new ingredient in functional food market. Probiotic containing foods exhibits diverse health ben-efits and the starter cultures employed for formulation of probiotic supplemented food must possess certain pre-requisite characteristics to exhibit prophylactic properties. Probiotic containing foods available in the market are often of poor quality and did not meet the desired level of viable microorganisms, required for exhibiting health benefits. In the present article an endeavor has been made to highlight the significance of probiotic viability and their population for exhibiting health benefits and the quality of probiotic containing foods available in the global market and prerequisites for identity of a product as a probiotic food have also been delineated. Production of probiotic supplemented food with prophylactic is emerging to build-up consumer's confidence for long-term sus-tainability of probiotic food industries.

Keywords: Probiotic; Health claims; Food; Starter cultures

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Received: February 20, 2013
Accepted: March 20, 2013
Published: April 19, 2013

Citation: S. Sarkar. (2013). Microbiological Considerations for Probiotic Supplemented Foods. Int J Microbiol Adv Immunol, 1(1), 1-5. **doi:** http://dx.doi.org/10.19070/2329-9967-130001

Introduction

Fermentation employing lactic acid bacteria is the old-est, simple and safest means of food preservation [1] and relatively recently their efficacy to exhibit health benefits have been explored [2] With the realization of the link between diet and health, a world-wide consum-er's inclination towards functional foods that possess certain health properties besides basic nutrition have been noted. An intense acceptance of functional foods due to consumer's demand, social attitudes, scientific evidence of the human health benefits of a particu-lar ingredient and commercially driven interest to add value to existing foods were observed [3].

Probiotics may be defined as "live microbial food in-gredient that, when ingested in sufficient quantities, exert health benefits on the consumer"[4]. Probiotics are now emerged as an important category of food supplement and could be found in conventional, di-etary supplements and medicinal foods [5] in many countries including Japan, Europe and USA [6]. Sig-nificance of human gut microbiota in health restora-tion and maintenance have led acceptance of probiot-ics as functional foods[2] in current era of self-care and complementary medicine [7,8] and comprises approximately 65% of the world's functional food mar-ket [9]. Owing to diverse prophylactic properties, pro-biotic foods could fall into the category of functional foods [3] and consumption of functional probiotic bacteria is increasing due to promotion of gut health, disease prevention and therapy [10]. Beneficial health effects extend by probiotics is due to maintenance of the equilibrium of indigenous microbiota [11] with the growth inhibition of pathogenic microorganisms and boosting of innate and acquired immunity [12]. It has been established that viability and metabolic activities of probiotics during food processing, at the point of sale [13,14] and in host gastro-intestinal tract [15] are essential for extending health benefits. For long-term existence of probiotic as functional foods in the world market, it becomes imperative to ensure their higher viability till consumption and ability to exhibit probiot-ic effect [3]. Further well-designed placebo-controlled studies are emerging for determining the optimal dose, duration of treatment, selection probiotic strains, their mode of actions [16] and efficacy of multi-strain prep-arations [17] prior to their recommendations for thera-peutic or preventive use. In the present endeavor, an attempt has been made to highlight the microbiologi-cal considerations for probiotic selection to ensure its safe application in probiotic supplemented foods capa-ble of exhibiting prophylactic characteristics.

Viability of Probiotic Cultures

Significance of Probiotic Viability

For exhibiting prophylactic properties, cultured milk products must retain sufficient population of viable organisms throughout its anticipated shelf-life. In-gestion of acidophilus milk containing 3x107cells/ml L. acidophilus for 30 day induced a fall in blood serum cholesterol level in human volunteers [18]. Ingestion of yoghurt containing 108 cfu/g B. longum [19] and hydrolyzed whey formulae containing 1x109 cfu/g B. lactis [20] induced significant reduction in total se-rum cholesterol in humans and modify infant's gut mi-crobiota, thereby alleviating allergic inflammation.

An elevation in bifidobacteria counts as well as a de-cline in enterobacteria in mice cherishing bifidus milk containing 107 cells of B. longum for 14 days was noted [21]. Animal and infant feeding trials revealed a decline in coliforms and an increment in faecal bifidobacte-ria and/or lactobacilli due to ingestion of bifidus milk [22], Propiono-Acido-Bifido [PAB] milk [23,24] and dietetic yoghurt [25] containing B. bifidum [108 cfu/ml]. Ingestion of fermented milk containing 5x107 cfu/ml L. acidophilus and L. casei by human volunteers [26] or bifidus milk containing 108 cfu/g B. bifidum by infants[22] induced an elevation in faecal lactobacilli [7.59-8.93 log cfu/ml] and bifidobacteria (2.2x108-19.8x108 cfu/g), respectively.

Lactobacilli supplemented in milk at a level of 109 via-ble/day [27] or 1011 cfu/day [28,29] were efficacious in reducing the faecal β-glucuronidase and β-glucosidase activity in human subjects responsible for carcinogen-esis. Decline in nitroreductase activity during ingestion and its retention at a low level after cessation of intake of fermented milk product containing L. acidophilus [107 cfu/g], B. bifidum ([108 cfu/g) and Lactococcus lac-tis (108 cfu/g) and Lactococcus lactis subsp. cremoris (108 cfu/g) were noted [30].

An improvement in lactose digestion due to ingestion of milk supplemented with L. acidophilus (2.5x106 to 2.5x108cfu/ml) was observed [31]. Human trials re-vealed lowest breath hydrogen (9.9, 22.8, 50.2 ppm) due to ingestion of cultured yoghurt in contrast to heated cultured yoghurt or direct acid yoghurt, respec-tively [32]. Decrement in viable population (3x108/g to 3.4x106/g) and lactase activity (0.64 to 0.07 units/g) due to pasteurization [33] and better tolerance of non-pasteurized yoghurt than pasteurized yoghurt by lactase-non-persistent individuals [34,35] indicate sig nificance of viable population.

It has been established that to achieve health benefits through ingesting cultured milk products especially yoghurt [36,37], probiotic cultures must retain its vi-ability at a level of >106 cfu/g [38]. It has been men-tioned that the viability of microorganisms must be retained both at the end of incubation as well as at the date of expiry of the product [39]. Suggested daily intake being >108/g [40,41], probiotic products must be consumed regularly in sufficient quantities to de-liver the relevant dosages of live bacteria to the gut [42]. The recommended intake is 300-400g/ week [43] or 100g/day [44]. Recommended viable population of probiotic to be present in food by different agencies is depicted in Table I.

Viability of Probiotics in Probiotic Foods

Several reports indicated poor viability of probiotics in health products [40,49] and often present at levels lower than those claimed on label [50,51]. Survey re-ports on fermented functional foods and health-care products indicated lower microbial contents than the labelled claim in few health-care products, whereas for bio-yoghurts no indication microbial content was fur-nished [52].

Bifidobacterium sp. could not be detected in drinking yoghurt-containing probiotics and reported that the identified strains do not always correspond to those declared on the label [53]. Bifidobacteria could be de-tected at a level 0.0 to 6.0 log cfu/ml only in 76% of the analyzed samples of bio-yoghurt containing Bifidobacteria sp. [54,55]and respectively in 90 and 50% of samples during purchase and date of expiry [56]. Recent market survey in Columbia on bio-yoghurts re-vealed that though the products had a total viable cell population of 107cfu/ml, however Bifidobacterium could be recovered only in 14.29 % samples [57].

Presence of viable population of L. acidophilus and Bi-fidobacterium sp. at a level lower than the recommended level (106cfu/g), by the expiry date of most of the market probiotic yoghurts have been mentioned [58,59]. Poor viability and large deviation in viability of bifidobacteria in yogurt have been mentioned [60] and are present at non-detectable levels or at a level of 104 -107 cfu/ml [58] or 106 cfu/ml [61]. Bifidobacte-ria were less acid tolerant than L. acidophilus [62] and were detected at a level of 106 cfu/ml, respectively in 14 and 24% of yoghurt samples [61] and both retained their viability at a level of >105 cfu/ml during storage [63]. Lower bifidobacterial population (<103 cfu/g) than L. acidophilus (<103-108 cfu/g) were detected in few Australian yoghurt containing probiotic cultures [64]. However, another investigation indicated B. bifi-dum was to be more resistant to yoghurt environment than L. acidophilus [65,66] and the counts declined from 1.54±0.45x109 to 0.38±0.02x109 cfu/ml during 15 days storage [67]. Stability of bifidobacteria and L. acidophilus in yoghurt environment is pH dependent.

Decline in viability of bifidobacteria and L. acidophilus were negligible at pH 5.0 but population declined by 0.1-7.6 log cycles and 1.6-6.2 log cycles, respectively at pH 4.0 [68]. Micro-aerophilic and anaerobic charac-teristics of L. acidophilus and Bifidobacteria sp. render them susceptible to oxygen contained in the yoghurt, resulting in their poor viability during its anticipated shelf-life [69] It has been annunciated that the initial concentration of yoghurt cultures must be maintained at 108-109 cells/ml in milk for sustaining therapeutic dosage up to 21 days/ 5°C [70]due to loss of viability by heat, pressure, low water activity and high acidity [71]

Besides instability of probiotic cultures in product it-self, viability is also lost during its transit through in-testinal tract. Viability of

Table 1: Recommended probiotic viability in probiotic foods

Viability Requirements (Min. cfu/ml)		Recommending Agencies	References
107	Lactobacillus acidophilus	International Dairy Federation	45
106	Bifidobacteria	International Dairy Federation	45
106	Lactic cultures	Australian Food Standards Code	46
108	Lactic acid bacteria	National Yoghurt Association	40
106	Bifidobacteria	Swiss Food Regulation	47
106	Bifidobacteria	Fermented Milk and Lactic Acid Beverages Association	40
107	Lactic acid bacteria	Spanish Yoghurt Quality Standards	48

lactic acid bacteria is report-ed to get influenced by gastric pH, digestive enzymes, bile salts [72] and must be adapted to the intestinal environment for its prolonged survival [73], as only 20-40% probiotic cultures survive the gastric transit [74]. Though appreciable growth of B. bifidum and L. acidophilus in presence of bile salts [75] and better sta-bility of former organism than Lactobacillus delbruekii subsp bulgaricus in the intestinal environment have been denoted but their survivality declined during passage through intestinal tract [25]. It has been annunciated that ingestion of fermented milk containing probiotic cultures resulted in survival of 23.5±10.4% bifidobac-teria [76], 30% B. bifidum, 10% L. acidophilus [77], 6.54-9.8% bifidobacteria and 4.4-7.45% lactobacilli [24,25] in the ceacum.

It is therefore necessary to ensure retention of viability of probiotic organisms both during processing, stor-age as well as transit through gastrointestinal tract with the objective of achieving prophylactic effects.

Factors Affecting Viability of Probiotics

Following factors affect the viability of probiotics in yoghurt during manufacture, storage and gastrointes-tinal tract transit.

- acid and hydrogen peroxide production by yoghurt cultures
- dissolved oxygen content of the product
- oxygen permeability through the package [78]
- concentration of lactic and acetic acids in the prod-uct [79].
- fat content of milk [66]
- heat-treatment of milk
- incubation temperature [80]
- concentration of buffers such as whey protein con-centrate [81]
- physiological status of probiotic cultures added
- physical condition of product storage
- possible interactions of the product with starter cul-tures [82].

Microbiological Considerations for Probiotic Supplemented Foods

Microbiological Considerations for Probiotic Selection

Selection of probiotic cultures intended for supple-mentation in foods should be based upon following criteria.

- must retain the functional health characteristics for which they were originally selected [83]
- beneficial effect on the host organism
- should adhere to the mucosal epithelial cells
- should exhibit enhancement and protection of the intestinal ecology [84]
- does not have the ability to invade the host intestinal tissues and cause any infection
- sensitive to broad spectrum and commonly used an-tibiotics [85]
- should be isolated from the same species as its in-tended host
- should be able to survive transit through the gastro-intestinal tract [86]
- every strain must exhibit efficacy of health benefits [87]
- must be non-pathogenic, non-toxic, and free of sig-nificant adverse side effects
- must retain stability during the intended shelf life of the product
- must contain an adequate number of viable cells to confer the health benefit
- must be compatible with product format to maintain desired sensory properties
- must be labeled in a truthful and informative manner to the consumer [88]

Microbiological Considerations for Health Claims

A 'health claim' is defined as "a statement, which char-acterizes the relationship of any substance to a disease or health-related condition, and these should be based upon well-established, generally accepted knowledge from evidence in the scientific literature and/or rec-ommendations from national or international public health bodies [89]. Probiotic can be commercialized either as nutritional supplement, pharmaceutical or foods but the marketing as a pharmaceutical product requires significant time, complex and costly research and demonstration of well-defined therapeutic targets [90]. Obstacles in providing probiotic therapy include selection of appropriate strains, poorly regulated probiotic quality, human biological factors which impair probiotic viability, difficulties in maintaining new bac terial population in the gut and local product [91]. Vari-ous clinically relevant steps required for the acceptance of probiotics by the medical community are enumer-ated underneath [92].

- implementation of Guidelines for the use of probi-otics
- phase I and II clinical trial data on strains and end products to prove health benefits
- use of Good Manufacturing Practices and produc-tion of high quality products
- studies which identify mechanisms of action of pro-biotic strains in vivo
- appropriate information dissemination about prod-ucts to physicians, health professionals and lay people
- development of probiotic organisms that carry vac-cines or other beneficial substances to the host
- development of anti-viral probiotics
- expansion of proven strains to benefit the oral cavity, nasopharynx, respiratory tract, stomach, vagina, blad-der, and skin as well as for cancer, allergies and recov-ery from surgery and injury

Microbiological Considerations for Safety Aspects

The usual approach for safety assessment for market-ing probiotic bacteria in the United States is presump-tion of safety, reasoned by a long history of safe use in fermented dairy products [93]. GRAS [Generally Recognized as Safe] substances are food substances judged by qualified subject experts as safe under the intended conditions of use. It should not be assume that all probiotics are GRAS, even if they are com-posed of species of Lactobacillus or Bifidobacterium [88]. In recognition of the importance of assuring safety, even among a group of bacteria that is GRAS, assess-ment of safety of a probiotic should be based upon the following documents.

- determination of antibiotic resistance patterns
- assessment of certain metabolic activities (e.g., D-lactate production, bile salt deconjugation)
- assessment of side-effects during human studies
- epidemiological surveillance of adverse incidents in consumers (post-market)
- strain must be tested for toxin production if the strain under

evaluation belongs to a species that is a known mammalian toxin producer

- determination of hemolytic activity of strain is re-quired if the strain under evaluation belongs to a spe-cies with known hemolytic potential [94]
- efficacy of the novel strains and the safety status of the traditional product in which they will be incorpo-rated must be evaluated prior to their incorporation [95]
- if applicable, establishing a history of safe use based on the intended use of the species in question
- conducting toxicity or pathogenicity assessments in validated laboratory or animal models that are relevant to the species being considered, as needed [88]

Conclusion

Recently, worldwide consumer's interest in probiotics as a functional food has increased dramatically ow-ing to its potential human health benefits. Viability of probiotics at a desired level at the end of shelf-life of the product is the key factor for exhibiting health beneficial effects; however recent market surveys indi-cate their poor viability. Microbiological considerations must be given for probiotic selection to ensure its safe application in probiotic supplemented foods capable of exhibiting prophylactic characteristics. Extensive clinical trials are indicated prior to clinical application.

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