Microbiological Considerations for Probiotic Supplemented Foods

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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-efts 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 microorgan-isms, 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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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 consumer’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-redient that, when ingested in sufficient quantities, exert health benefits on the consumer”[4]. Probiotics are now emerged as an important cat-egory of food supplement and could be found in con-tentional, 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 ac-

ceptance 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]. Ow-
ing 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 equilib-rium 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 impera-
tive to ensure their higher viability till consumption and ability to exhibit probiotic-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 yo-ghurt 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 in-fant’s gut mi-crobiota, thereby alleviating allergic inflammation.


An elevation in bifidobacteria counts as well as a decline in enterobacteria in mice consuming 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 fecal bifidobacteria and/or lactobacilli due to ingestion of bifidus milk [22], Propionio-Acido-Bifido (PAB) milk [23,24] and dietetic yoghurt [25] containing bifidus [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 fecal lactobacilli [7.59-8.93 log cfu/ml] and milk bifidobacteria (2.2x108-19.8x108 cfu/g), respectively.

Lactobacilli supplemented in milk at a level of 109 viable/day [27] or 1011 cfu/day [28,29] were efficacious in reducing the fecal 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, respecti-vely [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 suf-ficient 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 1.

**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]. Bifido-bacteria could be de-tested at a level 0.0 to 6.0 log cfu/ml only in 76% of the analyzed samples of bio-yoghurt containing Bifi-dobacteria 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-yogurths re-vealed that though the products had a total viable cell population of 107cfu/ml, however Bifidobacte-rium 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 vi-ability of bifidobacteria in yogurt have been mentioned [60] and are present at non-detectable levels or at a level of 104-107cfu/ml [58] or 106 cfu/ml [61]. Bifidobacteria were less acid toler-ant than L. acidophilus [62] and were detected at a level of 106cfu/ml, respectively in 14 and 24% of yoghurt samples [61] and both retained their viability at a level of >105cfu/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. bifidum 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 neg-ligible 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, re-sulting in their poor viability during its anticipated shelf-life [69]. It has been announced that the initial concentration of yoghurt cultures must be maintained at 108-109 cells/ml in milk for susta-ining 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**

<table>
<thead>
<tr>
<th>Viability Requirements (Min. cfu/ml)</th>
<th>Recommending Agencies</th>
<th>References</th>
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<tr>
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<tr>
<td>106 Bifidobacteria</td>
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<tr>
<td>106 Bifidobacteria</td>
<td>Fermented Milk and Lactic Acid Beverages Association</td>
<td>40</td>
</tr>
<tr>
<td>107 Lactic acid bacteria</td>
<td>Spanish Yoghurt Quality Standards</td>
<td>48</td>
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lactic acid bacteria is report-ed to get influenced by gastric pH, digestive enzymes, bile salts [72] and must be adapted to the intesti-nal environment for its prolonged survival [73], as only 20-40% probiotic cultures survive the gastric transit [74]. Though appreci-able growth of B. bifidum and L. acidophilus in presence of bile salts [75] and better sta-bility of former organism than Lactoba-cillus delbruekii subsp bulgaricus in the intestinal environment have been denoted but their survivality declined during passage through intestinal tract [25]. It has been announced 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% lacto-bacilli [24,25] in the caecum.

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

Factors Affecting Viability of Probiotics

Following factors affect the viability of probiotics in yoghurt dur-ing manufacture, storage and gastroin-testinal 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 Supple-mented Foods

Microbiological Considerations for Probiotic Selection

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

- must contain the 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 lit-erature and/or rec-ommendations from national or international public health bodies [89]. Probiotic can be commercialized ei-ther 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 enu-mer-at-ed 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, na-sopharynx, 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 probi-otic bacteria in the United States is presump-tion of safety, rea-soned 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 assumed that all probi-otics are GRAS, even if they are com-posed of species of Lactoba-cillus 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 pro-duction, bile salt deconjugation)
- assessment of side-effects during human studies
- epidemiological surveillance of adverse incidents in consum-ers (post-market)
- strain must be tested for toxin production if the strain under
evaluation belongs to a species that is a known mammalian pathogen. The determination of hemolytic activity of the strain is required if the strain under evaluation belongs to a species with known hemolytic potential.

Efficacy of the novel strains and the safety status of the traditional products in which they will be incorporated must be evaluated prior to their incorporation.

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.

**Conclusion**

Recently, worldwide consumer interest in probiotics as a functional food has increased dramatically owing 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 indicated 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.

**References**


