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PROBIOTICS AND ITS HEALTH BENEFITS

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Abstract

Probiotics are live microorganisms used as feed supplements to provide health benefits by improving intestinal microbial balance in the human body. LAB are the most important probiotics. Today they are incorporated into wide variety of foods.The quality of the product is based on the viability of the probiotic bacteria. We will discuss about their therapeutic role, suggested levels, viability, selection, mode of action, synbiotics and prebiotics.

Key words: Probiotics, LAB, Synbiotics, health benefits, viability, microencapsulation, mechanism of action.

INTRODUCTION

The term probiotic is derived from two Greek words which literally means “for life” [1]. The first scientist to discover health benefits of probiotics was the immunologist Dr. Eli Metchnikoff who was awarded the Nobel Prize in 1908 [2].

Generally, probiotics are live microorganisms that are used as feed supplements to provide health benefits by improving intestinal microbial balance in the human body [3].

Food and Agriculture Organization (FAO) of the United Nations and the World Health Organization (WHO) defines probiotics as “live micro organisms that when administered in adequate amounts confer a health benefit on the host” [4].

It includes a large range of microorganisms, mainly bacteria but also yeasts. LAB are the most important probiotic known to have beneficial effects on the human gastro-intestinal (GI) tract. These bacteria are Gram-positive and usually live in a non-aerobic environment but they also can survive aerobic conditions.

The examples of probiotics are *Lactococcus lactis, Enterococcus faecium, Escherichia coli, Saccharomyces cerevisiae, Saccharomyces boulardii*, bifidobacteria etc. [5, 6].

Most probiotic bacteria produce lactic acid. The lactic acid keeps the gut at a low pH, maintains the gut microflora and helps preserve the dairy products. Probiotics also combat the growth of harmful pathogens that cause foodborne illnesses (i.e. diarrhea) such as *Salmonella* and *Escherichia coli*. The probiotics prevent the attachment of these pathogens by competing for similar binding sites on the gut epithelium [7].

LAB are important in fermented foods for ripening and aroma development of certain cheeses and sausages, especially those produced in the Meditteranean [8], also used as starter cultures, co-cultures and bioprotective cultures in the food industry [9].

In fermented foods, LAB have been shown to produce antimicrobial compounds, such as organic acids, bacteriocins and antifungal peptides.

The United States Food and Drug Administration has categorized LAB as ‘generally regarded as safe’ (GRAS).

The global sales of probiotic supplements were predicted to rise 48% from $2.7bn in 2011 to $4bn in 2016 (www.nutraingredients.com/ Consumer-Trends). In US, per capita spending on probiotic supplements is expected to nearly double by 2016 and overtake Japan. Indeed, the market of probiotics and healthy food has great potential to grow, especially in Asia [10]. A bioactive compound influencing health that issynthesized by a probiotic culture is called ‘probioactive’ [11].

Why should we consume probiotics in foods?

The human gastrointestinal (GI) tract is “home” to a complex consortia of trillions (approximately 1×10^{13} to 1×10^{14}) of microbes, thousands of bacterial phylotypes, as well as

hydrogen- consuming methanogenic archaea, colonizing the entire length of the gut with a collective genome (also termed as microbiome) that contains at least 100-times as many genes as our own genome [12, 13]. Cutting edge research and vast accumulating data indicates that the gut microbiota is instrumental in energy metabolism and immune function of the host, and has a crucial role in the development of numerous conditions including obesity [14], diabetes [15], non-alcoholic fatty liver disease [16], inflammatory bowel diseases [17], and cancer [7].
The natural balance in our intestinal system can be disturbed by: bacteria infections, stress, antibiotic treatment, travelling. This may also result in intestinal disturbances such as diarrhoea, constipation, etc. Taking probiotics through food or supplements helps to balance the microbiota in the gut [18, 19].

Why Probiotics?

Why consider the value of probiotics to human health?

Boosting the body's ability to resist infection prevents morbidity, decreases antibiotic use (and possibly the spread of antibiotic-resistant pathogens), and decreases some primary infections. Exposure of the immune cells of the intestinal tract to the right types of microbes in infancy may be important to the prevention of allergy development later in life [20].

Probiotics play an important role in the control of irritable bowel syndrome and inflammatory bowel diseases, suppression of endogenous/ exogenous pathogens by normalization of the intestinal microbial composition, alleviation of food allergy symptoms in infants by immunomodulation, lowering serum cholesterol, improving lactose tolerance, and reducing risk factors for colon cancer by metabolic effects [21].

Probiotics play a therapeutic role by lowering cholesterol, improving lactose tolerance, nutritional enhancement and preventing some cancers and antibiotic associated diarrhoea [22].
Their health benefits may be due to the production of acid and/or
bacteriocins, competition with pathogenic bacteria preventing their adhesion to the intestine and enhancement of the immune system [23].

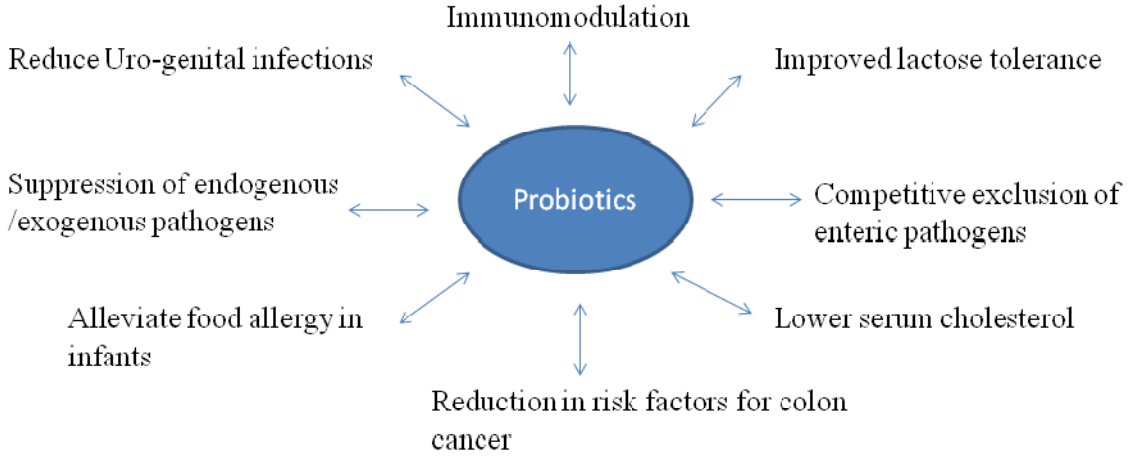

Figure 1. Health benefits of probiotics [24].

Table 1. Important studies for safety assessment of probiotic lactic acid bacteria (LAB) and other bacteria [25].

Type of property studied	**Safety factor to be assessed**
Intrinsic properties of lactic acid bacteria	Adhesion factors, antibiotics resistance, existence of plasmids and *Tra* genes, harmful enzyme profiles
Metabolic products	Concentrations, safety and other effects
Toxicity studies	Acute and subacute effects of ingestion of large amounts of tested bacteria
Mucosal effects	Adhesion, invasion potential, intestinal mucus degradation, infectivity in immunocompromised animals
Dose response effects	Dose response studies by oral administration in volunteers
Clinical assessment	Potential for side effects, careful evaluation in healthy volunteers and disease specific studies
Epidemiological studies	Surveillance of large populations following introduction of new strains and products

Food, particularly dairy products are considered as an ideal vehicle for delivering probiotic bacteria to the human gastrointestinal tract [26]. Dairy products may enhance microbial survival in gastric juice, most likely due to a buffering or protective effect [27].

In the United States, there are no approved probiotic drugs for human use, although one product, Preempt, which was developed by the United States Department of Agriculture, is available as an animal drug (the microbial preparation is used on newly hatched chicks to help prevent colonization by pathogens). Probiotics sold as pharmaceuticals are available in other countries. In the United States, food products containing probiotic bacteria are almost exclusively dairy products. Products such as breakfast cereal and nutrition bars containing probiotics are not currently available, but they present attractive possibilities for future probiotic products [20].

Probiotics were first commercialized via yogurts: Yakult was introduced in Japan in 1935, followed by Activia introduced in France in 1987. After 2006, the interest in probiotic products increased rapidly in the world (USA, Europe and Asia) and other fermented dairy products, as well as other foods, became food vectors to deliver probiotics to consumers. Among dairy products, yogurt is likely the most recognized product containing probiotics [28].

Examples of Some Commercially Available *Lactobacillus* and *Bifidobacterium* Probiotic Strains [20].

Strain	**Source**
L acidophilus NCFM	Rhodia, Inc. (Madison, WI, USA)
L acidophilus DDS-1	Nebraska Cultures, Inc. (Lincoln, NE)
L paracasei Shirota* (YIT 9018)	Yakult (Tokyo, Japan)
L paracasei Immunitas (DN 014001)	Danone (Paris, France)
B longum SBT-2928*	Snow Brand Milk Products Co., Ltd.
B breve strain Yakult*	Yakult
B lactis DR10 (HN019)	Fonterra Cooperative Group Ltd.

For the probiotics to be able to deliver their health benefits, the bacteria need to be viable in the gut [29]. The viability of probiotic cells is of paramount importance because to have their beneficial effects on the host's health they must stay alive as far as their site of action. The quality of the product is based on the viability of the probiotic bacteria, and the more is the viability of bacteria, the higher will be its quality [30, 31, 32]. They also should not affect the sensory characteristics of the food in any perceptible way, remain stable throughout processing and storage of the product and be resistant to the gastrointestinal environment. The efficiency of added probiotic bacteria depends on dose level and their viability must be maintained throughout storage, products shelf-life and they must survive the gut environment [33].

For probiotics delivered through foods, additional amounts of cells are likely required prior to processing to account for the loss of cells during the processing and/or storage phases.

Several factors have been reported to affect the viability of probiotics in fermented dairy products, including titratable acidity, pH, hydrogen peroxide, dissolved oxygen content, storage temperature, species and strains of
associative culture organisms, concentration of lactic and acetic acids and even whey protein concentration [34, 6, 35], strain variation, acid accumulation, interaction with starter cultures, and storage condition [36].

Analysis of probiotic products has confirmed that probiotic strains exhibit poor survival in traditional fermented dairy products [37, 28].

Suggested levels of probiotics frequently referred to as therapeutic levels ranged from10^6 [38] to over 10^7 or 10^8 cfu/ml [28]. The International Dairy Foundation (IDF) has recommended a minimum number of 10^7 CFU per gram of the product consumed [39]. The Canadian Food Inspection Agency [40] recommends a level of 10^9 CFU per serving to be able to present generic health claims.

Viability of probiotic bacteria can be improved by appropriate selection of acid and bile resistant strains, use of oxygen impermeable containers, two-step fermentation, stress adaptation and by sonication of yogurt bacteria [41].

Various other approaches have been attempted so as to increase the resistance of probiotic bacteria against adverse conditions like use of micronutrients such as peptides and amino acids, and micro-encapsulation [42].

Microencapsulation is a useful tool for improving the delivery of bio-active compounds in foods particularly probiotics [43]. Microencapsulation is a process by which live cells are packaged within a shell material, which confer them protection by preventing their direct exposure to unfavourable environment, but permits diffusion of nutrients in and out of the matrix, thereby supporting the viability of the cells [44]. Microencapsulation improves the survivability of probiotics and sensory attributes of product due to protective action against adverse conditions in fermented dairy foods like yoghurt. Micro-encapsulation confers protection to sensitive probiotic lactic acid bacteria from oxygen [45], freezing [46], acidic conditions during manufacture and storage [47], and gastrointestinal transit [48] and its efficacy is established by pH, composition and texture of food matrix, strains of culture employed [49], initial cell population [48] method of encapsulation and wall material used [50]. In dairy industry, microencapsulation has been applied to improve survival and delivery of bacterial cultures [51].

Electron microscopy is an effective technique to provide evidence of the presence of probiotics in capsules or beads and to assess the bacterial loading and size structure of the capsule [52]. The microbeads with diameters more than the special limit (>100 μm) can deteriorate mouth-feel properties of products such as liquid milk, yogurt and sour cream due to the appearance of the special sense of coarseness [53]. Various biopolymers have been utilized for coating probiotic cells. Typical biomaterials used for the purpose of probiotic encapsulation include: alginate, carrageenan, gelatin, chitosan and cellulose acetate phthalate [54]. Depending on the method used, the two methods are extrusion or droplet method and emulsion or two phase systemmethod. From various studies it has been concluded that encapsulation by both of these methods has increased the viability of probiotic bacteria more than 80% [55, 56, 57, 58].

Microencapsulation of probiotic bacteria also has widespread application during biomass production in the fermentors. For instance, microencapsulation enhanced the resistance of microorganisms against different conditions such as chemical poisoning agents, bacteriophage infections [59] and undesirable changes like genetic mutations. Hence, it resulted in increased biomass production of bacterial culture during fermentation and increased the metabolite production as well [60, 61].

Probiotics (usually 1-4 μm) are too big for nano-technology [43], so microencapsulation is a useful tool for improving the delivery of the active probiotics.

The mechanism of action of probiotic bacteria

The effects of probiotics can be classified in three modes of action.

The first is related with the modulation of the host's defences which is most likely important for the prevention and treatment of infectious disease and also for treatment of intestinal inflammation [62]. Probiotics may influence the immune system by means of products such as metabolites, cell wall

components or DNA. In fact, these products can be recognised by the host cells sensitive for these because of the presence of a specific receptor [63]. The main target cells are generally the gut epithelial and the gut-associated immune cells. Finally, the interaction between probiotics and the host's immune cells by adhesion might be the triggering signalling cascade leading to immune modulation [64].

The second mechanism of action can be described by a direct effect on other microorganisms which can be commensal and/or pathogenic. In this case, the therapy and the treatment of infections are concerned but restoration of the microbial balance in the gut is an important factor too [65]. Probiotics have the ability to be competitive with pathogens and therefore allow for preventing their adhesion to the intestine [66]. Eventually, probiotics have the ability to affect some microbial products such as toxins and host products like bile salts and food ingredients [67].

However, it is important to know that these three mechanisms of action are strain-dependent, and to date the modes of action of probiotic bacteria are not yet fully known [68].

Synbiotics

A synbiotic has been defined as "a mixture of prebiotics and probiotics that beneficially affects the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract, by selectively stimulating the growth and activating the metabolism of one or a limited number of health promoting bacteria, and thus improving host welfare" [69]. Synbiotics are not only a mixture of probiotics and prebiotics but a synergy between the two components [70].

Synbiotics can be used to modulate both composition and activity of the gut microbiota in a way beneficial to the human host [71].

Probiotic and prebiotic combination might improve the survival of the bacteria crossing the upper part of the GI- tract , thereby enhancing their effects in the

large bowel. In addition, their effects might be addidive or even synergistic.

The combination of suitable prebiotics with probiotics has been found (from both *in vitro* and *in vivo* experiments) to stimulate the survival and activity of the organism, for example a FOS in conjunction with a *Bifidobacterium* strain or lactitol in conjunction with *Lactobacillus*. Besides the synergistic effect in which the growth of beneficial bacteria (existing strains) in the colon is promoted, synbiotics also act to improve the survival, implantation and growth of newly added probiotic strains. The combination of *Bifidobacterium* and oligofructose has been found to act synergistically and retard colon carcinogenesis in rats compared to either given individually [72].

Prebiotics are non-digestible complex carbohydrates that selectively stimulate the growth and/or activity of bacteria in the colon and also beneficially affect the host [69]. They may exert a protective effect towards selected probiotic bacteria in improving their survival and activity during storage of the product containing probiotics as well as passage through the upper parts of the gastrointestinal tract (GIT). A range of oligosaccharides has been tested in this regard [73, 74] with inulin and other fructo-oligosaccharides frequently employed in studies as they resist digestion by gastric acid and pancreatic enzymes in vivo [75].

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